The transfer of environmental contaminants (Brominated and Chlorinated dioxins and biphenyls, PBDEs, HBCDDs, PCNs and PFAS) from recycled materials used for bedding to the eggs and tissues of chickens

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Abstract

Some types of poultry bedding made from recycled materials have been reported to contain environmental contaminants such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs, dioxins), polychlorinated biphenyls (PCBs) brominated flame retardants (BFRs) polychlorinated naphthalenes (PCNs), polybrominated dioxins (PBDD/Fs), perfluoroalkyl substances (PFAS), etc. In one of the first studies of its kind, the uptake of these contaminants by chicken muscle tissue, liver, and eggs from three types of recycled, commercially available bedding material was simultaneously investigated using conventional husbandry to raise day old chickens to maturity. A weight of evidence analysis showed that PCBs, polybrominated diphenylethers (PBDEs), PCDD/Fs, PCNs and PFAS displayed the highest potential for uptake which varied depending on the type of bedding material used. During the first three to four months of laying, an increasing trend was observed in the concentrations of ΣTEQ (summed toxic equivalence of PCDD/Fs, PCBs, PBDD/Fs, PCNs and polybrominated biphenyls), NDL-PCBs and PBDEs in the eggs of chickens raised on shredded cardboard. Further analysis using bio-transfer factors (BTFs) when egg production reached a steady state, revealed that some PCB congeners (PCBs 28, 81, 138, 153 and 180) irrespective of molecular configuration or chlorine number, showed the highest tendency for uptake. Conversely, BTFs for PBDEs showed good correlation with bromine number, increasing to a maximum value for BDE-209. This relationship was reversed for PCDFs (and to some extent for PCDDs) with tetra- and penta- chlorinated congeners showing a greater tendency for selective uptake. The overall patterns were consistent, although some variability in BTF values was observed between tested materials which may relate to differences in bioavailability. The results indicate a potentially overlooked source of food chain contamination as other livestock products (cow's milk, lamb, beef, duck, etc.) could be similarly impacted.

1. Introduction

A number of countries pursue economic policies aimed at the elimination or reduction of waste through the continuous use of resources. These policies promote the recycling of materials, simultaneously generating employment, reducing energy and resource consumption as well as waste (Stahel, 2016). The farming and food production industry is similarly encouraged to support these initiatives. Poultry products are currently more widely consumed than other animal products and the industry makes a significant contribution to the value of livestock farming – around 39 % of all meat produced globally (FAO, 2022), with a combined value expected to exceed $0.4 trillion by 2025 (Feed Additive Magazine, 2025). The industry increasingly uses a number of renewable services (energy) and goods such as bedding materials. Traditionally, sawdust and softwood shavings made from clean wood were most commonly used as bedding material (also referred to as litter) for chickens, but in recent years the increasing costs of this natural product combined with the pressure for greater sustainability of resources has led to an increase in the use of recycled materials that would previously have been disposed of as waste. This demand is also supported by regulation in different parts of the world, e.g., the European Union landfill directive (European Commission, 1999), itemised series of regulations in Japan (MOE, 1998), or regulations that are specific to some individual states as seen in North America.

The quality and type of bedding material is an important aspect of poultry husbandry and the welfare of the birds. In addition to providing comfort and warmth, bedding should absorb moisture from droppings, reducing faecal material in the upper layers by letting it permeate to the bottom. Upper layers of bedding should remain dry, allowing natural scratching behaviour and insulating the birds from the lower moister layers above packed soil or concrete flooring where they are generally housed. It should be non-toxic to the birds or handlers (Grimes et al., 2006), hygienic, and also minimise the build-up of ammonia based odour (Worley et al., 1999), which can be detrimental to the respiratory systems of the birds. Practically, most bedding types are organic materials (although sand can also be used) that are dry, absorbent, and from a commercial point of view, low-cost. Many types of bedding material are used depending on local availability and costs and include wood shavings, sawdust, shredded cardboard, dried paper sludge (DPS), peanut hulls, shredded sugar cane and straw.

Bedding materials produced through the recycling of waste wood are commonly used for raising poultry and other livestock, and are generally derived from clean grades of waste wood, e.g., Grade A (Defra, 2008). This grade includes “clean” solid softwood and hardwood, packaging waste, scrap pallets, packing cases etc. and is expected to use untreated wood. The selection of recycled wood however, is based on visual inspection which may not always be able to distinguish chemically treated wood from untreated material. This provides a potential source of contamination within recycled bedding products and earlier studies (Fries et al., 1999; Brambilla et al., 2009; Piskorska-Pliszczynska et al., 2016) have demonstrated raised polychlorinated dibenzo-*p*-dioxin and furan (PCDD/F) levels in milk and eggs resulting from the intake of pentachlorophenol treated wood (historically, pentachlorophenol was used as a wood preservative and was later found to be contaminated with PCDD/Fs).

Another material that is used for producing bedding for livestock including poultry is shredded cardboard (Fewell, 2019). Chopped corrugated cardboard can be mechanically cut into small pieces to produce a bedding material that is more absorbent than shredded paper. It is longer lasting than recycled paper or straw as the corrugation helps to resist the compacting that these materials suffer from. Additionally, it is easy to remove and as it is biodegradable it can be recycled further by addition as a feed to composting facilities.

DPS is a by-product that arises from the deinking of paper during recycling. The sludge is a nonhazardous solid waste that is removed by mechanical treatment and contains fibrous material and paper-making fillers. These generally comprise of cellulose and filler materials such as kaolin and calcium carbonate. The sludge is processed to remove excess water and then dried to yield a light friable material of 95% dry matter with good desiccant properties. DPS provides a highly absorbent bedding product, with good thermal properties and has been evaluated as a poultry bedding material (Villagra et al., 2011). It does not produce much dust, degrades quickly and from a hygiene viewpoint, tends to have low spore and pathogen levels.

Most recycled materials have an inherent degree of anthropogenicity which may arise either during production of the original material or during the recycling process or both. This introduces the potential for inadvertent inclusion of contaminants (Schlummer et al., 2004) as has been observed in the past (Rigby et al., 2015, 2021; Fernandes et al., 2019; Gerber et al., 2020; Mohajerani and Karabatak, 2020; Conesa et al., 2021). The type of contamination depends on the origins of the material or the recycling process, e.g., chicken pecking blocks made from recycled ash can contain PCDD/Fs, recycled paper and card materials can contain flame retardants and perfluorinated alkyl substances (PFAS) that were originally part of the printing and labelling material or a grease-repellent coating. These contaminants are of concern to animal and human health, with dietary intake being a primary pathway to human exposure. The most concerning effect of dioxin-like contaminants is the ability of the planar congeners within these groups to bind to the cellular aryl hydrocarbon receptor (AhR), which influences the initiation, promotion, and progression of carcinogenesis (Denison et al., 2002; Fernandes et al., 2022) Dioxin-like contaminants include PCDD/Fs, dioxin-like polychlorinated and polybrominated biphenyls (PCBs and PBBs), polychlorinated naphthalenes (PCNs) and polybrominated dibenzop-dioxins and furans (PBDD/Fs) (Van den Berg et al., 2013; Fernandes et al., 2021, 2022). The AhR mediated toxicity associated with each of these contaminant classes is cumulative, but other effects are also known and include thymic atrophy, teratogenesis, reproductive effects, chloracne, immunotoxicity, enzyme induction, etc. (McGregor et al., 1998).

The toxicity associated with the brominated flame retardants (BFRs) is mainly endocrine disruption, neuro-developmental and neuro-behavioural (delayed motor skill & lower IQ in humans), but also includes other effects such as synaptic plasticity, reproductive toxicity, estrogenic activity, etc. (Dishaw et al., 2014). BFRs include chemicals such as the polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCDD). PFAS which include perfluorooctanoic acid (PFOA) and perfluorooctane sulphonic acid (PFOS) are primarily hepatotoxic and immuno-toxic but have also been shown to induce carcinogenicity in rodents, and adverse reproductive effects in humans. An earlier opinion by the European Food Safety Authority (EFSA, 2018) proposed a huge reduction in the tolerable weekly intake (TWI) of PFOS and PFOA to around 13 ng/kg body weight (bw) per week for PFOS and 6 ng/kg bw per week for PFOA but more recently a TWI of 8 ng/kg bw per week was proposed for the sum of PFOA, PFOS, perfluorononanoic acid and perfluorohexane sulphonic acid (EFSA,2020). The occurrence of these four PFAS compounds in food is now regulated within the EU (European Commission, 2022). Many of the above contaminants, PCDD/Fs, PCBs, PCNs, PBDEs, HBCDDs, PFOA, PFOS, are listed as persistent organic pollutants (POPs) by the Stockholm Convention (Stockholm

Convention, 2019), for elimination of production and use or for minimisation of unintentional production.

Many of these contaminants are now known to occur in appreciable concentrations in some recycled materials (Rigby et al., 2015, 2021). These occurrences, combined with the increased use of recycled materials in livestock farming, create the potential for inadvertent contaminant uptake by animals during feeding. However, there is virtually no information on the proportion of bedding material consumed by livestock or whether the conditions under which animals are raised would influence the rates of ingestion. Poultry are known to consume bedding material (Malone, 1983; Lien et al., 1998; Foxall et al., 2004; Grimes et al., 2006; Fernandes et al., 2019) as part of their foraging and scratching behaviour although the extent of consumption can vary depending on the bedding and the behaviour. Consumption of around 2 percent of the dietary intake of feed has been reported (Fries et al., 1999; Foxall et al., 2004; Fernandes et al., 2011, 2019) although higher consumption rates (4% of intake) have also been reported (Malone, 1983; Lien et al., 1998).

Although a number of newer bedding materials have been introduced to the market, generally driven by the requirement to recycle resources, there has been no in-depth study to investigate whether the environmental contaminants that occur in these newer recycled products (Rigby et al., 2021) are assimilated by farmed animals. This is one of the first studies to address this deficit, and to investigate whether commercially available bedding made from recycled materials has the potential for contaminant transfer to laying chickens under routinely used husbandry conditions. It is also the first study investigating contaminant transfer that includes such a wide range of chlorinated, brominated and fluorinated environmental contaminants simultaneously. The results of this study would allow evidence-based evaluation of whether the use of recycled materials led to an increase in tissue and egg contamination. The data would also allow investigation of the biotransformation potential for contaminants where significant uptake was observed. The process of biotransformation reflects the ability of a particular species to enzymatically derivatise lipophilic chemicals to water-soluble forms which are more amenable to elimination from the body, e.g., via urine. Xenobiotic contaminants such as PCDD/F, PCBs, PCNs, etc. are metabolised, undergoing stages of hydrolysis, reduction and oxidation to yield e.g. –hydroxyl or -sulphonated derivatives. This is often a partial process which is dependent both on the species and the stability (chemical, enzymatic, etc.) or persistence of the chemical. Biotransformation is an indicative measure of contaminant transfer because of the variations in contaminant input and genetic variability. However, the uncertainty from inputs can be minimised by estimating the contaminant uptake at a point where contaminant inputs and animal growth are in equilibrium, i.e., a steady state in laying hens could be considered when feed is consumed at a steady rate balanced by regular production of eggs.

In order to achieve a more typical and realistic view of contaminant uptake through recycled bedding materials, this study was based on the use of established, current practices of poultry husbandry, using commercially available recycled materials and feed in the UK. The existing potential of these practices to contaminate the resulting food products should not however be ignored. Earlier examples such as the Belgian PCB/dioxins incident (Bernard et al., 1999) as well as more recent examples (Malisch, 2017; Pajurek et al., 2019; Hoogenboom et al., 2021) show feed and husbandry condition have a considerable influence on the occurrence levels of contaminants in chicken tissues and eggs.

2. Experimental

The evaluation of uptake potential of contaminants from the test recycled materials to chicken tissues and eggs was evaluated against a control bedding material. As per normal husbandry practice, the birds would be raised from day old hatchlings to maturity when egg-laying would begin. Tissues and eggs would be examined for contaminant content when egg laying commenced and biotransformation factors (BTFs) would be estimated when a steady rate of laying was reached.

2.1 Recycled materials

Although a number of materials that are suitable for poultry farming in the UK are available, the choice was based on commercial availability and current husbandry practices. Table 2.1 lists the materials considered for this study including the reasons for use, sources and potential contaminant occurrence. Three recycled materials were selected - recycled wood shavings, shredded recycled cardboard and DPS. The control material used for the study was wood shavings from wood that was untreated and unused. This material was established to have low levels of contamination from earlier studies (Fernandes et al., 2019). Another potential material that was considered for this study was gypsum but as it is no longer allowed as bedding material due to its toxicity, it was not included.

All study materials including the control were analysed on first receipt and prior to use as bedding material. They were homogenised, sub-sampled and milled (shredded cardboard and wood shavings were processed in a centrifugal mill) to yield particle sizes of < 1mm, prior to analysis.

2.2 Chicken housing and husbandry conditions

The chickens were raised in an established permanent enclosure of concrete construction. All study chickens were raised within the same enclosure. The concrete flooring was divided into four sections, separated by solid partitions, each of which was spread with one of the recycled and control materials (recycled wood shavings, recycled cardboard, DPS and clean wood shavings) to the normal depth as used in conventional practice. The partitions were sufficiently high so as to prevent birds from moving between sections. The four sections of the enclosure were populated on the same day with equal numbers (approximately 25) of female chickens. These were obtained from a single supplier as one day old pre-sexed hatchlings of the same strain. Ambient housing conditions such as light, temperature and humidity were monitored and recorded. Water and feed were provided *ad libitum*, and the physical well-being of the chickens was monitored for the duration of the study. These conditions were maintained over the study period (approximately 7 and a half months).

2.3 Sample Collection

The egg laying period commenced at approximately four months from the beginning of the study. The times were recorded and egg samples were collected regularly as produced. As individual eggs would not yield sufficient material for all the different analyses, eggs relating to each of the four materials were pooled separately on a weekly basis. The pools used for analysis were taken at the same intervals for each of the four materials to allow temporal parity for any influence of the materials. At the end of the planned intervals of egg collection, the chickens were anesthetised, killed and dissected. Tissue samples were collected from the same parts of the birds and included skin, breast and thigh muscle tissue and liver. Similar quantities of approximately

500 g of material were taken for each of the tissues (in case of liver, this was achieved by pooling livers from several animals because of the low organ weight – typically 35 g). Care was taken to keep the carcases from each of the four sections separated during dissection and sample collection. Feed samples were taken prior to distribution to the chickens and analysed with the other samples. These consisted of a starter feed for young chickens which was provided for the first six weeks, and was later replaced by a growers feed for the older birds that lasted until the study-end. Approximately 1 kg of each recycled material was representatively sampled for analysis from the provided bulk materials used as bedding.

2.4 Chemical Analysis

All of the samples collected above – tissues, eggs and feed, were analysed after homogenisation. Individual chicken livers have low mass and in order to allow all of the various analyses, these were pooled for each material group. All tissues and pooled egg samples were homogenised and aliquots for PFAS analysis which used fresh tissue were sub-sampled at this stage. The remaining homogenates were lyophilised by freeze-drying and re-homogenised. Feed was sub-sampled from the commercially obtained supply, mixed to homogenise, ground to < 1mm particle size and re-homogenised prior to analysis.

The following contaminants were analysed, as an earlier study (Rigby et al., 2015) had shown that they occurred in recycled animal bedding materials: Regulated contaminants and ECrecommended PBDEs are highlighted in bold.

- **PCDD/Fs - seventeen, 2378-Cl substituted PCDDs and PCDFs.** (EC, 2011)
- Dioxin like PCBs - IUPAC #: **77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189.**
- Non dioxin like PCBs - IUPAC #: 18, **28**, 31, 47, 49, 51, **52**, 99, **101**, 128, **138, 153**, **180**.

- Twelve PBDD/Fs including 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 (Fernandes et al., 2009)
- PBDEs - IUPAC #: 17, **28**, **47, 49**, 66, 71, 77, 85, **99, 100**, 119, 126, **138, 153, 154, 183, 209.**
- PBB congeners: IUPAC #: 15, 49, 52, 77, 101, 126, 169,153, 209.
- PCNs - PCNs-52/60, 53, 66/67, 68, 69, 71/72, 73, 74, & 75
- α-HBCDD, β-HBCDD and γ- HBCDD
- **Perfluorooctane sulfonate (PFOS), Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS)**

The analytical methodologies used for these determinations have all been extensively validated, described in detail (Driffield et al., 2008; Fernandes et al., 2004, 2008, 2010, 2012) and used in several published studies over the last fifteen years. All methods are based on isotope dilution or internal standardisation using ^{13}C-labelled analogues of target compounds with measurement by high resolution mass spectrometric (GC-HRMS) or tandem mass spectrometric analysis (LCMS/MS). Further details on the methods used are given in the supplementary information section. The analyses of the regulated and near-regulated contaminants (PCDD/Fs, DL-PCBs, NDL-PCBs and PBDEs and HBCDD) were carried out in accordance with published method performance criteria (European Commission,2011; Fernandes et al., 2022B). Rigorous quality control was incorporated during the analysis and more information on procedures, validation, details on quality control aspects (procedural blanks, use of in-house reference materials, successful participation in performance testing or inter-laboratory studies) have been reported earlier (Fernandes et al., 2019).

2.5 Data analysis

Basic statistical parameters such as minimum, maximum, mean and median are generally used to make comparative descriptions of the variations between the study data. However, arising from the purely pragmatic consideration of project costs, the volume of data obtained was insufficient to allow more sophisticated statistical analysis. Therefore, in order to establish whether the recycled materials had any impacts on the tissue and egg concentrations, a weights of evidence approach was used to support the data evaluation (Weed, 2005; Linkov et al., 2009). This is a quantitative approach that collates evidence to support a hypothesis and is increasingly used to analyse environmental and food contaminant data (Lake et al., 2014, Lehtonen et al., 2019; Fernandes et al., 2019; Eisenbrand, 2020). Practically, in this study the output of this analysis allowed comparison of tissue and egg concentrations across the different recycled materials (relative to the control) to ascertain whether the materials influenced the observed concentrations. The primary requirement for the evaluation was to establish significant occurrence of the contaminant in the recycled material being studied. This also provides validation for the presence of the contaminant in the tissues or eggs. Following this, the strength of evidence, illustrated in Figure 1, is represented for tissues as:

- **Θ Strong evidence of uptake** – when the lowest concentration from a recycled material group tissue exceeds the maximum concentration of the control group tissues
- Δ Some evidence of uptake – when the lowest concentration from a recycled material group tissue exceeds the median concentration of the control group tissues.

For the pooled egg samples that were collected at specific time periods, the strength of evidence is represented as:

- **Θ Strong evidence of uptake** – when all individual pooled egg concentrations from the recycled material groups exceeded the corresponding control concentrations

- **Δ** Some evidence of uptake – when the majority of pooled egg concentrations from the recycled material group were greater than the corresponding control concentrations.

2.6 Estimation of BTFs

BTFs should be calculated during a steady period of growth, ideally when there is equilibrium between contaminant uptake and output. In modern poultry farming there is an initial rapid phase of growth, but for egg laying chickens, a state of equilibrium can be assumed when the hens reach maturity, consume feed at a steady rate and regular egg laying is established. In this study, BTFs for PBDES, PCBs and PCDD/Fs in tissues and eggs were estimated at around three and a half months after egg laying commenced, which represented a reasonable approximation to a steady state phase. As described and used in other studies (Foxall et al., 2004; Fernandes et al., 2011, 2019; Lambiase et al., 2022) BTFs are estimated using the following equation which relates tissue (or egg) concentrations of a contaminant with the corresponding daily input flux.

$$\text{Bio-transfer factor (BTF)} = \frac{\text{Contaminant concentration in tissue (ng kg}^{-1}\text{ fat)}}{\text{Contaminant input flux (ng day}^{-1}\text{)}}$$

The input flux is determined by combining all the dietary inputs with an estimated component of recycled bedding material which is ingested during the course of the chicken's natural scratching behaviour. As these chickens were raised indoors, there is no soil component that would have been included as in the case of free-range chickens. As reported in other studies (Fries et al., 1982; Fries, 1999; Foxall et al., 2004; Fernandes et al., 2011), the quantity of bedding material ingested was conservatively estimated at 2% of the feed intake. The other potential contributors to the input flux – clean tap water and veterinary supplements were assessed as being insignificant (Foxall et al., 2004; Fernandes et al., 2019).

3.0 Results and Discussion

The selection of the contaminants (as listed in section 2.4) for the study was strongly influenced by existing regulations and recommendations by regulatory bodies or institutions that investigate chemical risks to the food chain, such as the European Commission and EFSA. Additionally, selection was also directed by the practical consideration of occurrence of the selected contaminants in the recycled materials. This practicality supports the main premise of the study, namely the transfer of contaminants that occur in recycled (or control) materials to the tissues and eggs of laying chickens. Moreover, the occurrence also validates the strength of evidence approach that was used to establish the transfer characteristics i.e., a contaminant found in significant amounts in the tissue or eggs of the chickens, must also be present in the materials used, or it is unlikely to originate from the material.

3.1 Contaminant occurrence in study materials

Occurrence data for the recycled materials (recycled wood shavings, shredded cardboard and DPS) and the control material (untreated wood shavings) are summarised in Table 3.1 on a whole (product) or fat weight basis (raw data has been presented earlier in a report (Fera, 2017), to the project sponsor). Conventional units as reported in the general literature or in regulations/recommendations have been used for all contaminants. As they elicit a common mode of toxic action (van den Berg et al. 2006) the concentrations of the dioxin-like contaminants – PCDD/Fs, dioxin-like PCBs and PBBs, PBDD/Fs and PCNs were expressed as a cumulative toxic equivalence (□TEQ) using previously established factors (TEFs) or relative potencies (REPs) that have been reported and used in several earlier studies (van den Berg et al. 2006; 2013; Fernandes et al., 2008, 2009, 2010, 2019, 2021, 2022; Zacs et al., 2021). The thirteen non-dioxin like PCBs measured in this study were summed (□NDL-PCBs) as were the eighteen PBDE congeners (□PBDEs), as well as alpha-, beta-, and gamma-HBCDD (Σα,β,γ-HBCDD). PFOS and PFOA were reported as individual concentrations as these are conventionally still reported separately, and the sum of the four regulated PFAS (Σ_4PFAS) are also included.

It is evident from the data in Table 3.1 that the shredded cardboard and DPS were more highly contaminated with all the measured contaminants than the recycled wood. With □TEQ values of approximately 10 ng/kg, these materials were around 50-fold more contaminated than the wood shavings, with even greater (multiple orders of magnitude) differences for the other contaminants, except for PFOS and PFOA. PCDD/Fs made the highest contribution to □TEQ, ranging from 50 to 79%, followed by PBDD/Fs plus PBBs (14 to 28%), PCBs (5 to 19%) and PCNs (1 to 2%). In terms of absolute concentrations, the highest levels of contamination were seen for the flame retardants and NDL-PCBs, in the order PBDEs > HBCDDs > NDL-PCBs. DPS and shredded cardboard showed the highest concentrations of any of the measured contaminants, with approximately 420 and 220 µg □PBDE/kg respectively.

As expected, from the experience of using this material in earlier studies, the untreated/unused wood shavings (control material) showed the lowest (and in some cases, undetectable) levels of contamination. The similarity in occurrence levels for both wood shaving materials was surprising as a recent report (Rigby et al., 2015) had shown significant contamination of recycled wood, particularly with contaminants such as PCBs, PBDEs, PCNs and HBCDDs. There is almost certainly variability in contaminant concentrations in recycled materials as the original products could derive from different sources, or could have been used for different purposes.

3.2 Contaminant uptake into chicken tissues and eggs

Contaminant uptake by the chickens was determined towards the end of the seven and a half month duration of the study after regular laying was established. Concentrations in eggs as well as tissues were investigated in order to assess contaminant disposition. Although egg production is the main reason for raising laying chickens, the meat from these birds is also used as food after the rate of egg production becomes commercially unviable. The median concentration values of the studied contaminants for the various tissues - muscle, liver and skin - and eggs of these hens are summarised in Table 3.1. The median □TEQ values ranged from 0.56 to 2.9 ng/kg fat. □TEQ for individual samples (data not shown) ranged from 0.38 to 4.1 ng/kg fat. On average, the major contribution to TEQ in eggs was from PCDD/Fs at 39%, with a contribution of 33% in the tissues, while the contribution from dioxin-like PCBs was lower (26% and 18% in eggs and tissues respectively). The average TEQ contribution from the brominated contaminants was 33 and 44% in eggs and tissues respectively (>96% of this was from PBDD/Fs), with lowest contribution from PCNs, at 2% for both tissues and eggs. These proportions are similar to the occurrence in the bedding materials used. Tissues and eggs derived from the shredded cardboard and DPS groups showed concentrations that were relatively higher (two to three-fold) than the control and were broadly similar, reflecting the recycled material concentrations. Although NDL-PCBs were relatively high in the tissues from the shredded cardboard group, the highest values were seen in the DPS group tissues/eggs, with a range of 14 to 25 ug/kg fat. These were approximately 25fold greater than the control group (range – 0.62 to 0.94 ug/kg fat). This comparative difference was broadly similar for the PBDEs, although the difference between tissue concentrations for DPS and control groups were not so pronounced, with an approximately seven-fold difference.

For the sum of three HBCDDs, the tissue distributions for the shredded cardboard and DPS groups ranged from 0.36 to 1.9 ug/kg fat, with 0.12 to 0.84 ug/kg fat for the control. However, the concentrations in the tissues from the recycled wood group are curiously high (range 10 – 22 ug/kg). The HBCDD concentration in the recycled material was low at 0.05 ug/kg, and the concentrations in the eggs were similar to the other groups. Contamination during dissection is a possibility, although this was done at the same time and using the same procedures, as for the other groups. Similarly, the same feed was used for all groups. The quality control data for the set of samples was also satisfactory, showing low procedural blank concentrations, good recoveries and reference material data. A possible reason for these high values may lie in the behaviour of HBCDD diastereomers and the measured analytes – alpha, beta and gamma- HBCDD. It is well established that the various diastereomers (others such as delta and epsilon are known as minor constituents) bio-transform to the alpha diastereomer which predominates in animal matrices (Law et al., 2005; Fernandes et al., 2008). It is possible that other diastereomers (or other potential precursors) that were not measured were present in the recycled waste wood shavings. To some extent this explanation may also be supported by the egg HBCDD concentrations. Despite high HBCDD concentrations in some recycled materials, egg concentrations are generally lower than the tissues, with the egg concentrations in the recycled wood group being the highest of all groups.

The concentrations of PFOS and PFOA were generally low (highest in DPS with median tissue range from 0.05 to 1.5 ug/kg whole), reflecting the concentrations in the recycled materials and control. The median tissue distributions in Table 3.1 also suggest that uptake is higher for PFOS than for PFOA which shows relatively low concentrations in tissues and eggs despite higher concentrations in the recycled materials compared to PFOS.

3.3 Evaluation of contaminant concentrations in tissues between recycled and control materials: Weight of evidence analysis

Data analysis based on the weights of evidence approach described in section 2.5 was used to examine the strength of association between contaminant concentrations in the recycled materials and those observed in the hen's tissues and eggs. Other inputs that may contribute to the observed tissue and egg concentrations were chicken feed and water. No medicinal inputs were used although veterinary care was provided for the birds throughout the study. The experience from previous studies (Foxall et al., 2004; Fernandes et al., 2011), has shown that the clean tap water used was not considered as a contributory source of contamination of the mainly lipophilic contaminants. The concentrations of the main contaminants in the starter and grower chicken feeds are summarised in Table 3.1, showing that concentrations were very low relative to the recycled materials, and the majority of the individual analytes in the raw data were below the limits of detection (Fera, 2017). Thus, the major source of contaminant uptake is likely to result from the inadvertent ingestion of bedding material.

The weight of evidence analysis is presented in Table 3.2. The numerical values that supplement the visual symbols described in section 2.5, are the ratios of median tissue or egg concentrations to the corresponding control median concentrations. No visualisation is shown for liver or for the PFAS analytes in tissues as these samples were pooled, but large differences (>100%) relative to the control are highlighted to indicate strongly divergent concentrations.

Table 3.2 shows evidence of uptake for all of the analytes measured in this study. Strong evidence is most frequently seen for □NDL-PCBs and □PBDEs for all tissues and eggs, particularly from chickens raised on shredded cardboard and DPS. The analysis showed similar strong evidence for dioxin-like TEQ for the same materials except for the eggs in the shredded cardboard group which showed some evidence. Strong evidence was also observed for PFOS egg concentrations in these two materials, with some evidence for PFOA. Conversely, for HBCDD, there was no evidence of uptake in the eggs, but strong evidence was observed for the tissues. This may indicate differences in tissue/egg disposition between these two contaminants. Table 3.2 also indicates evidence for the recycled wood shaving group, in particular for the ΣNDL-PCBs and ΣPBDEs and ΣTEQ. However, this observation could not be validated as these contaminants showed low levels of occurrence in the material and the visualisation was therefore excluded. The ratios of median values in this group were all generally above one, but usually lower than those observed for the other materials.

3.4 Trends in egg contamination

Eggs were collected from the recycled material and control groups at timed intervals, weekly from the onset of laying and then monthly, up to a period of three and a half months. The concentrations for some of the main chlorinated (ΣTEQ, ΣNDL-PCBs), brominated (ΣPBDEs) and fluorinated (PFOS) contaminants over this period have been plotted in Figure 2. HBCDD and PFOA were not plotted as a significant amount of the data was below the LOQ. The figure reveals a number of similarities as well as diverging trends. Firstly, in terms of magnitude, the control material always appears as the lowest trend line with the recycled wood generally in close proximity. The DPS trend line is at the top of the graphs denoting the highest concentrations.

These plots appear to reflect the analysis made in the previous sections and in general, although concentrations of the various contaminants vary over the collection period, they appear to return to near original values towards the end of the study. This would suggest that contaminant concentrations fluctuate following the onset of laying before stabilising. There are some exceptions, in particular the eggs from the recycled cardboard material group appear to increase in concentration over the duration of the study period, at least for □TEQ, □NDL-PCBs and □PBDEs. The upward trend is particularly emphasised for PBDEs. Of all the materials used in this study, the recycled cardboard was the most susceptible to physical disintegration over time, and this process may lead to greater bioavailability of the contaminants. The reverse is observed for PBDE concentrations in eggs in the DPS group with concentrations initially increasing for the first period, but then declining to approximately half the original concentrations. It is clear however, that concentrations in eggs that were obtained from the more contaminated materials show wide variations over the initial 3-4 months of egg laying. As hens raised commercially for egg laying would be considered viable egg producers for approximately ten additional months (RSPCA, 2020), a longer study period may help to establish longer term trends in contaminant concentrations.

The occurrence of PCDD/Fs, PCBs and PBDEs in hens eggs has been recorded before (Bernard et al., 1999; Brambilla et al., 2009; Rawn et al., 2011; Piskorska-Pliszczynska et al., 2016; Pajurek et al., 2022) and can sometimes be an indicator of local pollution, but the simultaneous investigation of such a wide range of environmental contaminants is rare. In recent years, conventional commercial husbandry and animal feed have rarely been contributory factors in poultry or egg contamination incidences as they have in the past (Bernard et al., 1999; Malisch et al., 2017); rather, the more recent incidents have related to contaminated materials, either within the housing, farm machinery or paints (Winkler, 2015; Piskorska-Pliszczynska et al., 2016; Pajurek et al., 2022).

3.5 Biotransfer of contaminants to chicken tissues and eggs

The assessment of contaminant bio-transfer ideally requires a steady state to be reached between the contaminant input flux and growth rate. The economic pressures on the farming industry result in animals being slaughtered as soon as they have reached an optimum weight (i.e., market-ready), so this stage is usually not reached in most conventionally farmed livestock. However, laying chickens could be considered to have achieved this equilibrium following a period of regular egg laying during which the contaminant inputs (feed, access to soil or bedding material, etc.) do not change. In this study, the final sampling period during which the chickens had been regularly producing eggs for approximately three and a half months, with a steady feed consumption rate, provides a practical representation of a steady flux of inputs balanced by egg production. This last set of egg data, together with concurrent tissue data was therefore used to estimate BTF values for PCDD/Fs, PCBs, PBDEs, PBDD/Fs and PBBs. However, a significant proportion of the tissue concentration data for PBDD/F and PBB congeners were below LOQs. As the use of LOQ values would introduce a high level of uncertainty to the BTF estimates (because the true concentration is unknown), PBDD/Fs and PBBs were excluded (similarly, BTFs for PCDD/Fs in skin were excluded for the same reason). Estimated BTF values for thirteen PCBs (six NDL- and seven DL-PCBs) fifteen PCDD/Fs and eight PBDEs congeners, as well as average values for these three contaminant groups are presented in Table 3.3. BTFs were estimated for all congeners, but this selection represents the most commonly measured compounds while also acknowledging literature observations that molecular configuration and the degree of halogenation can affect transfer rates (Fries et al., 1999; Thomas et al., 1999a; Thomas et al., 1999b; Fernandes et al., 2011). The selection excludes two PCDD/F and five DL-PCB congeners because they frequently occurred below the LOQ.

Table 3.3 reveals a number of trends in contaminant bio-transfer:

- The main observation is the greater magnitude of PCB BTFs relative to PCDD/Fs and PBDEs, with the order PCBs > PBDEs > PCDD/Fs, which may indicate a higher persistence for PCBs in chicken tissues
- Although higher BTF values appear to occur for both, DL- and NDL-PCBs, with (on average) little difference in magnitude, there are strong congener-specific differences. Larger PCB molecules with di-ortho substitution (PCBs 138, 153 and 180) have similar BTF values to smaller non-ortho substituted molecules (PCBs 28 and 81)
- On average, PCDFs show a greater tendency to biotransfer than PCDDs. There are distinct differences when compared to PCB uptake however, with the lower chlorinated PCDD/F congeners i.e. tetra-, penta- and some hexa-chlorinated congeners, showing much higher BTF values than the corresponding hepta- or octa-chlorinated compounds.
- BTF values for PBDEs show a strong correlation (r[Pearson] = 0.95 to 0.98) between the number of bromine atoms on the PBDE molecule and the absolute magnitude (Figure 3) for muscle, eggs and liver (but not for skin) with a near linear increase with increasing bromine number. An inverse, though less marked correlation is observed for PCDD/Fs and particularly for the PCDFs
- BTF values for PCBs and PCDFs are to some extent similar in tissues and eggs, and as would be expected there was generally good correlation between BTF values for liver and eggs for the different sets of contaminants (r[Pearson], 0.81 to 0.99). However, differences were more apparent for PBDEs, particularly between muscle and skin. For all contaminants, the mean BTF magnitude was greater in liver relative to other tissues or

eggs, which may be a result of the pharmacokinetics of lipophilic contaminant absorption and the role of the liver in processing lipids.

These observations suggest that contaminant biotransfer appears to depend on the molecular characteristics of configuration and the degree of halogenation. This is consistent with the results of other studies investigating PCDD/F uptake (Traag et al., 2006) and biotransfer (Fernandes et al., 2011; Lambiase et al., 2022) in laying chickens, that also report on the greater magnitude of BTFs for the more toxic lower chlorinated PCDD/F homologues. This relationship is not observed for the PCBs which show varying magnitudes of BTFs, suggesting a greater dependence of the uptake on molecular configuration. The range of BTF values between different contaminants also indicates a complexity of halogenated contaminant uptake that has not been noted before, but some common features remain such as the generally greater magnitude of BTFs in liver across all contaminants. This is likely related to the physiological function of this organ in detoxification, processing fat and also directing the deposition of these lipophilic contaminants to various body compartments. In avian eggs, the formation of the nutrient rich yolk relies on lipids synthesized by the liver which are transported as lipoproteins to the developing ovarian follicles. In laying chickens this is indicated by the generally good correlation between BTF values in liver and eggs due to the transfer of liver nutrients during egg formation. In general, these observations also confirm the potential usefulness of chickens as a biomarker for potential human exposure to persistent halogenated contaminants.

4.0 Conclusions

This is one of the first studies to provide evidence of the uptake of persistent environmental contaminants from recycled materials used as poultry bedding into the tissues and eggs of laying chickens. In particular, DPS and shredded cardboard contained elevated levels of flame retardants and PCBs in the order PBDEs > HBCDDs > NDL-PCBs. The uptake of all measured contaminants by chicken tissues and eggs, analysed using a weights of evidence approach, showed that the extent of uptake varied with the recycled material used. The strongest evidence of uptake was seen for □NDL-PCBs, □PBDEs and □TEQ in the tissues and eggs of chickens that used shredded cardboard and DPS as bedding. There was similar evidence for Σ α,β,γ – HBCDD uptake from these materials into the tissues, but not in the eggs. A study of the trend in contaminant concentrations in eggs that were produced using the more contaminated materials show that concentrations varied over the initial 3-4 months of egg laying Some egg concentrations e.g. for □TEQ, □NDL-PCBs and □PBDEs in the shredded cardboard group appeared to increase in concentration during the study period. Given that contaminant transfer from bedding to tissues and eggs was established, a longer study period would probably help to determine whether egg contaminant concentrations stabilise or continue to increase which is commercially relevant, as egg producing hens are considered viable for considerably longer periods than considered in this study. The magnitude of the estimated BTFs suggest that contaminant uptake varies between different groups and congeners, and appears to depend on molecular configuration and the degree of halogenation. A number of PCB congeners showed the highest BTF values especially for planar tri- and tetra-chlorinated, and non-planar hexa-and hepta-chlorinated congeners. On the other hand, the degree of halogenation appears to make the higher brominated PBDEs more susceptible to uptake with the highest BTF values for BDEs

183 and BDE-209.

As the pressure to use recycled materials increases, the diversity of materials used to produce animal bedding is likely to increase, and additionally, batch-to-batch variations in the production of existing materials can potentially lead to large variations in contaminant concentrations in the bedding materials. It would be prudent from the food safety point of view to investigate these issues further, considering both the material variability as well as longer periods of exposure not only in laying chickens but also in other species.

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Table (Editable version)

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The transfer of environmental contaminants (brominated and chlorinated dioxins and biphenyls, PBDEs, HBCDDs, PCNs and PFAS) from recycled materials used for bedding to the eggs and tissues of chickens

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Tables 3.1 to 3.3 and Table 2.1

Table 3.1: Median contaminant concentrations in recycled and control bedding, chicken tissues, eggs, and feed.

Contaminant concentrations	Σ DL-TEQ* (ng/kg)	Σ13 NDL-PCBs (µg/kg)	Σ17 PBDEs (µg/kg)	Σ α,β,γ - HBCDD (µg/kg)	PFOS µg/kg ww	PFOA µg/kg ww	Σ_4 PFAS µg/kg ww
	Concentrations: Recycled materials, feeds and PFAS data are on a whole weight (ww) basis; all other data (tissues/egg) are on a fat weight basis						
Recycled material	**1 - Shredded Ca dboard**						
Material (ww)	9.84	19	223	43	1.90	8.50	15
	1.42 2.21	6.07 7.27	11	0.39 1.43		0.04 0.06	
Eggs	2.19	8.41	3.5	1.23	0.60	0.69	0.83
Muscle	2.33	12	3.0	1.76	0.06	0.10	0.23
Skin			31		0.28		3.10
Liver					0.96		1.31
Recycled material	**2 - Dried Paper Sludge**						
Material (ww)	10	48	420	55	3.9	5.0	16
Eggs	2.07	19	16	0.36	1.5	0.06	1.79
Muscle	2.93	18	10	1.91	0.12	0.05	0.33
Skin	2.56	14	8.0	1.07	0.33	0.51	2.08
Liver	2.59	25	13	1.33	0.51	0.09	0.82
Recycled material	**3 - Shavings, Recycled wood**						
Material (ww)	0.26	0.39	0.36	0.05	<1.0	4.0	6.7
Eggs	0.7	1.32	1.24	0.48	0.09	0.03	0.48
Muscle	1.28	1.74	2.7	10	0.04	0.04	0.18
Skin	1.62	1.32	1.5	15	0.19	0.48	2.73
Liver	0.89	2.17	3.7	22	0.29	0.07	0.76
CONTROL mate ial - Shavings, Unused wood							
Material (ww)	0.33	0.37	0.43	0.10	0.60	2.8	6.3
Eggs	0.56	0.63	1.05	0.39	0.09	0.04	0.21

Muscle	1.04	0.78	1.61	0.84	0.03	0.02	0.10
Skin	0.79	0.62	0.46	0.12	0.27	0.6	2.22
Liver	0.90	0.94	3.4	0.72	0.22	0.07	1.22
Other potential inp ıts - feed							
Starter Feed (ww)	0.15	0.07	0.10	nm	nm	nm	nm
Grower feed (ww)	0.08	0.07	0.09	nm	nm	nm	nm

nm – not measured

*□DL-TEQ – summed Toxic equivalence (TEQ) of measured PCDD/Fs, dioxin-like PCBs and PBBs, PBDD/Fs and PCNs

Table 3.2 Weight of evidence (WoE) analysis showing strong Θ evidence or some Δ evidence of contaminant uptake into chicken tissues and eggs

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**		
Σ DL-TEQ	**2.1** Θ	**2.8** Θ	1.2	**2.8** Θ	**3.2** Θ	**2.1**	2.6	2.9	1.0	2.5 Δ	**3.7** Θ	1.3
Σ13 NDLPCBs	**9.3** Θ	**24** Θ	**2.2**	**14** Θ	**17** Θ	**2.1**	13	27	2.3	**9.4** Θ	**29** Θ	**2.1**
Σ17 PBDEs	**2.2** Θ	**6.3** Θ	**1.7**	**6.5** Θ	**22** Θ	**3.3**	9.1	3.9	1.1	**11** Θ	**15** Θ	**1.2**
PFOS	2.0	4.0	1.3	1.0	1.2	0.7	4.4	2.3	1.3	**6.7** Θ	**17** Θ	1.0
PFOA	3.0	2.5	2.0	1.2	0.9	0.8	1.4	1.3	1.0	1.0 Δ	1.5 Δ	0.8
Σ_4 PFAS	2.3	3.3	1.8	1.4	0.9	1.2	1.1	0.7	0.6	**4.0** Θ	**8.5** Θ	2.3
Σ α,β,γ - HBCDD	**1.7** Θ	**2.3** Θ	-	**10** Θ	**8.9** Θ	-	2.4	1.8	-	1.0	0.9	1.2

DL-TEQ – summed Toxic equivalence (TEQ) of measured PCDD/Fs, dioxin-like PCBs and PBBs, PBDD/Fs and PCNs

Note 1. Numerical values are the ratios of median tissue or egg concentrations to the corresponding control median concentrations

Note 2. Where WoE analysis was not possible (liver and PFAS) due to pooling of samples, highlighted cells represent strongly divergent concentrations which may indicate uptake by tissues.

Note 3: WoE visualisation symbols were not included for some wood shaving results (shown in bold font) as the analysis was not validated because contaminant levels in the recycled material were low

Table 3.3: Biotransfer factors (BTFs) for various contaminants estimated for chicken tissues and eggs

	Biotransfer Factors			
Contaminant	**Muscle**	**Skin**	**Eggs**	**Liver**
NDL-PCBs				
PCB28	241	207	233	192
PCB52	21	28	16	18
PCB101	19	29	13	20
PCB138	173	163	162	326
PCB153	220	216	194	271
PCB180	278	138	137	266
DL-PCBs				
PCB105	99	103	115	141
PCB118	146	169	171	206
PCB156	64	78	78	117
PCB77	131	244	145	145
PCB81	220	269	252	238
PCB126	143	211	157	177
PCB169	84	186	78	97
PCBs Mean	**141**	**157**	**135**	**170**
PCDD/Fs				
2,3,7,8-TCDD	58	NE	35	69
1,2,3,7,8-PeCDD	112	NE	43	45
1,2,3,4,7,8-HxCDD	31	NE	23	41
1,2,3,6,7,8-HxCDD	38	NE	52	72
1,2,3,7,8,9-HxCDD	27	NE	26	46
1,2,3,4,6,7,8-HpCDD	10	NE	10	19
OCDD	2	NE	6	7
2,3,7,8-TCDF	153	NE	150	216
1,2,3,7,8-PeCDF	73	NE	61	102
2,3,4,7,8-PeCDF	78	NE	63	91
1,2,3,4,7,8-HxCDF	59	NE	52	86
1,2,3,6,7,8-HxCDF	38	NE	36	55
2,3,4,6,7,8-HxCDF	48	NE	31	74
1,2,3,4,6,7,8-HpCDF	19	NE	26	42
OCDF	11	NE	9	17
PCDD/Fs Mean	**50**	NE	**42**	**65**

PBDEs				
BDE-28	16	21	7	11
BDE-47	66	97	39	85
BDE-49	32	71	12	34
BDE-99	60	107	38	80
BDE-100	83	107	59	123
BDE-153	98	125	81	103
BDE-154	93	119	63	119
BDE-183	132	86	104	137
BDE-209	188	49	146	251
PBDEs Mean	**85**	**87**	**61**	**105**

NE- not estimated as many concentrations were below the LOQ

Table 2.1. Recycled bedding materials and control material considered for the study.

Type of material	Frequency of use	Reasons for use	Materials derived from	Potential changes in use	Targeted contaminants (based on potential occurrence, persistence and bio-accumulative potential)	Toxicity characteristics of the contaminants
Recycled Wood (Grade A "clean") Shavings	Most common bedding	Cheap, absorbent, easy to use	Packaging waste, scrap pallets, packing cases, cable drums, off-cuts from manufacture of untreated products.	The proportion of waste wood going to animal bedding has consistently increased over the last few years. This is expected to continue.	PCDD/Fs, PCBs BFRs – PBDEs, PBBs, HBCD, PCNs PBDD/Fs, PXDD/Fs, PXBs PTEs	carcinogenic neurotoxic, endocrine disruptors (EDs) carcinogenic carcinogenic Carcinogenic, allergens
Shredded cardboard and paper	Readily available indicating high level of usage.	Absorbent, dust free, less compacting	Paper mills, newspaper, magazines, other waste paper, corrugated cardboard. Difficult to obtain from source, hence mostly from suppliers.	Reasonably large supply of beddings made from these materials so unlikely that will reduce.	Heavy PTEs PCDD/Fs, PCBs BFRs – PBDEs, PBBs, HBCD, PCNs PBDD/Fs, PXDD/Fs, PXBs PFOS?	v high -carcinogenic v high -carcinogenic neurotoxic, EDs high-carcinogenic v high-carcinogenic
De-inked paper pulp and dried deinked paper sludge	Dried paper sludge and PS are available from source	Low moisture content, no odour	Paper manufacturing	A new product combining paper crumb (sludge) and paper ash being developed. If successful, could increase usage of these products.	Heavy PTEs PCDD/Fs, PCBs BFRs – PBDEs, PBBs, HBCD, PCNs PBDD/Fs, PXDD/Fs, PXBs PFOS?	v high -carcinogenic v high -carcinogenic neurotoxic, EDs high-carcinogenic v high-carcinogenic
Gypsum **(not selected)**	Discontinued from March 2012	Use of this material is not allowed	plasterboard	If further studies show no risk; although unlikely as discontinued	Hydrogen sulphide(H_2S) Heavy PTEs	v high -carcinogenic, allergens
	Common bedding. **Used**		Untreated wood			-

Shavings from untreated wood	**as Control**	Cheap, absorbent, easy to use		As resources get scarcer, recycled wood may be used	Shown to have v low contamination, by previous testing	

Figure

The transfer of environmental contaminants (brominated and chlorinated dioxins and biphenyls, PBDEs, HBCDDs, PCNs and PFAS) from recycled materials used for bedding to the eggs and tissues of chickens A. R. Fernandes, I. R. Lake, A. Dowding, M. Rose, N. R. Jones, F. Smith, S. Panton

Figures 1 to 3

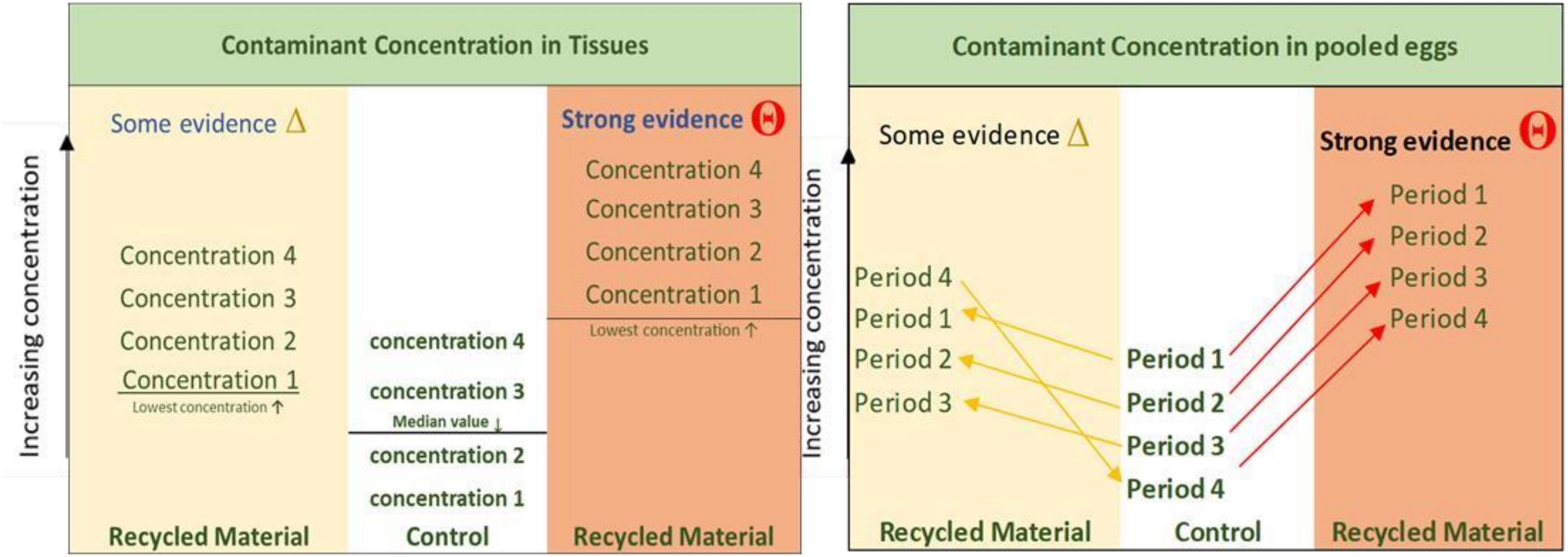
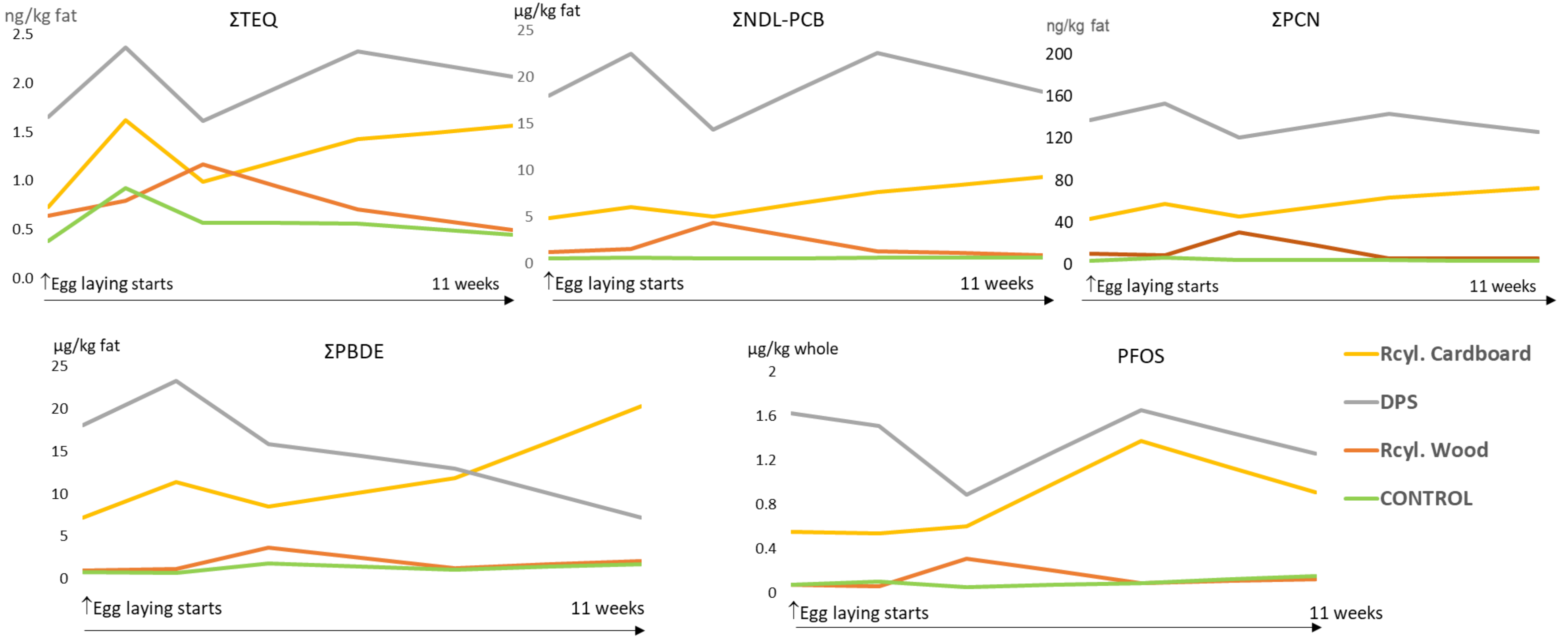
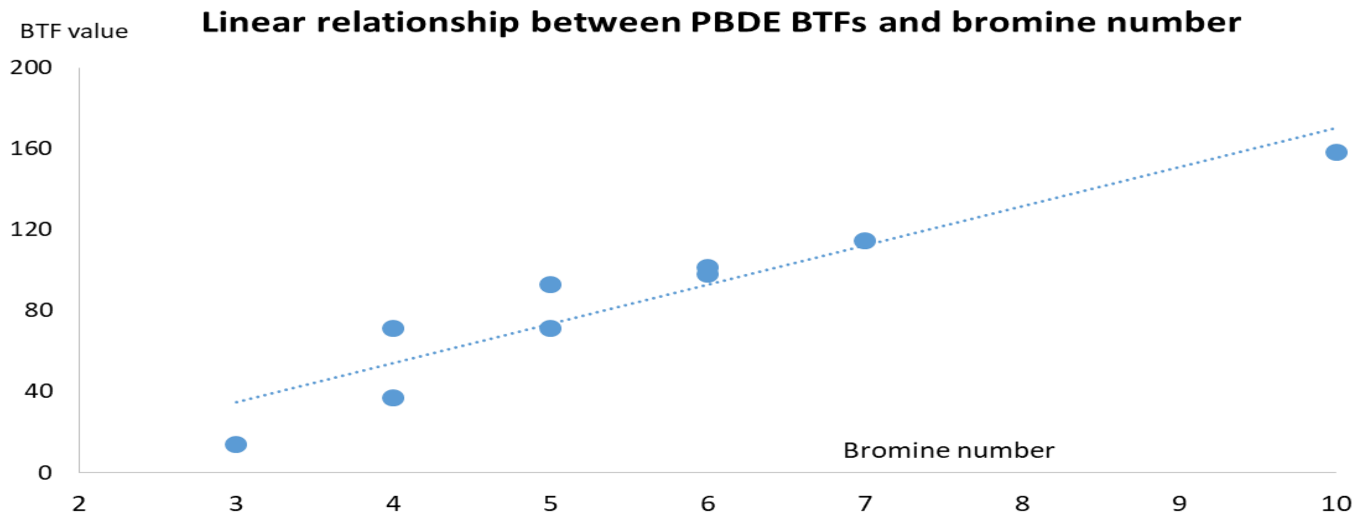


Figure 1. Strength of evidence visualisation: Examples of strong ϴ evidence or some Δ evidence conditions for tissues or eggs collected during specific periods



□TEQ is the summed toxic equivalence (TEQ) of measured PCDD/Fs, dioxin-like PCBs and PBBs, PBDD/Fs and PCNs

Figure 2: Trends in contaminant concentrations in eggs over the study duration



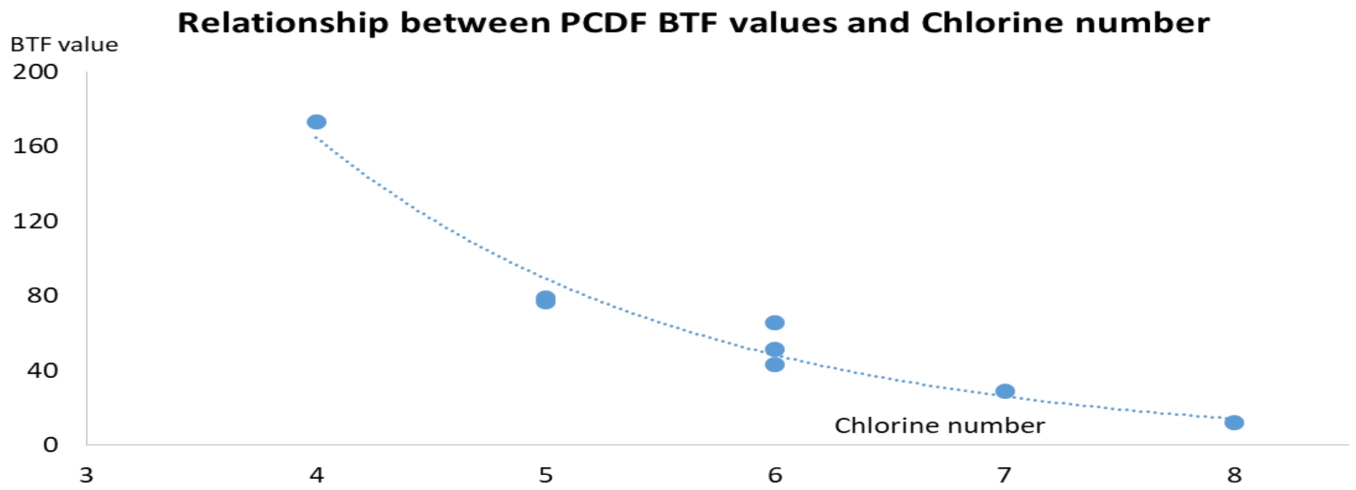


Figure 3: Relation between magnitude of BTFs and degree of halogenation in chicken tissues and eggs

<u>CredIT Author listing:</u>

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Alan Dowding: Resources, Writing – Review and Editing

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