Science of the Total Environment 886 (2023) 163546

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transfer of polychlorinated, polybrominated and mixed-halogenated dioxins, furans and biphenyls, polychlorinated naphthalenes and alkanes, polycyclic aromatic hydrocarbons and chlorobenzenes to the milk of dairy cattle from controlled ingestion of industrial and municipal bioresources recycled to agricultural land

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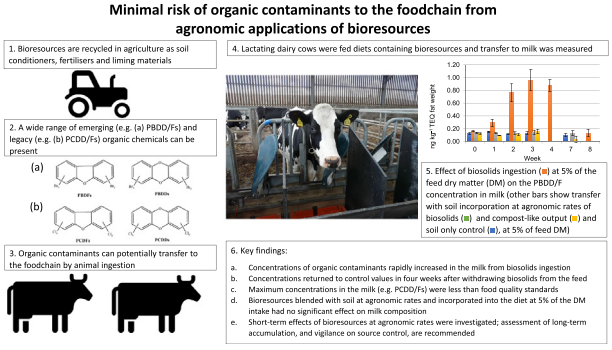
HIGHLIGHTS

- Bioresources are a source of organic contaminants to agricultural systems.
- Animal ingestion is a vulnerable pathway of transfer to the human foodchain.
- Organic contaminant concentrations in cow's milk increased from ingesting biosolids.
- Minimal additional transfer was seen at agronomic rates of bioresource application.
- Bioresource management practices minimise ingestion and risk to the foodchain.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Paromita Chakraborty

Keywords:
Ash
Agriculture
Bioresource
Biosolids
Cattle
Compost-like-output
Grazing
Milk
Organic contaminants
Waste

ABSTRACT

Recycled bioresources (biosolids, compost-like-output, meat and bonemeal ash, poultry litter ash, paper sludge ash) were added to the feed of dairy cattle to simulate incidental ingestion from agricultural utilisation, to investigate the transfer of organic contaminants from the ingested materials to milk. The bioresources were blended with a loamy sand soil at agronomic rates to simulate a single application to land, which was added to the diet at 5 % of the total intake on a dry matter (DM) basis. Biosolids, and control treatments consisting of unamended soil, were also added directly to the feed at 5 % DM. The cattle were fed the bioresource amended diets for a target period of three to four weeks, depending on material, and monitoring continued for four weeks after treatment withdrawal. Milk samples were taken weekly with chemical analysis of selected samples for a range of organic contaminants including: polychlorinated, polybrominated and mixed-halogenated dioxins, furans and biphenyls, polychlorinated naphthalenes and alkanes (often called chlorinated paraffins), polycyclic aromatic hydrocarbons and chlorobenzenes.
No statistically significant additional transfer of organic contaminants to the milk was detected due to the relatively low levels of contaminants present when the bioresources were incorporated with soil at agronomic rates. However,

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http://dx.doi.org/10.1016/j.scitotenv.2023.163546
Received 10 October 2022; Received in revised form 11 April 2023; Accepted 13 April 2023
Available online 18 April 2023

direct biosolids ingestion by cattle significantly increased the transfer of contaminants to milk in comparison to control animals. Although present in larger concentrations in biosolids than their chlorinated counterparts, the carry over rates and bioconcentration factors of brominated dioxins and furans were considerably smaller. Direct ingestion of biosolids resulted in most contaminants approaching, but not always completely reaching, steady state concentrations within the treatment feeding period, however, concentrations generally declined to control values within four-weeks after withdrawing the biosolids-amended diet.

1. Introduction

Large numbers of organic compounds with important operational properties are manufactured by the international chemical industry. Other organic compounds may form de novo, for example, during incomplete thermal combustion processes (e.g. polychlorinated or polybrominated dibenzo-p-dioxins/furans (PCDD/Fs or PBDD/Fs) and polyaromatic hydrocarbons (PAHs)). Many of these are persistent and bioaccumulative, and are found in environmental media, in industrial and municipal bioresources, and also in food (Amlinger et al., 2004; Rigby et al., 2021; Semple et al., 2001; Smith and Riddell-Black, 2007; Smith, 2009) and are therefore relevant to human health.

The UK Government's Clean Growth Strategy (HM Government, 2017), 25 Year Environment Plan (Defra: Department for Environment, 2018a) and the Waste and Resources Strategy for England (Defra: Department for Environment, 2018b) pledge to ensure resource efficiency and circularity to minimise waste and reduce its environmental impact. Recycling industrial and municipal bioresources in agriculture contributes to Clean Growth Strategy (HM Government, 2017) ambitions by: i) reducing pressure on virgin resources, minimising the negative environmental and carbon impacts associated with their extraction, and ii) diverting biodegradable and other residual waste streams that would otherwise be sent to landfill or incineration for disposal, contributing to the goal of zero food waste in landfill by 2035, and zero avoidable waste by 2050. This practice also supports the development of the circular economy for nutrients (Green Alliance, 2007; Green Alliance, 2011; Green Alliance, 2017), food security and underpins multiple UN Sustainable Development Goals (UN: United Nations, 2015).

For biosolids (treated sewage sludge), there is long-term experience of beneficial use as a soil amendment and the practice is closely regulated to protect human health and the environment. Currently, 3.5 million t fresh weight, equivalent to 87 % of biosolids that are produced in the UK are reported to be recycled to agricultural land (ABL: Assured Biosolids Limited, 2020). This is typically the preferred option for biosolids management as it takes advantage of the fertiliser value and soil improving properties of the material. The risks to public health and the environment from land-applied biosolids including pathogens, heavy metals, nutrient enrichment and 'established' organic contaminants (e.g. PCDD/Fs, polychlorinated biphenyls (PCBs) and PAHs) have received significant research attention (Smith, 1996; Clarke and Smith, 2011).

The residual ash from waste combustion processes, such as meat and bonemeal ash (MBMA), poultry litter ash (PLA) and paper sludge ash (PSA), also demonstrate agronomic benefit as soil amendments. A Quality Protocol for PLA has established the end of waste criteria for the full recovery of this material as a product for use as an agricultural fertiliser (WRAP, 2012). The management of industrial and municipal solid waste (MSW) by mechanical biological treatment is also expanding as a means of waste valorisation and landfill diversion, and the stabilised biodegradable output, described as compost-like-output (CLO), has value as a soil conditioning agent (Carbonell et al., 2011). In contrast to source-separated, biodegradable MSW streams, such as green or food waste, land application of CLO is currently not permitted in the UK. However, it is widely practiced elsewhere (Carbonell et al., 2011; Stretton-Maycock and Merrington, 2009), and the application of high-quality CLO to agricultural land could increase in the future.

Established organic contaminants, such as dioxin-like (DL) compounds, and the toxicological risks associated with their dispersal in the environment, food and water are relatively well understood (e.g. Huang et al., 2017; Rowlands et al., 2013). Clarke and Smith (2011) identified a number of organic contaminants as research priorities for agricultural use of biosolids due to their human toxicity, bioaccumulation and environmental persistence, amongst other factors, including polychlorinated alkanes (PCAs), used as lubricant additives and plasticisers (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2020) and polychlorinated naphthalenes (PCNs), historically used as engine oil additives (Fernandes et al., 2017). Less is known about the risks of other halogenated, persistent chemicals, such as brominated and mixed-halogenated dioxins, furans and biphenyls (Birnbaum et al., 2003; Olsman et al., 2009) and few measurements have been made of these contaminants in bioresources or their transfer to food products from agricultural utilisation. However, recent advances in analytical techniques (e.g. Fernandes et al., 2008a; Fernandes et al., 2011) enable a wide range of organic contaminants, including these priority groups, to be detected at trace levels in food and environmental media and specifically to assess their significance and the risk to human health from the recycling of bioresources to agricultural land. Rigby et al. (2021) recently reported the concentrations of these, as well as other brominated and perfluorinated organic contaminants in a range of bioresources used in agriculture.

Accumulation in dairy products or meat by grazing livestock is considered to be the most significant pathway of organic contaminant transfer to the foodchain from land application of waste-derived bioresources (Fries, 1995; Schowanek et al., 2004). Long-term accumulation of

Table 1
Rates of waste addition to the soil for the dairy cattle trials (dry solids (DS) basis).

Waste	Rate (t DS ha^{-1}/g DS kg^{-1} ds)[a]
Biosolids	10.7[b]
CLO	19.2[b]
MBMA	14.0[c]
PLA	3.3[c]
PSA	19.0[d]

CLO, compost-like-output; MBMA, meat and bonemeal ash; PLA, poultry litter ash; PSA, paper sludge ash; ds, dry soil.

[a] An incorporation depth of 10 cm was assumed (in practice plough depth may be 20–30 cm, but 10 cm was used to simulate an upper concentration range for incorporated materials). Soil density was assumed to be 1 (it may typically be closer to 1.2–1.8 for a sandy soil (Chaudhari et al., 2013), but this was chosen to reflect an upper range concentration).

[b] Based on a maximum application of 500 kg nitrogen (N) ha^{-1} over a two-year period in nitrate vulnerable zones. Biosolids = 4.68 % N dry solids (DS); CLO = 2.6 % N DS. Hence for biosolids, ((500/4.68)*100)/1000 = 10.7 t (t) DS ha^{-1} and for CLO, ((500/2.6)*100)/1000 = 19.2 t DS ha^{-1}.

[c] MBMA and PLA are applied to land for their phosphorus (P) and potassium (K) fertiliser value. Hence, application rates were calculated to maximise the agronomic application rate according to the P or K content of the recycled product (MBMA = 2.82 % K; 9.76 % P; PLA = 12.1 % K; 7.78 % P, all on a DS basis), whichever resulted in the greatest rate, and assuming a maximum rate of agronomic application of P or K to a previous crop of potatoes on soil with index 0, and an expected yield of 70 t ha^{-1} requiring 476 kg K_2O ha^{-1} (395 kg K ha^{-1}) and 250 kg P_2O_5 ha^{-1} (109 kg P ha^{-1}). Hence, both materials were applied according to their K content. For MBMA, ((395/2.82) X100)/1000) = 14 t ha^{-1}. For PLA, ((395/7.78)/1000) = 3.3 t ha^{-1}.

[d] Calculated according to the neutralising value of the PSA (47 %), and assuming a maximum agronomic application rate, which is for an arable, acidic soil, requiring 50–55 t ha^{-1} when neutralising value is 55 %.

contaminants in body tissues occurs over the lifespan of an animal, but turnover and elimination from this pool is not as rapid as for contaminants in milk (Sadler et al., 2005). Cow's milk is particularly sensitive and responsive to bioaccumulation of organic contaminants and, since dairy foods also represent a major component of the human diet (Fries, 1982), monitoring the concentrations in milk may be an effective experimental strategy to identify potential risks to health from organic contaminants in bioresources recycled to agricultural land. For example, Fries (1982) and Eisele (1985) reported uptake and transfer to milk was typically rapid and that contaminant concentrations could reach steady state within two to three weeks. Hoogenboom et al. (2015) and Lorenzi et al. (2020) also found that milk responded rapidly although, in this case, steady-state concentrations required slightly longer periods for cows exposed to elevated amounts of PCDD/Fs and PCBs in the feed. For example, Hoogenboom et al. (2015) indicated that at least 30 days might be required to reach steady state and, for a few PCB and PCDD/F congeners, Lorenzi et al. (2020) reported longer durations of up to 35 or 42 days were necessary. However, examination of the Σ Toxic Equivalent (TEQ) values presented by Lorenzi et al. (2020) for PCDD/Fs and DL-PCBs in milk suggests that concentrations are at steady state within about 21 and 35 days, respectively. Nevertheless, both Hoogenboom et al. (2015)) and Lorenzi et al. (2020) show that contaminant concentrations in milk increased rapidly within 3 weeks of dietary exposure and approached approximately 75–80 % of maximum levels by day 21.

Direct contamination of pasture represents a principal route of potential short-term exposure to grazing livestock from the surface application of bioresources to grassland (Smith, 1996). However, under field grazing conditions, pasture contamination rapidly declines and the main long-term potential risk of exposure to consumers of animal food products from organic contaminants in land applied bioresources is through livestock ingestion of contaminated soil when grazing (Carrington et al., 1998a, 1998b; Smith, 1996). It is generally recognised, however, that incorporation into the soil essentially eliminates the animal ingestion of organic contaminants from applied bioresources (Wild et al., 1994), but data are required to experimentally confirm this observation, and to examine the transfers of a wider range of identified contaminants in bioresources to animal products from the agricultural utilisation route. Therefore, the aim of the research reported here was to determine the potential transfer and uptake of a range of established and emerging persistent organic contaminants into milk from bioresources applied to agricultural land simulated under controlled conditions in lactating Holstein cows fed a total mixed ration (TMR). The objective was to quantify the effects of the direct addition (biosolids) to the TMR and of bioresource incorporation into soil at agronomic rates on the transfer or organic contaminants to milk.

2. Materials and methods

2.1. Experimental approach

Two trials were conducted at the University of Reading Centre for Dairy Research (CEDAR) to examine and represent the uptake of organic contaminants via ingestion of bioresources from soil or foliar contamination. Each trial used separate groups of 16 lactating Holstein cows. Mid-lactation cattle were selected for the study to: i) minimise mobilisation or deposition of large amounts of contaminants in body adipose tissue, and ii) determine the representative transfer of contaminants to milk from the experimental diets (Hardie and Spurlock, 2015).

The ingestion experiments were conducted using housed livestock with prepared feeds containing the bioresources, to carefully determine the intake of contaminants. This approach allowed for much greater control of the experimental variables than is possible in comparative field studies or by relying on voluntary, incidental animal ingestion. Bioresources blended with soil were added directly to the feed to simulate the incidental ingestion by cattle grazing amended pasture of: i) bulky biowastes: biosolids via soil or foliar and CLO via soil contamination (referred to as Trial 1), and ii) ash via soil contamination (referred to as Trial 2). The dairy ingestion experiments were designed to provide a reasonable upper intake by dairy animals, therefore, bioresources-amended soil was incorporated at 5 % of the diet dry matter (DM), consistent with the upper soil ingestion rate for cattle (Thornton, 1974; Smith, 1996); bioresource applications to soil followed normal maximum agronomic rates (Defra: Department for Environment, 2010; Table 1). A rate of 5 % DM addition to the diet was also used for the biosolids-only treatment, without soil incorporation, representing a reasonable upper estimated sward contamination rate for pasture after surface application and a waiting period of 21 days (Smith, 1996), as required by the Sewage Sludge in Agriculture: Code of Practice (Defra: Department for Environment, 2018c). The animals in the control treatments (without bioresource addition) received the same rate of soil-amendment into the feed (5 % DM) to match the other experimental diets. The bioresource-soil mixtures were supplied to the animals daily for a target period of three weeks, which was designed to exceed the minimum (two weeks) necessary for contaminants to approach or at least achieve a significant proportion of steady state concentrations in the milk (Fries, 1982; Eisele, 1985; Hoogenboom et al., 2015; Lorenzi et al., 2020). To allow for rumen adaptation, the materials were introduced gradually into the diets over a period of four days. However, the Biosolids treatment had a low DM content and challenging physical properties and was given a longer period of introduction of 16 days up to the target feed rate of 5 % DM to avoid rejection by the cattle, therefore, the feeding regime was extended to four weeks in this case.

Animals and milk concentrations were monitored for a further four weeks following the cessation of bioresource inclusion in the diet to assess the residual persistency of contaminants from the ingested waste materials. Diets were fed ad-libitum in the form of a TMR to maximise the intake of bioresources by reducing the ability of the cow to avoid consumption of the bioresources through sorting and physical de-selection. Animals were group-housed throughout, bedded on wheat straw and milked twice daily through a conventional herringbone parlour. Access to diet and individual feed intakes were facilitated and measured using an electronic Calan Broadbent feeding system (American Calan, Northwood, New Hampshire, USA). The measurements taken included diet composition, feed intake, live weight and milk yield and standard composition parameters, in addition to chemical analysis of the milk for organic contaminants detected in the bioresources. All animal procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

2.2. Selection of bioresources and soil

Several suppliers were identified of representative examples of the different categories of bioresources typically applied as fertilisers and soil improvers on agricultural land. These included: biosolids (2 examples); CLO (2 examples); MBMA (2 examples); PLA (2 examples) and PSA (1 example). Further details of the selection process, a description of the full set of materials collected, and methods of collection, transport and storage and sub-sampling for physico-chemical and organic contaminant analysis are provided by Rigby et al. (2021). One example from each category was selected for inclusion in the experimental programme, however, each bioresource was collected in sufficient quantities to complete the feeding trials so that the material selected for the experiments was equivalent to that used for the chemical characterisation. The bioresources were analysed for the suite of organic contaminants shown in Table 2.

The analytical methodology and the general physico-chemical properties of the different bioresources are described by Rigby et al. (2021). A material from each category was selected with generally the largest overall organic contaminant concentrations based on the preliminary chemical analysis.

Table 2
Organic contaminant groups analysed and detected in the bioresources and soil blends and which were consequently analysed in the milk.

Contaminant group	Contaminants analysed in milk for the bioresource treatments					
	Biosolids	Biosolids-soil	CLO-soil	MBMA-soil	PLA-soil	PSA-soil
PCDD/Fs; PCBs[a, b]	✔	✔	✔	✔	✔	✔
PBDD/Fs; PBBs[a, b]	✔	✔	✔	✔[h]	✔[h]	X
PXDD/Fs; PXBs[c]	✔	X	✔	X[h]	X	X
PCNs[d]	✔	✔	✔	✔	✔	X
PAHs[e]	✔	X[i]	✔	✔	✔	✔
PCAs[f]	✔	✔	✔	X	X	X
CBs[g]	✔	✔	✔	X	X	X

PCDD/Fs, polychlorinated dibenzo-*p*-dioxins/dibenzofurans; PCBs, polychlorinated biphenyls; PBDD/Fs, polybrominated dibenzo-*p*-dioxins/dibenzofurans; PBBs, polybrominated biphenyls; PXDD/Fs, mixed halogenated dibenzo-*p*-dioxins/dibenzofurans; PXBs, mixed halogenated biphenyls; PCNs, polychlorinated naphthalenes; PAHs, polycyclic aromatic hydrocarbons; PCAs, polychlorinated alkanes; CBs, chlorobenzenes.
✔, contaminant group detected in the bioresource and soil mixture, therefore milk samples was examined for this group; X, contaminant group below analytical limit of quantification (LoQ) in bioresource or soil mixture and was not determined in milk samples.
Milk from the cows in the control group of each trial was analysed for the full range of contaminants that were measured in the corresponding treatments in that trial.
[a] Fernandes et al., 2004.
[b] Fernandes et al., 2008a.
[c] Fernandes et al., 2011.
[d] Fernandes et al., 2010.
[e] Rose et al., 2007.
[f] Fernandes et al., 2008b.
[g] Fernandes et al., 2016.
[h] Only PXBs 105 and 156 were detected in the MBMA-soil treatment at concentrations close to the LoQ, therefore, PXBs were not measured in the milk from this treatment.
[i] PAHs were detected in the biosolids-soil blend, but concentrations in the milk were similar to the control.

However, in the case of CLO, the less contaminated material was used for palatability reasons as this contained fewer physical contaminants. For reference, Rigby et al. (2021) denoted the specific batch of each waste type used in the ingestion experiments as: Biosolids2, CLO2, MBMA1, PLA2 and PSA.

The bioresources were blended with a specified slightly acidic (pH 6.7) coarse-textured, loamy sand soil (Bourne Amenity Ltd., Newenden, Kent, UK). The soil type was selected specifically for its low fertility status and poor sorption characteristics to maximise the potential bioavailability and transfer of contaminants. The physicochemical properties of the soil are presented in Table S1 of the Supplementary Information (SI).

2.3. Animal selection and dietary treatments

Sixteen animals in mid-lactation were selected for each of the ingestion experiments. The physiological condition of dairy animals is a critical factor in the design of ingestion experiments investigating the effects of dietary components on milk composition. This is because cows at different stages of the lactation cycle can either mobilise contaminants from or deposit contaminants in body fat (Thomas et al., 1998). However, cows in mid-lactation demonstrate an approximately constant intake of contaminants and, hence, steady state concentrations in milk (Hardie and Spurlock, 2015). At commencement of Trial 1, cows averaged 170 days in milk (standard deviation (S.D.) = 42.6) with an average body weight (bw) of 714 kg (S.D. = 50.9) and parity (number of offspring) equivalent to 3.6 (S.D. = 1.49). At the start of Trial 2 cows averaged 241 days in milk (S.D. = 50.6) with an average bw of 724 kg (S.D. = 60.5) and parity of 3.8 (S.D. = 1.68). Animal health and condition were observed on a daily basis during both experiments.

A basal diet was fed as a TMR in each trial with forage DM composed of maize silage (29.1 %) and grass silage (21 %). Other ingredients in the diet were straw, concentrates, supplemental fat and a vitamin and mineral mixture as 3.2 %, 44 %, 1.6 % and 1.1 % of diet DM, respectively. The formulated nutritional composition of the basal diet was: metabolisable energy, 12.2 MJ kg; crude protein, 17.2 %; neutral detergent fibre, 35.2 %; starch, 28.1 %; oil, 4.2 % and sugars, 3.4 %, all on a DM basis.

Four animals were randomly assigned to four experimental diets within each trial as follows: Trial 1 basal diet was blended with: i) biosolids, ii) biosolids incorporated into soil (Biosolids-soil), iii) CLO incorporated into soil (CLO-soil), and iv) soil, as a control diet; Trial 2 treatments were as follows: i) MBMA incorporated into soil (MBMA-soil), ii) PLA incorporated into soil (PLA-soil), iii) PSA incorporated into soil (PSA-soil), and iv) soil control. Bioresource-soil mixtures were prepared using a cement mixer and a hand-held mortar mixer to thoroughly incorporate the materials into the soil and were equilibrated for six weeks prior to the feeding experiments to simulate the minimum potential time period likely between incorporation of amendments and grazing under UK farming conditions. The separate ingredients of each diet were weighed into a complete diet mixer (Data Ranger, American Calan, Northwood, New Hampshire, USA) to ensure a homogenous presentation. The diets were prepared each morning and offered ad libitum (with a target rate of 5 % refusal) once daily at 09:00 h, and the cattle were milked twice daily at approximately 06:30 h and 16:30 h.

Animals were bedded on straw and housed in a covered yard during the feeding period of the experimental diets in separate pens (6 m × 10 m) according to treatment to minimise cross contamination. The individual feeding behaviour of each animal was recorded using Calan gate equipped feed troughs. Cows were maintained in their treatment groups for one week after cessation of the treatment diets.

2.4. Measurements and sample collection

2.4.1. Animal production parameters and feed and milk composition

Cows were weighed initially, and subsequently at weekly intervals until the end of the four-week withdrawal period. During the bioresource feeding phase, samples of the TMR and the main dietary ingredients were taken on a daily basis and a representative weekly bulk sample was prepared. These were stored at −20 °C until analysis of chemical composition. Total mixed ration refusals and their corresponding DM concentration were measured daily or weekly, respectively, and DM intake was calculated on a daily basis. Particle size distribution of offered feed and feed remaining at 24 h after feeding was determined for all cows using a Penn State Particle Separator over two days in each week of feeding the amended diets, to estimate the potential for preferential selection of TMR components by the cows (Heinriches and Kononoff, 2002).

Milk production was determined daily throughout the experiments and milk samples (30 ml) were taken from six successive a.m. and p.m. milkings at weekly intervals and preserved with potassium dichromate (1 mg ml^{-1}; Lactabs, Thomson and Capper, Runcorn, UK) for the determination of milk composition during weeks 1 to 7 (or 8 in the case of the Biosolids treatment).

Offered diets were analysed by wet chemistry as detailed by Hammond et al. (2014). Samples were stored at −20 °C until thawed and coarsely chopped with dry ice before analysis for total N (macro Kjeldahl method) to estimate crude protein (CP) concentration (N multiplied by 6.25). In addition, representative sub-samples were dried at 60 °C and ground through a 1 mm screen before analysis for concentrations of neutral and acid detergent fibre (following the procedures of Mertens (2002) and Robertson and Van Soest (1981)), starch (enzymatic conversion to glucose and glucose measured using amyloglucosidase), oil (ether extraction following acid hydrolysis), and ash (by combustion at 600 °C). Milk samples were analysed using mid-infrared spectroscopy (Foss Electric Ltd., York, UK) to determine fat, protein and lactose concentrations.

2.4.2. Sample collection for organic contaminant analyses

Bulk milk samples of approximately 2 l per cow were collected from the two milkings over a 24 h period from each animal, immediately prior to feeding the amended diets, and then weekly until the end of the withdrawal period. Each sample was taken using an automated sampling system that took a representative sample across the milking period. The milk samples were immediately frozen at -20 °C. Four replicate milk samples taken in the week prior to feeding the amended diets (week 0), and at the end of week 3, after cessation of the amended diets (week 4 for the Biosolids treatment) for each treatment were submitted for organic chemical analysis. The organic contaminants detected in the biosolids or bioresource-soil blend supplied in a particular treatment diet were measured in the milk samples from that treatment, and also in the milk from the corresponding group of control cattle (Table 2). If one or more compounds in a contaminant group were elevated at week 3 (or week 4 for the Biosolids treatment) in comparison to week 0, or in comparison to the control at week 3 (or week 4 for the Biosolids treatment), the milk samples from weeks 1, 2 and 7 were also analysed (week 1, 2, 3 and 8 for the Biosolids treatment).

A 2 kg bulk sample of the diets fed from each week of each experiment was collected and stored frozen (−20 °C) and the week 2 composites were submitted for chemical analysis. Due to the longer acclimatisation period for the Biosolids treatment, the chemical analysis was conducted on the week 3 feed composite sample.

Straw bedding was replenished at intervals of two days when clean bedding was also collected to create a sample (2 kg) representative of each week of the 3 (or 4) week period that the cows were fed the recycled bioresource materials. Composite weekly bedding samples were stored frozen (−20 °C) and a sample from week 1 for each trial was submitted for contaminant analysis.

2.5. Organic contaminant content of the bioresources and the diets, dietary constituents, and straw bedding

Rigby et al. (2021) provided a detailed description of the organic contaminant profiles of the selected bioresources (Biosolids2, CLO2, MBMA1, PLA2 and PSA). Briefly, PCDD/Fs were found in the bioresources selected for the ingestion trials with the following WHO_{2005}-TEQ (Van den Berg et al., 2006) values: biosolids, 12.4 ng kg^{-1} DS; CLO, 11.2 ng kg^{-1} DS; MBMA, 83.1 ng kg^{-1} DS; PLA, 12.3 ng kg^{-1} DS and PSA, 0.12 ng kg^{-1} DS. The largest ΣWHO_{2005}-TEQ values obtained for non-ortho DL-PCBs (PCBs 77, 81, 126 and 169) were measured in the biosolids and MBMA samples at 1.7 ng kg^{-1} DS for both materials compared to 0.77 ng kg^{-1} DS for CLO, 0.36 ng kg^{-1} DS for PLA and 0.03 ng kg^{-1} DS for PSA. The ΣWHO_{2005}-TEQ values for ortho DL-PCBs (PCBs 105, 114, 118, 123, 156, 167 and 189) were relatively small for the biosolids and CLO samples supplied in the diets at 0.29 and 0.09 ng kg^{-1} DS, respectively, and were below the limit of quantification (LoQ) for the ashes (<0.01 ng kg^{-1} DS).

The PBDD/F concentration in biosolids was approximately six times larger than for the PCDD/Fs with a TEQ equivalent to 77.9 ng WHO_{1998}-TEQ kg^{-1} DS. The PBDD/Fs were also more abundant than PCDD/Fs in CLO, equivalent to 18.0 ng TEQ kg^{-1} DS. The concentrations of PBDD/Fs in the ashes were significantly smaller compared to the biosolids and CLOs, with an upper bound WHO_{2005}-TEQ generally <1 ng kg^{-1} DS, with the exception of PLA, which had a WHO_{2005}-TEQ value of 5.1 ng kg^{-1} DS. Unlike the biosolids and CLOs, the concentrations of PBDD/Fs in the ashes were less than their chlorinated counterparts. `For non-ortho PBBs, the bioresources had WHO_{1998}-TEQ values between 0.003 ng kg^{-1} DS (PSA) - 0.04 ng kg^{-1} DS (CLO). For the ortho PBBs, PBB 15 was detected in low concentrations in biosolids and CLOs at 0.02 μg kg^{-1} DS in both materials, which also contained 0.03 and 0.32 μg kg^{-1} DS, respectively, of PBB 153, but the other congeners were below the LoQ, and no ortho PBBs were detected in the ash samples.

Mixed-halogenated dibenzo-p-dioxins/dibenzofurans (PXDD/Fs) and mixed-halogenated biphenyls (PXBs) were found in relatively smaller concentrations in the bioresources compared to the PCDD/Fs and PBDD/Fs, although this is partly because fewer congeners could be measured due to the availability of analytical standards (Rigby et al., 2021). For PSA, all the individual PXDD/F congeners were below their LoQs. However, for biosolids, CLO, MBMA and PLA, between 7 and 12 of the congeners were detected. CLO had the greatest upper bound estimated TEQ value (see Rigby et al., 2021 for the basis to estimating TEQs for PXDD/Fs and PXBs) of 0.29 ng kg^{-1} DS, compared to values of 0.27 and 0.24 ng kg^{-1} DS for biosolids and MBMA, respectively, and PLA and PSA contained significantly lower values equivalent to 0.06 and 0.10 ng kg^{-1} DS, respectively.

Biosolids and CLO contained the largest Σ_{11}PCN concentrations of 541 ng kg^{-1} DS and 680 ng kg^{-1} DS, respectively. The upper bound Σ_{11}PCN values measured in the ash materials used in the ingestion experiments were in the range: 39.4 ng kg^{-1} DS (PSA) to 108 ng kg^{-1} DS (MBMA).

Biosolids had the largest overall ΣPAH_{16} (16 EPA PAHs, US ATSDR: Agency for Toxic Substances & Disease Registry, 2013), equivalent to 9415 μg kg^{-1} DS, followed by CLO with a ΣPAH_{16} of 4938 μg kg^{-1} DS. In contrast, the ΣPAH_{16} was up to three orders of magnitude smaller in the ash materials and in the range: 8.7 μg kg^{-1} DS (MBMA) to 117.8 μg kg^{-1} DS (PLA).

The concentrations of PCAs (sum of short and medium chain) in biosolids and CLO were equivalent to 140,688 μg kg^{-1} DS and 11,079 μg kg^{-1} DS, respectively, and in MBMA, PLA and PSA, they were 243.8, 27.6 and 5.9 μg kg^{-1} DS, respectively. Chlorobenzenes in biosolids and CLO had upper bound sums of 3.11 and 2.68 μg kg^{-1} DS, respectively, and concentrations in the recycled ashes were very small or below the LoQ.

In general, the concentrations of organic contaminants in the soil, straw bedding and the basal diet were low, although the organic contaminant content in the soil tended to be slightly above that in the straw or the basal diet (Tables S2-S13, SI). For example, the WHO_{2005}-TEQs for PCDD/Fs were 0.16 ng kg^{-1} dry soil (ds) and were 0.05 and 0.04 ng kg^{-1} DM for the straw and basal diet, respectively. For PAHs, the sum of Σ_{16}PAH was 156 ng kg^{-1} ds for soil, and 46.9 and 32.9 μg kg^{-1} DM for straw and basal diet samples, respectively, for Trial 1 and 72.0 and 22.8 μg kg^{-1} DM, respectively, for Trial 2. Hence, in contrast to the other organic contaminants examined, concentrations of PAHs in the soil and, in some cases, the straw and diet materials, were greater than in the ash samples (ΣPAH_{16}: 8.7–118 μg kg^{-1} DS), although they were significantly lower than in biosolids (9415 μg kg^{-1} DS) or CLO (4938 μg kg^{-1} DS).

The concentrations of organic contaminants measured in the diets fed to the cattle were generally consistent with expected calculated concentrations given the amounts in the dietary constituents and the amendments and the rates of addition to the diet, with some exceptions. The lower contaminant

concentrations in the Biosolids-soil and CLO-soil treatments compared to the Biosolids treatment were as expected, because in these treatments the biosolids and CLO were blended with soil at a rate of approximately 1 % and 2 % DS (on an agronomic N loading basis, Table 1), respectively, before blending with the basal feed at 5 % on a DM basis, whereas the Biosolids treatment contained biosolids blended directly with the feed at 5 % DM. In cases where concentrations were close to or below the LoQ (in these cases the LoQ was used), wider variation was observed between the calculated and observed concentrations in the diet. Differences between measured and calculated values could also be attributed to sub-sampling effects and the physico-chemical behaviour of certain contaminants groups.

For instance, in Trial 1, calculated WHO_{2005}-TEQ values for PCDD/Fs in the diets for the Control and Biosolids-soil diet matched the measured value at 0.05 ng TEQ kg^{-1} DS (Table S2, SI), and the calculated value for the CLO-soil diet was similar and equivalent to 0.06 ng TEQ kg^{-1} DS. However, the calculated value for the Biosolids diet was 0.66 ng TEQ kg^{-1} DS and more than twice the measured value of 0.25 ng TEQ kg^{-1} DS (Table S2, SI). This was mainly due to differences between the concentrations of PCDDs (rather than PCDFs) measured in the diet and the calculated values; in particular, 2,3,7,8-TCDD measured in the feed at 0.01 μg kg^{-1} DM was only 23 % of the expected concentration of 0.04 μg kg^{-1} DM. The sub-sample of the bulk biosolids sample (1.1 t fresh weight) collected for the trials was a composite consisting of 10 smaller sub-samples, which were well-mixed prior to analysis. It is possible that variability in the stockpile that the biosolids were collected from was not entirely represented by the sub-sample. However, the feed sample analysed for the Biosolids treatment was a composite of the daily feed blend from the third week of the trial when the cattle were offered the target rate of biosolids of 5 % DM intake, and was therefore expected to be representative of the concentrations of contaminants consumed by the cattle. Concentrations of PCAs measured in the diets were also below expected values; concentrations in the Biosolids, Biosolids-soil and CLO-soil feed blends were equivalent to 2871, 83.3 and 43.5 μg kg^{-1} DM, respectively, which were 30–40 % of the calculated values, whereas the concentration in the Control feed (which included soil incorporated at a rate of 5 % in the DM) was 90 % of the calculated value. Some short-chained PCAs are volatile (Tomy et al., 1999), and volatilisation after waste collection (and sampling for analysis), during soil equilibration, and in the feed mixing process could potentially account for the reduced amounts of other contaminant groups measured in the waste-amended feeds compared to the calculated mass balance.

2.6. Statistical analysis, calculations and data presentation

2.6.1. Statistical analysis of animal production data

Data were analysed using the Mixed Procedure of SAS (2015; version 9.4) and a model testing for fixed effects of treatment, week, and their interaction, random effects of cow within treatment, and repeated effects of week within cow using the covariance structure (compound symmetry, heterogeneous compound symmetry, autoregressive, heterogeneous autoregressive or unstructured) giving the best fit based on the lowest Bayesian Information Criterion value. Least squares means were compared using Dunnett's adjusted comparisons of treatments relative to the control.

2.6.2. Statistical analysis and calculations of organic contaminant concentrations in milk

World Health Organization toxic equivalencies (WHO-TEQs) take into account the concentration and the toxicity of each PCDD/F or DL-PCB congener relative to 2,3,7,8 TCDD. WHO-TEQs for PBDD/Fs, DL-PBBs and PXDD/Fs were calculated using Toxic Equivalency Factors (TEF) from analogous PCDD/F congeners, as proposed by Van den Berg et al., 2013, to provide an indicative overall TEQ. The earlier TEFs assigned by the WHO in 1998 for dioxin and DL substances were also used to allow comparison with other studies estimating WHO_{1998}-TEQs for PBDD/Fs and DL-PBBs. This approach was taken by Fernandes and Falandysz (2021) with the caution that any current assessments of the toxicity contribution from PBDD/Fs have a level of underestimation as not all contributing congeners are included. There is also a degree of uncertainty due to the use of analogous chlorinated TEFs.

For each compound, the 'explore' function of the IBM SPSS Statistics programme was applied to produce descriptive statistics and determine the normality of the data, and homogeneity of variances for contaminant concentrations in milk samples. If the data met the assumptions of analysis of variance (ANOVA), steps 1–4 below were followed:

1. Two-way ANOVA with repeated measures was conducted to examine the effects of treatment and time on the concentration of each congener in milk, and where significant differences ($P \leq 0.05$) were found, post-hoc tests (least significant difference: LSD) were performed.
2. One-way ANOVA was used to compare the different treatments at week 3 (week 4 for the Biosolids treatment), and where significant differences ($P \leq 0.05$) were found, post-hoc tests (LSD) were performed.
3. *t*-tests were used to compare week 3 (week 4 for the Biosolids treatment) to week 0 for each treatment using a significance level of 0.05.
4. If a significant difference ($P \leq 0.05$) between week 3 (or week 4 for biosolids) and week 0 data was found, t-tests were used to compare week 7 and week 3 (or week 8 and 4 for biosolids) data using a significance level of 0.05

If the assumptions of ANOVA were violated the following steps were followed:

1. A Kruskal-Wallis test was applied to compare the different treatments at week 3 (week 4 for Biosolids). The Kruskal-Wallis test does not assume a normal distribution of the residuals, and tests whether the medians of each group are equal. Where significant differences ($P \leq 0.05$) were found, Mann Whitney *U* tests were used for pairwise comparisons.
2. Paired tests for two related samples (Wilcoxon Signed Rank tests) were used to compare week 3 data (week 4 for Biosolids) to week 0 data using a significance level of 0.05.
3. If a significant difference between week 3 (or week 4 for Biosolids) and week 0 data was found, paired tests for related samples were used to compare week 7 and week 3 (or week 8 and 4 for Biosolids) data using a significance level of 0.05.

Bioconcentration factors (BCFs), to indicate the extent of transfer of contaminants from the feed to the milk, were calculated for a subset of analytical data according to Eq. (1):

$$\text{BCF} = \text{contaminant concentration in milk}\left(\mu g \text{ or ng } kg^{-1} \text{ fat}\right)/\text{contaminant concentration in feed}^{a}\left(\mu g \text{ or ng } kg^{-1} \text{ DM}\right) \quad (1)$$

[a]concentration in the composite feed sample from week 2 of the feeding period (week 3 for biosolids)

Carry Over Rates (CORs) (or transfer rates), which are considered by some authors to be a more appropriate metric than BCFs as they are not strongly influenced by lactation rate, body fat weight or livestock diet (Costera et al., 2006), were also calculated for the same subset of data, according to Eq. (2):

$$\mathrm{COR}(\%) = \left(\text{contaminant concentration in the milk}\left(\mu g \text{ or ng kg}^{-1} \text{ fat}\right) \text{x daily milk fat yield}^{a}\left(\text{kg fat day}^{-1}\right)\right) \div \left(\text{contaminant concentration in feed}^{b}\left(\mu g \text{ or ng kg}^{-1} \text{ DM}\right) \text{x daily dry matter intake}^{c}\left(\text{kg day}^{-1}\right)\right) \text{ x } 100 \quad (2)$$

[a]mean daily milk fat yield from week 3 (week 4 for the Biosolids treatment); [b]concentration in the composite feed sample from week 2 of the feeding period (week 3 for the Biosolids treatment); [c]mean daily dry matter intake during week 3 (week 4 for the Biosolids treatment).

The rate of organic contaminant uptake and the maximum concentration in milk at steady state were estimated by fitting the asymptotic relationship to the concentration data with the general form shown in Eq. (3):

$$y = a + b^{*}\left(1 - \exp^{-cx}\right) \quad (3)$$

where *y* is the organic contaminant concentration in milk at a given time ($\mu g\ kg^{-1}$ or $ng\ kg^{-1}$ milk fat, units depend on the concentration range of the contaminant); *a* is the initial concentration; $a + b$ is the concentration at steady state ($\mu g\ kg^{-1}$ or $ng\ kg^{-1}$ milk fat, units depend on the concentration range of the contaminant); *c* is the time rate constant and *x* is the time period (days) to reach the steady state concentration (Costera et al., 2006). The regression coefficients: *a*, *b* and *c*, were estimated using the constrained non-linear curve estimation procedure in SPSS.

Eq. (3) was rearranged to estimate the number of days required to achieve 95 % of the steady state concentration in milk fat as follows:

$$x = \ln(1 - (y/(a+b)))/-c \quad (4)$$

This procedure was followed for treatments and contaminant groups where statistically significant ($P \leq 0.05$) uptakes of contaminants to milk were detected in comparison to the control.

3. Results and discussion

3.1. Animal production data

The nutritional composition of the diets is presented in Table S14 and showed that they were broadly similar overall (note that the control diet also included soil incorporated into the TMR at a rate of 5 % DM). A full description of the animal production data is included in the SI (-Section S2.1) and includes feed intake, nutritional composition of the diets, animal production values (milk yield, composition, and liveweights; see Tables S15 and S16) and particle size analysis of the feed and the refused feed. Particle size analysis (Tables S17 and S18) indicated that the animals showed a degree of active avoidance of the bioresources in the feed. Indeed, biosolids were physically challenging to directly incorporate into the TMR and thus the material was partially susceptible to diet sorting. Nevertheless, in all cases, the majority of feed presented was consumed (see SI Section S2.1). Any trends or significant reductions in performance were most probably explained by the dilution

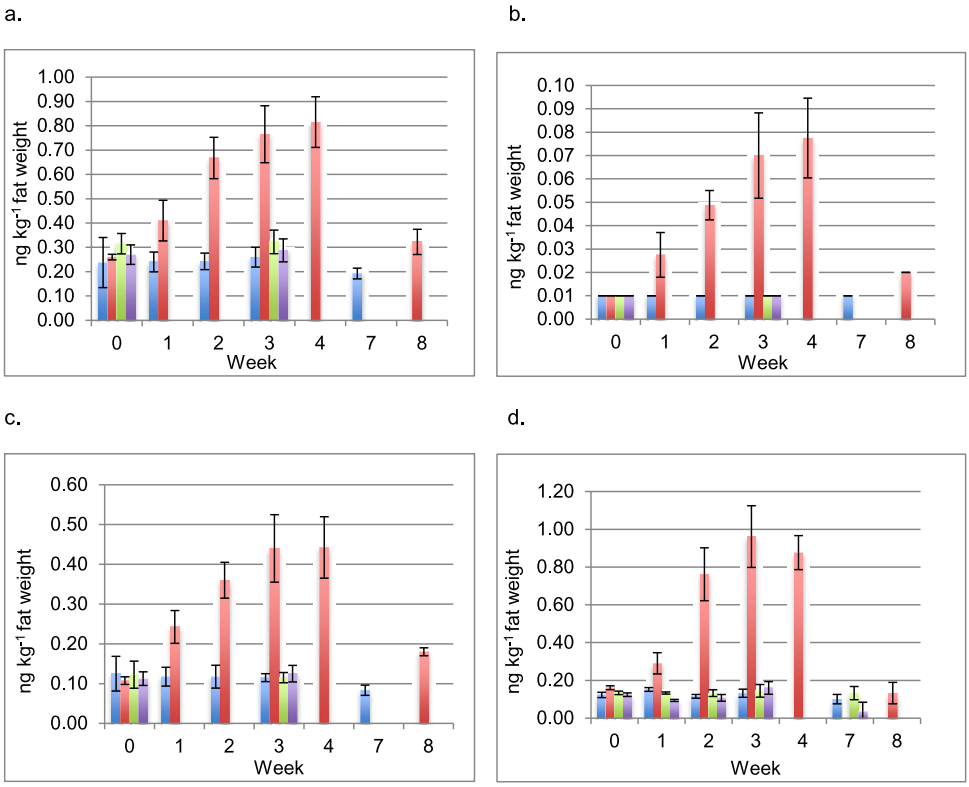

Fig. 1. Upper bound WHO_{2005}-TEQ concentrations for a) PCDD/Fs, b) dioxin-like ortho PCBs, c) non-ortho PCBs, and d) upper bound WHO_{1998}-TEQ concentrations of PBDD/Fs in milk (fat weight basis) for cattle ingesting diets amended with the following treatments: ■, Control (soil), ■, Biosolids, ■, Biosolids-soil and ■, CLO-soil; Where values were < LoQ the LoQ was used to calculate the mean; Error bars show the standard deviation of the mean.

of nutrients in the diet. However, animal performance was unaffected by incorporating the bioresources into the diets overall and was consistent with typical production values for commercial dairy cattle.

3.2. Organic contaminant transfer to milk from ingestion of bulky biowaste materials

3.2.1. Polychlorinated dibenzo-p-dioxin/furans

The maximum WHO_{2005}-TEQ for PCDD/Fs measured in milk for the Biosolids treatment was equivalent to 0.82 ng kg^{-1} fat at week 4 (the final week of feeding for the biosolids-amended diet) and was the only condition where a significant increase in overall PCDD/F WHO_{2005}-TEQ was observed in comparison to milk of Control cattle, which contained 0.24–0.26 ng TEQ kg^{-1} fat (Fig. 1a). A reduction in fat content of milk in the Biosolids treatment by approximately 15 % (Table S15, SI) may partially explain the increase in PCDD/F concentration, nevertheless, the results demonstrated the additional transfer of PCDD/F to the milk from the biosolids-amended feed was >3 times larger relative to the Control.

UK Total Diet Studies (TDSs) measure the concentrations of a range of contaminants in composite samples of different food groups across the UK and, hence, whilst not proving a benchmark, provide a snapshot of the average concentrations of various contaminants in different food groups available from UK retail outlets. The results showed the PCDD/F TEQ of milk for the Control was similar, but the Biosolids treatment was increased by a factor of ~3, compared to the Food Standards Agency (FSA) UK TDS value for milk of 0.25 ng TEQ kg^{-1} fat (Fera: Food and Environment Research Agency, 2012) and the European Food Safety Authority (EFSA) lower bound mean value in milk/milk products of 0.28 ng TEQ kg^{-1} fat (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2018). However, it was only increased by factor of two relative to the EFSA upper bound mean of 0.43 ng TEQ kg^{-1} fat (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2018) and was less than the lower and upper bound 95 percentile European background concentrations in milk of 0.92 and 1.06 ng TEQ kg^{-1} fat, respectively (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2018). Furthermore, the TEQ for the Biosolids treatment was almost 70 % smaller than the EU Maximum Level of 2.5 ng TEQ kg^{-1} fat for PCDD/Fs in milk (EC: European Commission, 2011, as amended).

Significantly ($P \leq 0.05$) increased concentrations of the 7 PCDD and 7 of 10 PCDF congeners determined in the milk at week 4 were observed in the Biosolids treatment in comparison to the Control (Table S19 and Fig. S1, SI). No additional transfer of PCDD/Fs to the milk was observed for the Biosolids-soil and CLO-soil treatments, which may be expected given that the concentrations in the diets were equivalent to those in the Control (Table S2, SI). Carry Over Rates and BCFs for each congener for the Control and treatments amended with bulky biowastes from Trial 1 are presented in Table 3. Under the conditions of the investigation, BCFs were expected to provide a reliable estimate of bioavailability given the diets were carefully controlled and the animals were selected to be a consistent stage of lactation, nevertheless, CORs were also calculated for comparison with other published literature. The metrics were calculated based on the contaminant concentrations measured in the milk after feeding the experimental diets for 3 weeks (or 4 weeks for Biosolids). The CORs and BCFs were generally consistent with previous reports (e.g. McLachlan et al., 1990; Fries et al., 1999; Amutova et al., 2021; Driesen et al., 2022) and demonstrated that transport of PCDD/Fs to milk decreased with increasing level of chlorination. For the Control, Biosolids-soil and CLO-soil treatments, the patterns observed in each group of COR and BCF values were generally similar, whereas they were smaller for the Biosolids treatment, being reduced by approximately 50 % in the case of PeCDDs. The mean COR of 2378-TCDD was 39.5 % in the Biosolids treatment (Table 3), similar to the mean transfer rate of 34 % ± 6.3 found for 2378-TCDD in a meta-analysis of persistent organic pollutants in food (Amutova et al., 2021). The concentrations of 2378-TCDD were < LoQ in the feed of the Control, Biosolids-soil and CLO-soil treatment so CORs and BCFs were not calculated for these treatments.

Table 3
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for PCDD/F congeners in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

Congener		Control		Biosolids		Biosolids-soil		CLO-soil	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
2378-TCDD	COR	–	–	39.5	9.02	–	–	–	–
	BCF	–	–	8.25	1.50	–	–	–	–
12378-PeCDD	COR	54.2	4.82	21.9	3.37	73.1	21.3	–	–
	BCF	8.75	0.96	4.63	0.96	12.0	2.45	–	–
123478-HxCDD	COR	37.1	7.80	15.2	4.44	45.5	10.2	–	–
	BCF	6.00	1.41	3.15	0.66	7.50	1.00	–	–
123678-HxCDD	COR	58.1	10.1	20.8	4.50	50.4	7.38	50.4	7.38
	BCF	9.33	1.44	4.32	0.35	8.38	0.63	9.00	1.41
123789-HxCDD	COR	29.1	4.35	11.7	2.85	22.8	5.08	22.8	5.08
	BCF	4.67	0.54	2.44	0.47	3.75	0.50	3.50	1.73
1234678-HpCDD	COR	7.33	2.00	3.13	0.76	8.18	4.13	8.18	4.13
	BCF	1.19	0.36	0.65	0.08	1.33	0.58	1.05	0.13
OCDD	COR	1.40	0.49	0.37	0.09	–	–	–	–
	BCF	0.23	0.09	0.08	0.00	–	–	–	–
2378-TCDF	COR	–	-	–	–	–	–	–	–
	BCF	–	-	–	–	–	–	–	–
12378-PeCDF	COR	–	-	–	–	30.2	20.2	30.2	20.2
	BCF	–	-	–	–	5.63	3.61	5.63	3.61
23478-PeCDF	COR	38.9	7.27	22.1	4.42	48.6	11.4	48.6	11.4
	BCF	6.25	1.10	4.60	0.45	8.00	1.18	6.50	0.58
123478-HxCDF	COR	26.7	5.49	17.3	3.16	23.5	6.11	23.5	6.11
	BCF	4.30	0.89	3.61	0.35	3.92	1.00	5.25	0.65
123678-HxCDF	COR	30.2	4.51	18.2	3.28	31.7	6.08	31.72	6.08
	BCF	4.88	0.75	3.81	0.55	5.25	0.65	5.88	1.38
123789-HxCDF	COR	–	-	3.42	1.90	–	-	-	-
	BCF	–	-	0.67	0.27	–	-	–	-
234678-HxCDF	COR	29.4	6.12	13.2	1.94	35.9	11.3	35.9	11.3
	BCF	4.75	1.04	2.77	0.31	5.88	1.31	4.63	1.11
1234678-HpCDF	COR	6.37	2.57	2.61	0.49	10.8	6.60	10.8	6.60
	BCF	1.03	0.43	0.54	0.06	1.75	0.95	1.17	0.37
1234789-HpCDF	COR	–	-	3.11	1.54	–	-	–	-
	BCF	–	-	0.63	0.28	–	-	–	-
OCDF	COR	–	-	0.30	0.04	4.30	0.74	4.30	0.74
	BCF	–	-	0.06	0.01	0.71	0.05	0.78	0.45
WHO_{2005}-TEQ lower	COR	39.1	5.17	15.6	2.58	46.5	6.94	46.5	6.94
	BCF	6.29	0.62	3.26	0.42	7.71	0.40	9.00	1.52
WHO_{2005}-TEQ upper	COR	32.2	4.50	15.6 [22.0][a]	2.58	39.2	9.65	39.2	9.65
	BCF	5.20	0.82	3.26 [4.32][a]	0.42	6.45	0.97	5.75	0.94

Carry Over Rate (COR) (%) = daily contamination in the milk (ng day^{-1})/daily contamination in the feed (ng day^{-1}) x100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (ng kg^{-1} fat)/contaminant concentration in the feed (ng kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation; −, COR or BCF was not determined either because all four of the replicates were < LoQ in the milk or the feed concentration was <LoQ.
[a] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis (Table 4).

The COR of 12378-PCDD for the Biosolids treatment was 21.9 % and was in a similar range to the mean transfer rate reported by Amutova et al. (2021) for this congener of 26.7 % ± 7.1. However, the CORs for the Control and Biosolids-soil treatments were considerably larger and equivalent to 54.2 and 73.1 %, respectively. The reduced bioavailability may be explained by the binding of lipophilic organic contaminants to organic matter in biosolids, which markedly reduces bioaccessibility (Aigars et al., 2017). These results agree with previous research indicating the reduced bioavailability of PCDD/Fs from non-food matrices when compared with food matrices (Theelen and Van Laar, 1992; Slob et al., 1995; Huwe and Smith, 2005; Wittsiepe et al., 2007). For example, Beck et al. (1996) reviewed the forms and bioavailability of non-ionic organic compounds in biosolids-amended agricultural soil and found evidence that organic contaminants were less available in amended in comparison to unamended soils. It is also well established that extrapolation of research on the behaviour of organic contaminants in unamended soils overestimates the plant

Table 4
Parameter estimates, including estimated steady state concentration and days to reach 95 % of steady state, from asymptotic regression of data for cattle ingesting biosolids at 5 % of their total dietary DM intake.

Contaminant group	Parameter estimates			r^2	Time to reach 95 % steady state (days)
	a	*b*	*c*		
ΣPCDD/Fs (WHO_{2005}-TEQ) (ng kg^{-1} fat)	0.24	0.84	0.044	0.87**	69
Σ$ICES_6$ PCBs (μg kg^{-1} fat)	0.52	6.96	0.051	0.91**	59
ΣDioxin-like ortho PCBs (WHO_{2005}-TEQ) (ng kg^{-1} fat)	0.01	0.16	0.021	0.84**	143
ΣNon-ortho PCBs (WHO_{2005}-TEQ) (ng kg^{-1} fat)	0.10	0.42	0.065	0.84**	46
ΣPBDD/Fs (WHO_{1998}-TEQ) (ng kg^{-1} fat)	0.11	1.17	0.047	0.83**	64
ΣPCNs (ng kg^{-1} fat)	5.91	17.5	0.072	0.81**	42
ΣPCAs (μg kg^{-1} fat)	46.5	8800	0.015	0.88**	200

$a + b$ is the concentration at steady state (μg kg^{-1} or ng kg^{-1} milk fat, units depend on the concentration range of the contaminant); *c* is the time rate constant.
** $P \leq 0.01$.

availability and uptake of compounds from biosolids-amended soils (O'Connor et al., 1991). The soil used in this research (Table S1, SI) was selected specifically for its relatively low organic matter (4.2 % ds) and high sand particle size fraction (84 % ds), conditions which can increase the bioavailability of organic contaminants in agricultural soil (Gao et al., 2013).

An alternative explanation, or at least a contributing factor, to the lower CORs and BCFs in the Biosolids treatment is that the increasing concentration of an organic contaminant in the feed may itself reduce the rate of transfer to milk regardless of the matrix. Fries (1996) presented BCF data for the milk fat of cattle that indicated that the BCF for the PCB mixture Aroclor 1254 decreased from 3.6 to 1.5 with increasing concentration of the PCB in the feed from 0.22 to 51.2 mg kg^{-1}. However, contaminant concentrations considered by Fries (1996) were many orders of magnitude greater than those measured in the diets or contemporary bioresources investigated here. Therefore, it is possible that such a diminishing uptake response in relation to concentration may only operate under very extreme exposure conditions and may, thus, not be relevant to the interpretation of the results presented here.

A number of the PCDD/F congeners appeared to be reaching a steady state in the milk of the Biosolids treatment at week 4 of the feeding study (eg 123789-HxCDD and 23478-PeCDF; Fig. S1, SI); however, the transfer of others, such as 1234678-HpCDD and 234678-HxCDF was still increasing when the amended experimental diet was withdrawn. Hence, the lower COR and BCF for the Biosolids treatment may also partially reflect that steady state had not been achieved for some congeners due to the higher concentrations of contaminants in the feed compared to the other diets. Furthermore, for the cattle ingesting the Biosolids treatment, there was a 16-day transition from feed containing 0 % biosolids to feed containing 5 % biosolids in the DM, compared to a far shorter transition period of 4 days for the Control (soil only) and bioresource-soil treatments. Hence, by the end of week 4 the cattle ingesting the Biosolids treatment had been consuming the maximum rate of 5 % biosolids DM in their diet for 12 days, whereas the cattle ingesting the other treatments had been consuming the maximum amendment rate for 17 days by the end of week 3.

Hoogenboom et al. (2015) investigated the transfer and uptake of dioxins and DL-PCBs from smoke contaminated maize or beet forage to cow's milk. The upper bound TEQs were 0.79–1.9 ng kg^{-1} for PCDD/Fs in the feed and 0.98–2.05 ng kg^{-1} for DL-PCBs. However, the concentrations of PCDD/Fs and PCBs were still increasing in milk fat after day 33 of the study and the initial rapid increase that occurred over the first 1–2 weeks was followed by a slower rise until the end of the exposure period. Hence, although the concentrations of PCDD/Fs in the milk of the cattle ingesting the Biosolids treatment may have been approaching the maximum concentration by the end of week 4, it may have required a longer period of ingestion to reach full steady state. It should be noted, however, that the feed supplied by Hoogenboom et al. (2015) contained approximately 2–8 times more PCDD/Fs than the direct addition of biosolids to the diet prepared here.

An asymptotic regression procedure, described in Section 2.6.2, was therefore used to estimate the rate of uptake of PCDD/Fs to the milk in the Biosolids treatment, the steady state concentration and days to reach steady state; the regression parameters for PCDD/Fs, along with the other contaminant groups, are presented in Table 4 and were highly statistically significant ($P \leq 0.01$, $r^2 = 0.87$). The predicted maximum steady state TEQ concentration for PCDD/Fs for cattle ingesting the Biosolids treatment was 1.08 ng kg^{-1} fat (approximately 60 % smaller than the EU Maximum Level; EC: European Commission, 2011, as amended), and the statistical model estimated that the exposure time to achieve 95 % of the steady state value was 69 days. However, this is likely to be an overestimate of the time period required to reach a steady state concentration given that, for animal husbandry reasons, the target maximum rate of biosolids ingestion of 5 % DM of the TMR was not received until day 16 of the feeding experiment.

As Hoogenboom et al. (2015) also observed, there was an initial rapid rise in PCDD/F concentrations in milk over approximately a three-week period of feeding, followed by a slower rate of increase, thus the maximum ΣPCDD/F WHO_{2005}-TEQ concentration at 4 weeks for the Biosolids treatment, of 0.82 ng kg^{-1} fat (Fig. 1a), was equivalent to 76 % of the estimated maximum steady state. A BCF of 4.32 was calculated using the steady state concentration estimated by the regression model (Table 3), which, although higher than the value obtained using the week 4 data, was, nevertheless, lower than the BCFs for the other treatments, indicating reduced bioavailability in the biosolids-amended diet compared to the soil blended treatments, including the Control.

3.2.2. Polychlorinated biphenyls

As observed for PCDD/Fs, ortho DL-PCBs were also only transferred to the milk in statistically significantly ($P \leq 0.05$) greater amounts for the Biosolids treatment compared to the Control (Table S20 and Fig. S2, SI) due to the greater concentrations of DL-PCBs in the Biosolids diet treatment (Table S3). Thus, the WHO_{2005}-TEQ value for the ortho DL-PCBs (105,

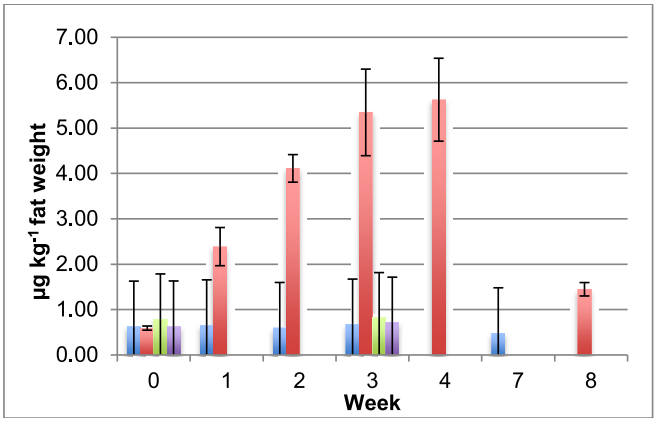

Fig. 2. Upper bound Σ$ICES_6$ PCB concentrations in milk (fat weight basis) for cattle ingesting diets amended with the following treatments: ■, Control (soil), ■, Biosolids, ■, Biosolids-soil and ■, CLO-soil; Where values were < LoQ the LoQ was used to calculate the mean; Error bars show the standard deviation of the mean.

114, 118, 123, 156, 167 and 189) in the Biosolids treatment at week 4 was 0.08 ng kg^{-1} fat (Fig. 1b) and was significantly greater than the mean Control value of 0.01 ng kg^{-1} fat (Table S20, SI). All four of the measured non-ortho PCB congeners (77, 81, 126 and 169) were also transferred to the milk of cattle in the Biosolids treatment group in significantly greater concentrations than for Control animals (Table S21 and Fig. S3, SI). The WHO_{2005}-TEQ value for non-ortho PCBs was 0.44 ng TEQ kg^{-1} fat for the Biosolids treatment at week 4 compared to 0.12 ng TEQ kg^{-1} fat in the Control (Fig. 1c).

Transfer of non-DL ortho PCBs to the milk significantly above Control concentrations was observed only in the Biosolids treatment for 7 of 13 measured non-DL congeners (49, 99, 101, 128, 138, 153, 180) (Fig. S3, SI). The $\Sigma ICES_6$ was 5.6 μg kg^{-1} fat for the Biosolids treatment at week 4, compared to 0.63–0.67 μg kg^{-1} fat in the Control (weeks 0–3) (Fig. 2 and Table S20, SI).

Mean CORs and BCFs for ortho PCBs and non-ortho PCBs are presented in Tables 5 and 6, respectively, and were generally in a similar range to those reported previously (Thomas et al., 1998). As observed for the PCDD/Fs, the CORs and BCFs for the Biosolids treatment were typically smaller than for the other dietary amendments, for example, the COR for PCB 180 was 79.4 % for the Control and 38.6 % for the Biosolids treatment, compared to a mean reported value of 51.8 % ± 17.7 % (Amutova et al., 2021). It is possible that the lower values for the Biosolids treatment compared to the Control, CLO-soil and Biosolids-soil treatments were a consequence of the reduced bioavailability of organic contaminants in biosolids. However, again, it is also possible that this was partly explained because the concentrations of PCBs in the milk fat of the Biosolids treatment had not yet reached a steady state by week 4, especially for certain congeners, such as 114, 189 and 77, that were still increasing (Figs. S2 and S3, SI).

Indeed, the predicted maximum steady state concentration for cattle ingesting the Biosolids treatment was 0.17 ng WHO_{2005}-TEQ kg^{-1} fat for ortho DL-PCBs (Table 4), and the estimated time period to reach 95 % of the maximum concentration was 143 days. Thus, the results indicated the ortho DL-PCB concentration measured at 4 weeks of 0.08 ng WHO_{2005}-TEQ kg^{-1} fat was less than half the potential maximum value. In the study by Lorenzi et al. (2020), ortho DL-PCBs reached steady state within 45 days (also determined via kinetic modelling). This period was shorter than typically reported for naturally contaminated diets, which can reach steady state within 3 months of continued exposure McLachlan and Richter (1998) and was explained due to the high concentration of contaminated corn oil offered to the cattle. This was fed daily by supplying each cow in the treatment group with 20 ml of contaminated corn oil (control cows received uncontaminated corn oil) mixed in 1 kg of TMR prior to feeding 22 kg of TMR. The Σ of ortho DL-PCBs: 105, 114, 118, 123, 156, 167, 189 in the corn oil was 53.3 ng g^{-1}, approximately equivalent to 1 μg in the 20 ml fed daily. However, this was less than the daily amount of ortho DL-PCBs supplied in the feed to cattle in the Biosolids treatment in this investigation. The upper bound Σ of the same seven DL-PCBs (105, 114, 118, 123, 156, 167, 189) in the feed was 0.36 μg kg^{-1} (Table S3). Hence, with an average daily intake of 22.3 kg DM day^{-1} (Table S15) for this treatment, this was equivalent to a daily consumption of 8.03 μg. Differences in the time intervals to achieve steady state could be explained by a number of reasons, however, the physico-chemical nature and properties of the source of dietary contamination could be an important factor. For example, PCBs could be more uniformly incorporated into the feed in corn oil and cattle may therefore receive a more consistent daily intake compared to semi-solid aggregates in biosolids.

Table 5
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for $ICES_6$ and dioxin-like ortho PCB congeners in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

		Control		Biosolids		Biosolids-soil		CLO-soil	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
PCB 18	COR	–	–	4.93	2.28	–	-	–	–
	BCF	–	–	1.00	0.31	–	-	–	–
PCB 28	COR	–	–	4.12	0.84	-	-	-	-
	BCF	–	–	0.86	0.12	–	-	–	–
PCB 52	COR	–	–	1.03	0.21	11.4	1.34	–	–
	BCF	–	–	0.21	0.03	1.90	0.20	–	–
PCB 101	COR	11.4	9.06	1.89	0.32	10.2	7.00	7.56	4.87
	BCF	1.83	1.48	0.39	0.04	1.67	1.15	1.50	1.08
PCB 105	COR	–	-	-	-	–	-	–	–
	BCF	–	-	–	-	–	-	–	–
PCB 114	COR	-	-	–	-	-	-	–	–
	BCF	–	-	–	-	–	-	–	–
PCB 118	COR	41.5	4.83	36.2	8.41	80.5	25.3	57.2	9.91
	BCF	6.67	0.54	7.57	1.65	13.3	3.30	10.8	1.26
PCB 123	COR	–	-	–	-	-	-	–	–
	BCF	–	-	–	-	–	-	–	–
PCB 138	COR	53.6	4.98	33.1	6.85	64.7	22.1	48.6	9.12
	BCF	8.63	0.63	6.94	1.34	10.63	2.87	9.13	1.11
PCB 153	COR	68.4	6.74	40.5	8.76	84.5	30.5	61.9	11.5
	BCF	11.0	0.71	8.46	1.48	13.8	4.03	11.6	1.49
PCB 156	COR	–	-	38.1	8.02	–	-	–	–
	BCF	–	-	8.00	1.70	–	-	–	–
PCB 167	COR	–	-	37.0	8.39	–	-	–	–
	BCF	–	-	7.75	1.71	–	-	–	–
PCB 180	COR	79.4	12.9	38.6	7.57	119.7	68.4	82.8	18.7
	BCF	12.8	1.71	8.03	0.97	19.5	9.81	15.5	2.38
PCB 189	COR	–	–	–	–	–	–	–	–
	BCF	–	–	–	–	–	–	–	–
$\Sigma ICES_6$[a] lower	COR	32.8	4.17	20.5	4.19	40.1	15.9	28.3	5.80
	BCF	5.28	0.48	4.28	0.68	6.57	2.17	5.32	0.79
$\Sigma ICES_6$[a] upper	COR	36.3	1.32	20.6 [27.7][b]	4.16	39.7	14.3	27.1	4.04
	BCF	5.85	0.24	4.29 [5.7][b]	0.70	6.52	1.88	5.11	0.44

Carry Over Rate (COR) (%) = daily contamination in the milk (μg or ng day^{-1})/daily contamination in the feed (μg or ng day^{-1}) x 100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (μg or ng kg^{-1} fat)/contaminant concentration in the feed (μg or ng kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation; −, COR or BCF was not determined either because all four of the replicates were < LoQ in the milk or the feed concentration was <LoQ.
CORs/BCFs not reported for PCB 189 as all feed concentrations were <LoQ.
COR/BCFs were not calculated for WHO_{2005}-TEQ values as these values were very low and <0.01 ng kg^{-1} in the feed samples.
[a] $\Sigma ICES_6$ congeners: 28, 52, 101, 138, 153 and 180.
[b] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis.

Table 6
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for non-ortho PCB congeners in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

		Control		Biosolids		Biosolids-soil		CLO-soil	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
PCB 77	COR	5.78	2.18	1.03	0.31	5.99	1.16	5.09	1.66
	BCF	0.93	0.37	0.21	0.02	0.99	0.11	0.94	0.23
PCB 81	COR	13.26	1.90	8.07	2.30	16.3	4.52	18.15	4.83
	BCF	2.15	0.40	1.66	0.25	2.67	0.52	3.39	0.66
PCB 126	COR	59.3	5.88	30.0	6.02	66.2	14.2	68.6	14.7
	BCF	9.55	0.79	6.29	1.13	10.93	1.44	12.9	2.15
PCB 169	COR	46.6	11.7	38.6	8.39	49.9	16.4	43.2	9.39
	BCF	7.50	1.91	8.06	1.38	8.17	2.08	8.17	1.84
WHO_{2005}-TEQ lower	COR	71.4	6.82	30.2	5.97	69.6	13.7	66.6	14.8
	BCF	11.5	1.00	6.32	1.10	11.50	1.29	12.5	2.08
WHO_{2005}-TEQ upper	COR	71.4	6.8	30.2 [36.1][a]	5.96	69.6	13.7	66.6	14.8
	BCF	11.5	1.00	6.32 [7.25][a]	1.10	11.50	1.29	12.5	2.08

Carry Over Rate (COR) (%) = daily contamination in the milk (ng day^{-1})/daily contamination in the feed (ng day^{-1}) x100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (ng kg^{-1} fat)/contaminant concentration in the feed (ng kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation.
[a] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis.

Hoogenboom et al. (2015) fed dairy cows smoke contaminated maize silage or sugar beet pulp with an upper bound Σ of the same seven ortho DL-PCBs between 0.2 and 1.4 μg day^{-1}, providing a similar daily consumption to Lorenzi et al. (2020). In this case, near steady state conditions for dioxins and DL-PCBs occurred at approximately day 29, however, physiologically based kinetic modelling showed the true steady state required 200 days, more consistent with the 143 days corresponding to 95 % of the maximum concentration calculated here.

The predicted steady state concentration for the upper bound $\Sigma ICES_6$ PCBs was 7.47 μg kg^{-1} fat, hence, the amount measured in milk fat at the end of week 4 represented 79 % of the estimated maximum. The regression model estimated that the $\Sigma ICES_6$ PCBs would increase to 95 % of the steady state concentration in 59 days (Table 4). The maximum predicted concentration of non-ortho PCBs in milk was 0.52 ng WHO_{2005}-TEQ kg^{-1} fat; the concentration at week 4 of 0.44 ng WHO_{2005}-TEQ kg^{-1} fat was therefore equivalent to 83 % of the estimated maximum steady state. The rate of accumulation of non-ortho PCBs in the milk was also more rapid compared to the ortho PCBs (Fig. 1b and c) and was estimated to increase to 95 % of the maximum steady state concentration in 46 days, compared to 143 days for the ortho DL-PCBs, despite the smaller predicted maximum concentration of ortho DL-PCBs (Table 4).

Carry over rates and BCFs determined for PCDD/Fs tended to decrease with increasing level of chlorination, in contrast, for the PCBs, some of the more highly chlorinated PCBs, such as 138, 153 and 180, apparently had larger CORs and BCFs compared to less chlorinated congeners. This is consistent with the observations of other authors and the possible metabolism of some of the lower chlorinated PCBs (Thomas et al., 1998; Tremolada et al., 2014; Driesen et al., 2022).

3.2.3. Polybrominated and mixed halogenated dibenzo-p-dioxin/furans and biphenyls

A statistically significant ($P \leq 0.05$) increase in the transfer of PBDD/Fs to milk was observed in the Biosolids treatment compared to the Control for 10 of 11 congeners (Table S22, SI), and particularly for PBDFs (Fig. S4, SI), but there was no significant effect of biosolids and CLO amendment to soil on the PBDD/F concentration in milk. Earlier TEQ values assigned by the WHO in 1998 (Van den Berg et al., 1998) were used to examine the PBDD/F concentration data for consistency with other studies that have estimated TEQs for PBDD/Fs (Fernandes et al., 2018; Falandysz et al., 2020), and because there is not yet sufficient evidence to indicate that WHO_{2005}-TEQs are more applicable to the brominated congeners (Van den Berg et al., 2013). The WHO_{1998}-TEQ at weeks 3 and 4 were equivalent to 0.96 and 0.88 ng TEQ kg^{-1} fat in the Biosolids treatment, respectively. These values were more than seven times those measured in the Control of 0.12–0.15 ng TEQ kg^{-1} fat during weeks 0–3 (Fig. 1d). However, the maximum WHO_{1998}-TEQ value determined for PBDD/Fs in milk for the Biosolids treatment was similar to the maximum WHO_{2005}-TEQ due to the PCDD/Fs (Fig. 1a). Given that the WHO_{1998}-TEQ for PBDD/Fs in the biosolids was five times larger than the WHO_{2005}-TEQ for PCDD/Fs (Section 2.5), the results strongly suggested that PBDD/F transfer to milk was considerably less than for PCDD/Fs.

Carry Over Rates and BCFs for PBDD/Fs are presented in Table 7. The PBDD/Fs in the milk and/or the feed in the Control, Biosolids-soil and CLO-soil treatments were generally <LoQ with the exception of 2378-TBDD/F, 23478-PeBDF and 1234678-HpBDF. In these cases, the CORs and BCFs for PBDD/Fs were smaller than the corresponding PCDD/Fs. For example, the Control treatment BCF for 23478-PeBDF, of 2.3, and the COR of 14.5 %, were almost three times smaller than the corresponding

Table 7
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for PBDD/F congeners in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

		Control		Biosolids (3)		Biosolids (4)		Biosolids-soil		CLO-soil	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
237-TriBDD	COR	–	–	–	–	–	–	–	–	–	–
	BCF	–	–	–	–	–	–	–	–	–	–
2378-TBDD	COR	–	–	11.6	2.96	4.06	1.54	–	–	–	–
	BCF	–	–	2.00	0.33	0.83	0.17	–	–	–	–
12378-PeBDD	COR	–	–	–	–	–	–	–	–	–	–
	BCF	–	–	–	–	–	–	–	–	–	–
123478/123678-HxBDD	COR	–	–	–	–	–	–	–	–	–	–
	BCF	–	–	–	–	–	–	–	–	–	–
123789-HxBDD	COR	–	–	–	–	7.47	4.21	–	–	–	–
	BCF	–	–	–	–	–	–	–	–	–	–
238-TriBDF	COR	–	–	1.34	0.60	0.28	0.11	–	–	8.34	2.76
	BCF	–	–	0.24	0.13	0.06	0.02	–	–	1.59	0.56
2378-TBDF	COR	–	–	1.83	1.59	1.13	0.07	–	–	–	–
	BCF	–	–	0.09	0.03	0.86	0.04	–	–	–	–
12378-PeBDF	COR	–	–	3.35	0.94	1.70	0.27	–	–	–	–
	BCF	–	–	0.58	0.11	0.37	0.11	–	–	–	–
23478-PeBDF	COR	14.5	6.80	8.93	2.48	8.12	1.24	–	–	–	–
	BCF	2.30	0.93	1.54	0.29	1.70	0.19	–	–	–	–
123478-HxBDF	COR	–	–	0.69	0.15	0.21	0.06	–	–	–	–
	BCF	–	–	0.13	0.06	0.05	0.02	–	–	–	–
1234678- HpBDF	COR	1.01	0.60	0.12	0.04	0.06	0.01	0.60	0.50	–	–
	BCF	0.17	0.10	0.02	0.00	0.01	0.00	0.09	0.07	–	–
WHO-TEQ[a] lower	COR	1.20	1.04	1.24	0.27	0.96	0.15	1.07	0.40	13.0	10.4
	BCF	0.19	0.15	0.22	0.04	0.20	0.02	0.18	0.05	2.44	1.85
WHO-TEQ[a] upper	COR	6.00	1.01	1.34	0.33	1.01[1.7][b]	0.16	3.00	0.61	9.89	3.39
	BCF	0.97	0.19	0.23	0.04	0.21[0.3][b]	0.02	0.50	0.05	1.85	0.55

Carry Over Rate (COR) (%) = daily contamination in the milk (ng day^{-1})/daily contamination in the feed (ng day^{-1}) x100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (ng kg^{-1} fat)/contaminant concentration in the feed (ng kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation; −, COR or BCF was not determined either because all four of the replicates were < LoQ in the milk or the feed concentration was <LoQ.
[a] Calculated using WHO_{1998}-TEQs for PCDD/Fs.
[b] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis.

BCF and COR values obtained for 23478-PeCDF, which were equivalent to 6.3 and 38.9 %, respectively.

At week 4, the CORs of PBDD/Fs for the Biosolids treatment (Table 7) were generally between 1 and 82 times smaller than the corresponding PCDD/Fs and the BCF values were 2–72 times less than for their PCDD/F counterparts (Table 3). For the majority of PBDD/F congeners, with the exception of 23478-PeBDF, the concentrations in the milk decreased between week 3 and week 4 (Fig. S4, SI), hence, the BCFs for week 3 are also presented in Table 7. Nevertheless, CORs and BCFs determined at week 3 were also smaller than the corresponding PCDD/Fs, for example by a factor of 2.5–28 times in the case of BCFs. As observed for the PCDD/Fs, when concentrations of PBDD/Fs were > LoQ and CORs and BCFs could be calculated for the Control, Biosolids-soil and CLO-soil treatments, they were greater than those calculated for corresponding congeners in the Biosolids treatment, potentially indicating the lower bioavailability of PBDD/Fs in biosolids. It is possible that the PBDD/Fs had not reached a maximum steady state by week 4, although the data indicated that the concentration of PBDD/Fs in the milk fat of the Biosolids treatment was showing a diminishing response with time (Fig. S4, SI). In particular, the more highly brominated furans had significantly smaller CORs and BCFs than the chlorinated furans, for example, the BCF for 123478-HxBDF at week 4 was 0.05, 72 times smaller than the BCF for 123478-HxCDF of 3.6 (Table 3).

The estimated steady state concentration for the PBDD/Fs in the milk of the Biosolids treatment was 1.28 ng WHO_{1998}-TEQ kg^{-1} fat (Table 4); hence, the week 4 result of 0.88 ng WHO_{1998}-TEQ kg^{-1} fat (Table S22) was equivalent to 69 % of the maximum predicted concentration. The regression analysis indicated that the concentration would increase to 95 % of the maximum steady state in 64 days, similar to the 69 days determined for PCDD/Fs. The COR and BCF of 1.7 % and 0.3 (Table 7), respectively, calculated using the estimated steady state concentration for ΣPBDD/Fs TEQ (Table 4), were higher than the COR and BCF of 1.01 and 0.21, respectively, determined by measurement at week 4, but the values were still significantly smaller compared to the other treatments (Table 7). This pattern of behaviour thus indicated the potentially reduced bioavailability of PBDD/Fs due to the biosolids matrix, although the significantly larger and therefore more reliable measurement of the concentration in this treatment compared to the control and soil-blended treatments cannot be discounted.

Ortho DL-PBBs were not detected and most of the non-ortho PBBs were <LoQ so statistics could not be conducted (Table S23, SI). However, one ortho PBB congener, PBB 153, was detected in significantly ($P \leq 0.03$) increased concentrations in the Biosolids treatment compared to the Control (Fig. S5, SI). PXDD/Fs were also <LoQ in milk (Table S24, SI). Neither PXDD/Fs or PXBs were detected in the blended biosolids-soil mixture, therefore, analysis of these compounds was not taken forwards to the milk (Table 2). However, PXBs 105 and 118 were present in milk in statistically significantly greater concentrations in the Biosolids treatment compared to the CLO-soil and Control treatments, and were also elevated in the CLO-soil treatment relative to the Control, albeit not significantly ($P > 0.05$) (Table S25, SI).

3.2.4. Chlorobenzenes, polycyclic aromatic hydrocarbons and polychlorinated naphthalenes

Chlorobenzenes and PAHs were present in biosolids and CLO, nevertheless, concentrations in the milk were < LoQ for chlorobenzenes (Table S26, SI) and generally <LoQ for PAHs, or, when detected in milk, no transfer occurred above the Control values (Table S27, SI). Thus, the results indicated that chlorobenzenes present in bioresources recycled to land have a very low degree of bioavailability to livestock. PAHs were not detected in milk with direct biosolids addition to the diet, therefore, PAHs were not analysed in the milk of the Biosolids-soil treatment (Table 2). The minimal transfer of PAHs to milk may be explained because PAHs are subjected to metabolic degradation in the animal and therefore do not bioaccumulate (Abdel-Shafy and Mansour, 2016). However, PAH metabolites may be carcinogenic (Moorthy et al., 2015), hence, it remains important to be aware if animals are exposed to sources of PAHs in recycled waste products applied to land.

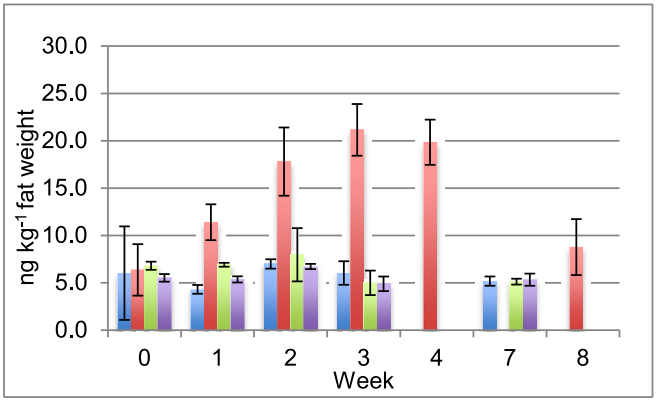

Fig. 3. Upper bound ΣPCN concentrations in milk (fat weight basis) for cattle ingesting diets amended with the following treatments: ■, Control (soil), ■, Biosolids, ■, Biosolids-soil and ■, CLO-soil; Where values were < LoQ the LoQ was used to calculate the mean; Error bars show the standard deviation of the mean.

Milk contained a ΣPCN of approximately 5 ng kg^{-1} fat in the Control and blended bioresource-soil conditions, and up to 20 ng kg^{-1} fat for milk from cattle ingesting the diet amended directly with biosolids at 5 % of the DM intake (Fig. 3). By comparison, the ΣPCN reported in a composite milk sample in the FSA TDS was considerably smaller and equivalent to 0.37 ng kg^{-1} fat weight (Fera: Food and Environment Research Agency, 2012). Thus, background concentrations of certain organic contaminants in milk can potentially vary under typical agricultural husbandry practices by at least an order of magnitude. The PCN concentration in milk of cattle ingesting the Biosolids treatment was significantly increased at week 4 compared to the Control treatment (Fig. 3 and Table S28, SI) with the most important PCN congeners being: 52, 66/67, 71/72, 73 and 75 (Fig. S6). Thus, the ΣPCNs in the milk at week 4 with direct biosolids ingestion was 19.8 ng kg^{-1} fat compared to 4.3–7.0 ng kg^{-1} fat in the Control treatment (Fig. 3). The mean CORs and BCFs for the PCN congeners that were mostly >LoQ for all treatments at week 3 (week 4 for the Biosolids treatment) are presented in Table 8. Results pointed to the reduced bioavailability of PCNs in the Biosolids treatment compared to others; for

Table 8
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for selected PCN congeners in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

		Control		Biosolids		Biosolids-soil		CLO-soil	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
PCN 52	COR	12.9	5.45	4.57	0.59	13.8	8.53	13.8	6.53
	BCF	2.10	0.96	0.96	0.16	2.22	1.27	2.69	1.50
PCN 66/67	COR	31.0	6.02	23.9	5.61	–	–	24.2	4.49
	BCF	4.96	0.66	4.99	1.03	–	–	4.55	0.50
PCN 73	COR	–	–	20.1	4.13	–	–	29.8	9.32
	BCF	–	–	4.24	1.06	–	–	5.55	1.37
Σ lower	COR	–	–	4.82	0.81	–	–	7.79	2.54
	BCF	0.95	0.26	1.01	0.14	1.08	0.45	1.46	0.45
Σ upper	COR	19.6	4.72	5.09[6.11][a]	0.83	13.0	3.76	13.7	2.55
	BCF	3.14	0.65	1.07[1.25][a]	0.13	2.14	0.56	2.59	0.41

PCN congeners were included where values >LoQ were measured in the milk.
Carry Over Rate (COR) (%) = daily contamination in the milk (ng day^{-1})/daily contamination in the feed (ng day^{-1}) x100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (ng kg^{-1} fat)/contaminant concentration in the feed (ng kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation; −, COR or BCF was not determined either because all four of the replicates were < LoQ in the milk or the feed concentration was <LoQ.
[a] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis.

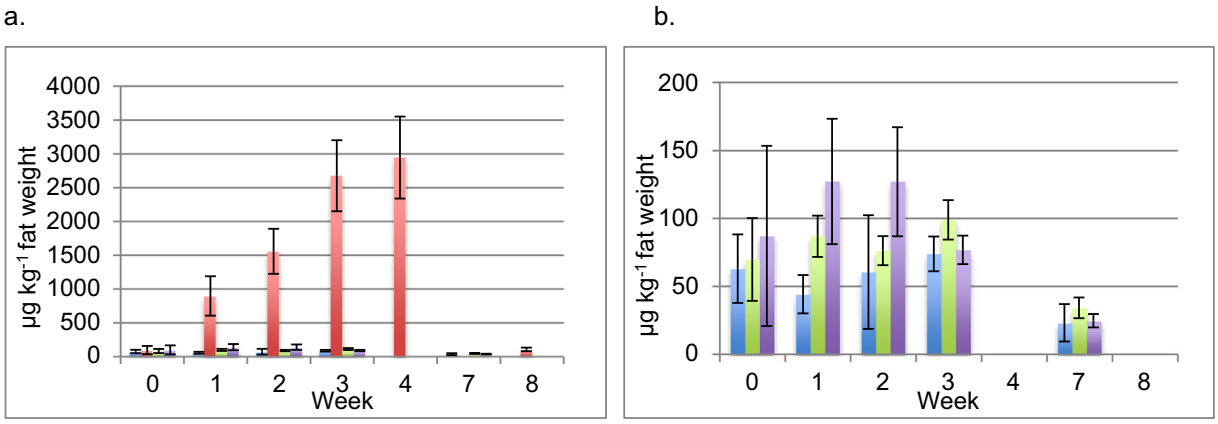

Fig. 4. Polychlorinated alkane concentrations in milk (fat weight basis) for cattle ingesting diets amended with the following treatments: ■, Control (soil), ■, Biosolids, ■, Biosolids-soil and ■, CLO-soil. All treatments are shown in a.; Biosolids-soil, CLO-soil and control treatments are also presented separately in b. due to differences in scale Where values were < LoQ, the LoQ was used to calculate the mean; Error bars show the standard deviation of the mean.

example, the BCF for PCN 52 was 2.1–2.7 for the Control, Biosolids-soil and CLO-soil treatments compared to 0.96 for the Biosolids treatment. Similarly, the COR for PCN 52 was 12.9–13.8 % for the Control, Biosolids-soil and CLO-soil treatments compared to 4.57 % for the Biosolids treatment (Table 8).

The predicted maximum steady state concentration for ΣPCNs in milk for the Biosolids treatment was 23.4 ng kg^{-1} fat (Table 4) compared to a measured concentration of 19.4 ng kg^{-1} fat at week 4 and it was estimated that 95 % of the steady state concentration would occur at day 42. The BCF for the Biosolids treatment calculated from the estimated steady state concentration (1.25) remained significantly below the BCFs for the other treatments (2.14–3.14), as did the COR (6.11, compared to 13.0–19.6, respectively).

3.2.5. Polychlorinated alkanes

Direct addition of biosolids to the diet at a rate of 5 % of the TMR DM significantly ($P \leq 0.05$) increased the concentrations of PCAs in milk (Fig. 4) and the concentration at week 4 was 2946 µg kg^{-1} fat compared to 43.8–73.6 µg kg^{-1} fat for the Control during weeks 0–3. PCAs were also present in greater concentrations in the milk of cattle ingesting the Biosolids-soil and CLO-soil treatments compared to the Control, although, interestingly, the concentrations were smaller in the diets for these treatments in comparison to the Control diet (Table S13, SI). This indicated that the background concentrations of PCAs in the diet may be relatively variable. For example, the concentration of PCAs in the milk of the Biosolids-soil treatment at week 3 was equivalent to 98.8 µg kg^{-1} fat, significantly greater than the Control value of 73.6 µg kg^{-1} fat ($P \leq 0.05$). In the CLO-soil treatment, the concentration at week 3 was similar to the Control at 76.6 µg kg^{-1} fat, but was greater at weeks 1 and 2, equivalent to 127 ng kg^{-1} fat at both time points. A worst-case estimate of short-chain PCAs in cows' milk of 16 µg kg^{-1} fat and 63 µg kg^{-1} fat for medium-chain PCAs (a total of 79 µg kg^{-1} fat) was previously reported by the Committee on Toxicology (COT, 2009), which is in a similar range to the concentrations measured here for the Control treatment. Mean CORs and BCFs for the total of the short- and medium-chain PCAs in each treatment are presented in Table 9 and were in the range 3.83–9.44 and 0.62–1.76, respectively.

The estimated maximum steady state concentration of the total sum of the short- and medium-chain PCAs in the milk of the Biosolids treatment was 8846 µg kg^{-1} fat; hence the concentration measured at week 4 potentially represented only 33 % of the maximum steady state value for cows ingesting the 5 % biosolids DM diet (Table 4). The regression model indicated an exposure equivalent to 200 days would be consistent with the 95 % maximum steady state concentration. The maximum predicted concentration was >100 times larger than the Control and much greater than for any other contaminant group, corresponding with the considerably larger concentrations of PCAs found in biosolids and CLO in comparison to the other types of organic contaminants examined (Section 2.5).

The concentration of PCAs in the milk of the Biosolids treatment represented a worst-case as, in practice, it is very unlikely that cattle would graze on pasture contaminated with up to 5 % DM biosolids for a 4 week period. Ingesting biosolids at 5 % of the DM intake could reflect an upper exposure condition, for example, following the surface application of biosolids to grazed pasture. This practice is allowed for enhanced treated biosolids types, but in this case a no-grazing period (three weeks) applies for residual pathogen attenuation reasons (ADAS: Agricultural Development and Advisory Service, 2001; Defra: Department for Environment, 2018c), which would diminish direct ingestion rates in practice; soil injection techniques also protect pasture quality. The Biosolids-soil treatment, on the other hand, may be a more representative example of biosolids application in practice, and in this case the concentration of PCAs in the milk was only slightly elevated compared to the Control treatment. Nevertheless, these findings highlight the very large concentrations of PCAs present in biosolids and the potential for transfer to the human diet through the livestock ingestion route in animal food products. Despite the very high concentrations, however, PCAs may not be the most significant group of contaminants in terms of toxicity. For example, the no-observed adverse effect level (NOAEL) for short- and medium-chain PCAs is 10 mg kg^{-1} bw day^{-1}, the lowest observed adverse effect level (LOAEL) reported for two low chlorinated long-chain PCAs was 100 mg kg^{-1} bw day^{-1} and the NOAEL of high chlorinated long-chain PCAs was 900 mg kg^{-1} bw day^{-1} (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2020). These compare to NOAEL and LOAEL values of 10–406 pg kg^{-1} bw day^{-1} for 2378-TCDD (EFSA: European Food Standards Agency, 2015). Nevertheless, the findings indicated there should be greater attention to

Table 9
Mean carry over rates (CORs) (%) and bioconcentration factors (BCFs) for PCAs (sum of short- and medium-chain) in Trial 1 based on the concentrations in milk at the end of feeding the experimental diets for 3 weeks (or 4 weeks for the Biosolids treatment).

	Control		Biosolids		Biosolids-soil		CLO-soil	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
COR	3.83	0.46	4.81[15.0][a]	0.26	7.13	1.26	9.44	2.24
BCF	0.62	0.11	1.03[3.08][a]	0.21	1.19	0.18	1.76	0.24

Carry Over Rate (COR) (%) = daily contamination in the milk (µg day^{-1})/daily contamination in the feed (µg day^{-1}) x100.
Bioconcentration Factor (BCF) = contaminant concentration in the milk (µg kg^{-1} fat)/contaminant concentration in the feed (µg kg^{-1} DS).
CLO, compost-like-output; SD, standard deviation.
[a] Value in parentheses is an indicative COR or BCF based on the estimated steady state concentration from the regression analysis.

source control of PCAs to reduce emissions to protect the environment, human health and valuable resource recovery routes. Indeed, EFSA recently published a scientific opinion on the risk for animal and human health from chlorinated paraffins (PCAs) in feed and food (EFSA CONTAM Panel: EFSA Panel on Contaminants in the Food Chain, 2020), highlighting them as an important group of concern, and the need for further information to accurately determine the risk to health from their release into the environment.

3.3. Organic contaminant status of milk from ingestion of recycled ash from the thermal combustion of different bioresource materials

With the exception of PCNs, there was no evidence of additional transfer to milk of the other organic contaminant groups that were detected in the recycled ash materials (PCDD/Fs, PCBs, PBDD/Fs, non-ortho PBBs, PXDD/Fs, PXBs or PAHs) (Tables S29-S37, SI). Consequently, milk samples collected at weeks 1, 2 and 7 were not analysed for these contaminant groups (Section 2.4.2). Neither ortho PBBs nor PCAs were not detected in the recycled ash materials and therefore were not analysed in the milk (Section 2.5).

In contrast, PCNs form de novo through a number of pathways during combustion processes, which may explain their presence in detectable amounts in some of the recycled ashes (Jansson et al., 2008). The results also demonstrated the potential for transfer from ash-amended soil to milk through animal ingestion. Thus, the chemical analysis of milk samples collected in week 0 and week 3 indicated that PCNs were elevated in the MBMA-soil and PLA-soil treatments in comparison to the Control at week 3. Therefore, PCNs were the only group of compounds measured in week 1, 2 and 7 samples in Trial 2 (Table S37, SI); PCNs were not detected in the PSA sample and were therefore not measured in the milk for this treatment. The upper bound ΣPCNs in milk during weeks 0–7 were: 4.5–7.1, 4.9–6.3 and 4.5–6.5 ng kg^{-1} fat in the MBMA-soil, PLA-soil and Control treatments, respectively. Statistical analysis indicated there was no significant difference ($P > 0.05$) between the week 0 and week 3 concentrations for each treatment or between the Control and the different treatments at week 3, for any of the congeners. However, the overall ΣPCNs was significantly ($P \leq 0.05$) increased for the MBMA-soil treatment at 7.06 ng kg^{-1} fat compared to the Control (4.48 ng kg^{-1} fat); though slightly larger than the Control, the PLA-soil treatment (5.18 ng kg^{-1} fat) was not significantly different to either of the other treatments ($P > 0.05$) (Table S37, SI). This behaviour was consistent with the upper bound ΣPCN concentration measured in MBMA, which was equivalent to 45.4 ng kg^{-1} DS and greater than the upper bound sum for PLA of 8.8 ng kg^{-1} DS. However, this pattern was not reflected in the concentrations measured in the blended feeds, which were 1.93 and 1.17 ng kg^{-1} DM for the PLA-soil and MBMA-soil treatments, respectively, similar to the Control value of 1.33 ng kg^{-1} DM (Table S12, SI). The PCN status of the milk therefore apparently followed the pattern in PCN composition of the ash materials although the diets contained relatively similar amounts of ΣPCNs. The significant reduction ($P \leq 0.05$) in milk production and fat output observed for the PLA-soil treatment, but not for the MBMA-soil treatment, compared to the Control (Table S16), was unlikely to be related to the PCN supply or concentration in the milk.

3.4. Concentrations of organic contaminants in milk following withdrawal of bioresource-amended feed

The cows were returned to a standard diet and monitored during the four-week withdrawal period when the concentrations of all the elevated contaminants declined approximately to control or pre-feeding values. For example, PCDD/F concentrations at week 4 in the Biosolids treatment were significantly greater than the Control with an overall mean TEQ equivalent to 0.82 ng kg^{-1} fat compared to 0.26 ng kg^{-1} fat in the Control (averaged over the trial period), by week 8, however, the concentration in the milk of the Biosolids treatment had significantly fallen to 0.32 ng kg^{-1}, and was similar to the Control (Fig. 1a). The PCA concentration was also highly elevated for the Biosolids treatment at week 4, equivalent to 2946 μg kg^{-1} fat, compared to a trial average of 52.6 μg kg^{-1} fat for the Control. Nevertheless, despite being present in significantly larger amounts than any of the other contaminant groups, PCAs in the milk of the Biosolids treatment rapidly declined by two orders of magnitude during the withdrawal period to 93.2 μg kg^{-1} fat at week 8 to a level broadly similar to the Control (Fig. 4).

4. Conclusions

Several important groups of organic contaminants were investigated for the potential to transfer to milk of cattle from the agricultural use of different bioresource materials on land, including biosolids from municipal wastewater treatment, CLO from the mechanically segregated organic fraction of MSW, and three ash products from biomass combustion. The transfer of organic contaminants to milk was most consistently observed for cattle ingesting biosolids at 5 % DM in the diet, simulating the intake of biosolids adhering to foliage or from the soil surface. This represented potentially a 'worst-case' as biosolids management practices on grassland normally avoid direct ingestion by grazing livestock. In practice, biosolids are typically incorporated into soil prior to pasture establishment, rather than being surface-applied to an established crop. Experimental diets presenting the different bioresource materials to cattle by blending at agronomic application rates with soil were therefore more representative of the potential intake by grazing cattle. Agronomic application was simulated using a shallow incorporation depth (10 cm) to a potentially vulnerable soil type and addition of the blended bioresource and soil to the diet at 5% DM. Nevertheless, no clear evidence of an increase in the baseline level of transfer of contaminants to milk was found. PCAs were the exception and transferred to the milk of dairy cattle ingesting both the Biosolids-soil and CLO-soil treatments, a behaviour explained by the concentrations of PCAs present in these materials, which were much larger than any of the other contaminant groups examined.

PCDD/Fs in milk fat were significantly increased for cattle ingesting biosolids directly introduced into the diet at 5 % DM of the TMR due to the greater concentrations of PCDD/Fs present compared to soil incorporated treatments, and the control, nevertheless, the WHO_{2005}-TEQ for PCDD/Fs was <30 % of the EU maximum food limit in milk and was less than the 95 percentile background range of concentrations reported for milk in Europe. PBDD/Fs were present in considerably larger amounts in biosolids and CLO compared to PCDD/Fs, but the CORs, BCFs and transfer to milk of cattle fed biosolids at 5 % DM intake were much smaller, suggesting reduced bioavailability of PBDD/Fs relative to their chlorinated counterparts. Nevertheless, the contribution of PBDD/Fs to the overall DL toxicity should be considered in risk assessments of the agricultural use of bioresources.

Carry Over Rates and BCFs were used to determine the magnitude of the transfer and bioavailability of organic contaminants to milk from different bioresources incorporated into the diet of lactating cows. The results indicated that the bioavailability in biosolids may be reduced, possibly due to strong physicochemical binding with the organic matter matrix, compared to the soil only control treatments and the diets amended with bioresources blended with soil at agronomic rates. However, this behaviour may also be explained because the concentrations were larger in the biosolids only diet and linked to this, organic contaminants in the Biosolids treatment had not reached a steady state in the milk. In some cases, the contaminant concentrations in the control and soil-blended treatments were also very small or <LoQ and CORs or BCFs could not be calculated. Indeed, CORs and BCFs may be influenced by the dose of an organic contaminant, regardless of the matrix. Hence, to fully assess the relative bioavailabilities of organic contaminants in different bioresources and the effect of soil incorporation would require experiments with equivalent total concentration and congener profiles for each type of organic contaminant and bioresource material. As this would be very difficult in practice, the results presented here provide a best overall estimate of the relative bioavailabilites of organic

contaminants to milk from biosolids and different soil incorporated bioresources.

The effects of a single agronomic application of different types of bioresources on transfers of organic contaminants to milk by grazing dairy cattle were investigated. In practice, however, bioresources may be applied repeatedly to the same area of land leading to potential accumulation of persistent organic contaminants in the soil in the long-term. Thus, further research is necessary to investigate the accumulation and transfer of contaminants to milk under representative, long-term agricultural application. However, contaminants that transferred to milk from the biosolids-only diet returned, or were very close, to background control values within 4 weeks of removing biosolids from the feed. Thus, for short periods of exposure from worst-case conditions of direct in ingestion of land applied bioresources by grazing livestock, the results showed there is limited risk of long-term accumulation, or transfer to the human diet, in milk.

Data availability

Data is provided in the supplementary information

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Alan Dowding, co-author, is also an employee of the Food Standards Agency who funded the research. He was involved in the selection of contaminants for analysis, conceiving the scope of the research programme, and in reviewing and editing the paper.

Acknowledgments

The Authors gratefully acknowledge the Food Standards Agency for funding the research. The opinions and conclusions expressed in this article are solely the views of the authors and do not necessarily reflect those of the Food Standards Agency. We would like to thank Sophia Acker and Radu Rautiu, Imperial College London Consultants for project management and gratefully acknowledge staff at the Centre for Dairy Research (CEDAR) for their technical contributions and care of the experimental animals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.163546.

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