Supply of data requirement to assess the safety of currently non-permitted waste streams to be used for rearing insects for feed

Project FS900220

Report by Fera Science Ltd.




Executive Summary

There has been increasing attention on the role that insects can have in the production of protein for inclusion in animal feed, whilst also reducing the volume of organic waste streams. Currently in the UK and the EU the material that can be used as a rearing substrate for insects for production of protein for feed and food is regulated and waste streams that contain or may potentially contain animal by-products (ABPs) are not permitted to be used to rear the insects. The aim of this study was to provide chemical and microbiological data from model insect rearing systems using four currently non-permitted rearing substrates as a basis to assess the potential risk from use of these materials.

Selection of the materials to be tested was based on the results of a questionnaire to key stakeholders with interest in insect bioconversion and discussion with the FSA. The materials selected for testing were:

- Supermarket surplus containing animal by-products (ABPs) (Supermarket)
- Food processing surplus containing ABPs (Manufacturing)
- Kitchen waste from hospitality sector containing ABPs (Catering)
- Broiler poultry manure (Poultry manure)

These materials were used to rear black soldier fly (BSF) larvae and samples of the rearing substrate, the larvae and the frass were taken for analysis of chemical and microbiological contaminants. Samples obtained from a UK insect producer using currently permitted rearing substrates were also included.

Analytical methods screened for 745 chemical analytes (metals, veterinary medicines, pesticides, mycotoxins, polycyclic aromatic hydrocarbons (PAHs) (kitchen waste only), nitrate/nitrite, perfluoroalkyl and polyfluoroalkyl substances (PFAS)), the presence of key microbial organisms was assessed, and non-targeted screens were used to assess the presence of natural toxins and viral RNA that were present in the samples.

Of the chemical analytes screened for, a total of 101 were found in the larvae. The majority of these were metals (58). Of the analytes found, there were no exceedances for any of the chemicals where maximum limits are specified in feed materials of animal origin. However, some pesticide residues in larvae reared on supermarket surplus and poultry manure exceeded the MRLs for terrestrial invertebrates which may have implications if the larvae were to be used as food.

The only regulatory limit exceeded for feed ingredients of animal origin was for the presence of Enterobacteriaceae in larvae reared on all four of the currently non-permitted substrates. The regulatory limit for Enterobacteriaceae in feed materials of animal origin is 300 cfu/g. However, in this study for the currently non-permitted substrates only minimal processing was used to kill the larvae. The regulatory levels specified refer to samples taken after the application of a processing method. The larvae reared on the baseline samples complied with the regulatory limits for Enterobacteriaceae for feed ingredients of animal origin. These larvae were culled by blanching in boiling water (approx. 100°C) and cooking until core temperature exceeded 75°C according to the producer’s protocols.

The results from this study illustrate that further processing of larvae is required to reduce the microbial load. It is likely that processing methods typically used by industry for the production of insect protein would significantly reduce the level of these organisms, as demonstrated by the results from the baseline samples, which were supplied by a UK insect producer using their standard protocols. However, this would need to be confirmed and supported by HACCP and GMP procedures.

There was evidence of bioaccumulation in the larvae for some compounds. Cadmium was shown to bioaccumulate in BSF larvae as previously reported. There was also evidence of bioaccumulation of other metals including magnesium, calcium, and phosphorus in larvae reared on both currently permitted and currently non-permitted substrates. Although this may not result in a direct safety concern, this may have implications for some metals that serve as macro- or micronutrients in animal feeds.

There was also evidence of bioaccumulation of didecyldimethylammonium chloride (DDAC) and haloxyfop in larvae reared on poultry manure. Other contaminants such as mycotoxins and PAHs also gave indication for potential bioaccumulation but variation between samples means that further testing would be needed to ascertain whether this is the case.

As far as the authors are aware this is the first study that has examined the presence of PFAS through the insect bioconversion process using naturally occurring levels of these compounds. Some PFAS were found in the larvae and the frass, but a more extensive study is recommended to confirm these findings.

Commission Regulation (EU) No 142/2011 specifies that processed manure taken during or immediately after processing should comply with limits Salmonella spp. and *Escherichia coli* or Enterococcaceae. The maximum value of 1000 cfu/g was exceeded for Enterococcaceae in frass samples from all rearing substrates but was only exceeded for *E. coli* in frass from catering waste. These results indicate that further processing of the frass from currently permitted and non-permitted substrates is required to conform to regulatory requirements.

The non-targeted viral screen confirmed the presence of RNA from plant and animal pathogens in larvae and frass, but the infectivity of these viruses could not be elucidated from this study. Infection studies should be carried out to determine if this is a risk, particularly for plant pathogens when frass is used as a fertiliser.

The non-targeted toxin screen demonstrated the presence of natural toxins such as solanidine. Currently there are no regulatory limits for the presence in animal feed and an EFSA risk assessment concluded that a risk characterisation of potato glycoalkaloids in feed for farm and companion animals was not possible due to insufficient data on potential adverse effects in these species (EFSA, 2020). Data on the presence of these compounds in a range of animal feeds is therefore required before an assessment of the levels found in insect larvae can be evaluated.

It was noted that several compounds were not detected in the rearing substrate but were detected in the larvae and/or the frass. It is considered that this may be due to the greater homogeneity of the larvae and the frass samples compared with the rearing substrate such that there may have been a more even distribution of any contaminant present.

Examination of the variation in results between replicates showed that between replicate variation occurred mainly for the rearing substrate. This is likely to be due to the rearing substrate being less homogenous than the larvae and the frass. It is therefore recommended that when assessing contaminants in rearing substrate a greater number of samples are assessed.

Finally, it is important to note that only a single source from each category was evaluated in this study and the samples obtained were taken over a short time period. More extensive testing across a wider range of suppliers and to reflect potential changes in seasonality is recommended.

Contents

Introduction

There has been increasing attention on the role that insects can have in the production of protein for inclusion in animal feed, whilst also reducing the volume of organic waste streams. The focus for insect-derived protein for inclusion in animal feed has predominantly been on use of the black soldier fly (BSF; *Hermetia illucens*) and the yellow mealworm (*Tenebrio molitor*). Studies in the field of insect bioconversion have expanded over the past two decades leading to the funding and build of commercial scale operations in North and South America, Europe, Southeast Asia and Australia.

In many countries materials to be used in animal feedstuffs are subject to regulations to ensure safety for the animals and human consumers. In the EU and the UK there are regulations that define the species of insect that can be used to produce insect protein for feed and food, the waste streams that the insects can be fed, and the animals that the resultant insect protein can be fed to.

Insects reared for inclusion in animal feed are defined in the EU Animal By-Products' legislation (Article 3(6) of Regulation (EC) No 1069/2009) as farmed animals. Only feed ingredients that can be used for feeding of other farmed animals can be fed to insects. This restricts the rearing substrates for the insects to vegetal based materials. Animal derived products, with a few exceptions, are not currently permitted. Exceptions to the use of animal by-products (ABPs) as insect rearing substrates include dairy products (e.g., milk (if pasteurised), cheese and eggs (if cooked), fishmeal, gelatine, collagen, hydrolysed proteins and blood products (non-ruminant).

Although vegetal based substrates such as brewery by-products and fresh fruit and vegetables are a good rearing substrate for certain insect species, species such as the BSF are able to develop on a wide range of organic substrates including those containing meat and fish and animal manures. The substrate on which the larvae are reared affects many aspects of the insect bioconversion process in addition to the nutritional composition of the larvae. For example, the resultant frass will have different properties depending on the waste source (Klammsteiner *et al*., 2020). It has also been shown that the life cycle assessment for the production of insect protein is dependent on the rearing substrate and it has been concluded that the use of food processing by-products, wastes or manures may be needed to reduce the environmental impacts of the insect bioconversion process (Smetana *et al*., 2021).

Potential contaminants (chemical and microbiological) and the associated risk will also be related to the material used as a rearing substrate and could affect the safety for use as an animal feed ingredient of the resultant larvae and the use of the frass.

Maximum limits for certain chemicals in animal feed are provided by the EU Directive on Undesirable Substances and Products (Directive 2002/32/EC) and the EU Regulation on Pesticides Maximum Residue Levels (MRLs) (Regulation (EC) No 396/2005). There are also limits specified in (Regulation (EC) 142/2011) for the presence of Salmonella and Enterobacteriaceae in feed materials of animal origin.

The safety of insect derived products with regard to chemical contaminants has been recently reviewed by Meyer *et al*. (2021). The review concluded that the heavy metals cadmium, mercury, lead and arsenic can bioaccumulate in insects, whilst mycotoxins and PAHs do not. A lack of data for PAHs, plant toxins, dioxins and PCBs was also highlighted. Vandeweyer *et al*. (2021) reviewed safety with regard to biological contaminants and it was concluded that data on prions, foodborne viruses and foodborne parasites is still needed and their fate during insect processing requires investigation. The presence of microorganisms and the need to assess these in production areas in addition to present in the rearing substrate was also highlighted (Vandeweyer *et al*., 2021). More recent studies have also examined physical contaminants such as microplastics (Lievens *et al*., 2023).

There are several studies that have shown that some insect species will bioaccumulate certain heavy metals. The degree of bioaccumulation depends on the insect species and the metal. For example, BSF will bioaccumulate cadmium (Charlton *et al*., 2015; Purschke *et al*., 2017; Gao *et al*., 2017), whilst yellow mealworm larvae have been reported to bioaccumulate arsenic (van der Fels-Klerx *et al*., 2016).

The uptake of mycotoxins has also been studied although studies have tended to focus on specific mycotoxins, mainly aflatoxin B1, zearalenone, deoxynivalenol, ochratoxin A and fumonisin B1 and B2 (Meyer *et al*., 2021 and references therein) and research looking at a wider range of potential mycotoxins is limited. Generally, spiked materials have been used in these studies and it has been concluded that BSF larvae do not bioaccumulate mycotoxins from the rearing substrate (Purschke *et al.*, 2017; Bosch *et al.*, 2017; Camenzuli *et al.*, 2018). The levels of mycotoxins found in larvae in these studies were very low or below the limit of quantification. Metabolism of certain mycotoxins has been demonstrated in *T. molitor* larvae (Camenzuli *et al*., 2018; Purschke *et al*., 2017). However, in some studies examining the mass balance, an inability to account for all the material, either as the native mycotoxin or known metabolites, led to the conclusion that further research on the metabolism of mycotoxins by different insect species is required (Camenzuli *et al*., 2018).

There is limited research on uptake of pesticides, veterinary medicines, PAHs (Meyer et al., 2021) and of other contaminants such as microplastics, but data is lacking on the risk from different rearing substrate types. For example, some contaminants such as veterinary medicine residues may be more likely to be found in materials containing animal derived products than in those of plant origin.

Ffoulkes *et al*. (2021) examined 22 potential substrates that could be used for rearing insects such as BSF and concluded that at least ten by-product streams could be used by the UK insect industry. Some of these such as brewers' grains and vegetable by-products are currently permitted for use and are already used by insect producers. Other waste streams identified are not currently permitted as they contain or may potentially contain ABPs. The waste streams identified were further divided by the authors into two categories; achievable and aspirational based on consideration of legal, social, and practical challenges (Ffoulkes *et al*., 2021).

The aim of this study was to provide chemical and microbiological data from model insect rearing systems using four currently non-permitted rearing substrates.

Analytical data from the study provides a key data set to be used in subsequent risk assessments.

Key objectives of the study were:

- To obtain representative material from the chosen streams and to rear the chosen insect species on the selected streams.
- To compare with baseline samples provided by a commercial insect supplier that have been reared on a permitted substrate.
- To conduct comprehensive chemical and microbiological analysis of the rearing substrate, larvae, and the frass.
- To complete a literature review on the potential allergen presence and subsequent risk that the allergens could be carried through the process from the substrate to the larvae and subsequently to the farmed animal.

1. Selection of substrates for testing

The current project allowed for the assessment of four rearing substrates. To aid in the selection of the four substrates an online questionnaire was used to seek the opinions of key stakeholders, including insect producers, insect protein users and academics, with interests in insect bioconversion. Opinions were sought for ten categories, which were based on the achievable and aspirational categories identified by Ffoulkes *et al*. (2021). The ten categories were:

- Mixed food surplus from retail containing ABPs
- Mixed food manufacturing surplus containing ABPs
- Mixed food surplus from hospitality and food service containing ABPs
- Domestic food surplus containing ABPs
- Poultry manure – Layers
- Poultry manure – Broilers
- Pig manure
- Cattle manure
- Anaerobic digestate – Food-based
- Anaerobic digestate – Manure-based

For each of these categories the following questions were posed with the selections for responses, if provided, also shown:

- If approved, would your business consider using this substrate to rear insects or including insects reared on it into your supply chain?

Options for response: Yes, No, Maybe

- Do you have any concerns for the use of this substrate from the point of view of:
    - Market acceptance
    - Substrate availability
    - Safety

Options for response: Yes, No

- If you answered yes, please give your reasons.

- Are there any specific examples of this substrate type that you would like to see tested in this category?

Additional questions not confined to the potential substrate categories were:

- Are there any substrate types, which haven't been mentioned, that you would ideally like to be examined?

- Do you have any further thoughts or considerations on this project that you would like to share with us?

The questionnaire was sent to eleven stakeholders, either individuals or representatives of larger interested bodies, representing insect production companies, industry lobbying groups, the broader farming community, retailers and academics.

Following compilation of the responses the data was reviewed by project stakeholders and the decision on the four substrate categories to be included in the study was made.

Results and observations

All 11 consultees responded with completed questionnaires. Whilst it is noted that 11 responses are a low number, all responses were from individuals, companies or member groups with knowledge of insect bioconversion and the current opportunities and challenges in the UK.

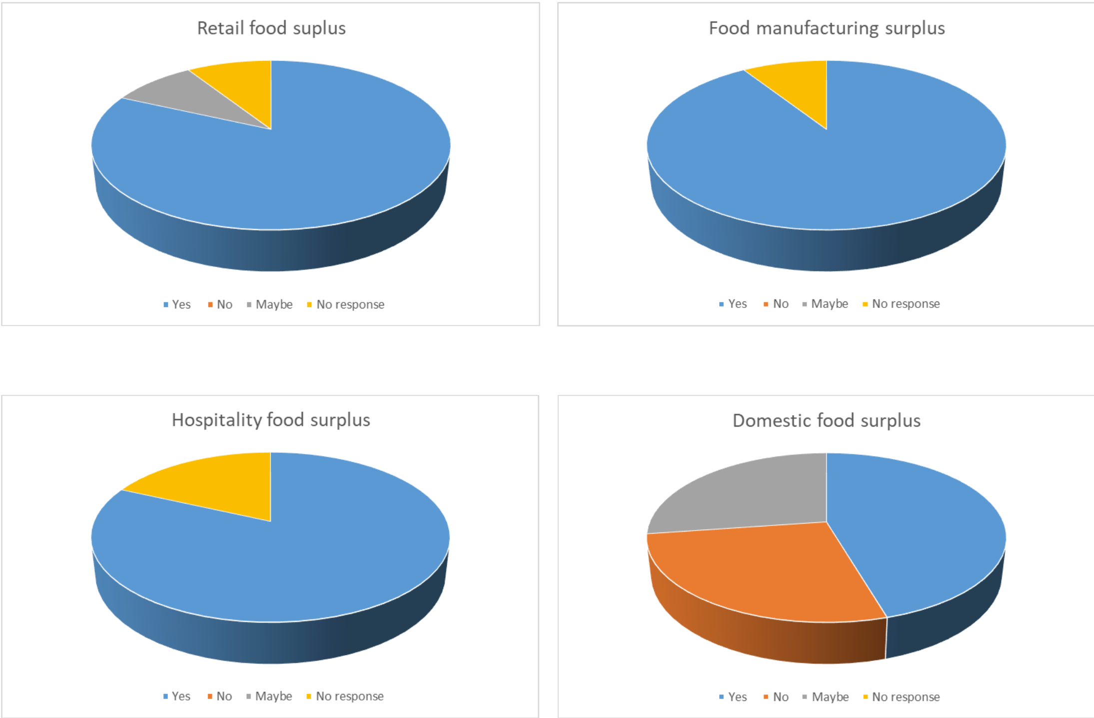
The main observations from the responses are summarised below by the source category.

Food based categories

Respondents indicated that they would consider the use of the food surplus categories as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain. The exception to this was domestic food surplus where 55% of respondents indicated that they would not use this or were undecided (Figure 1A).

For all four categories substrate availability was generally not considered to be of concern. Differences in the responses to concerns from the point of view of market acceptance and safety were observed with respondents indicating concerns for using domestic food surplus, particularly for safety (73% of respondents; Figure 1B).

A.



B.

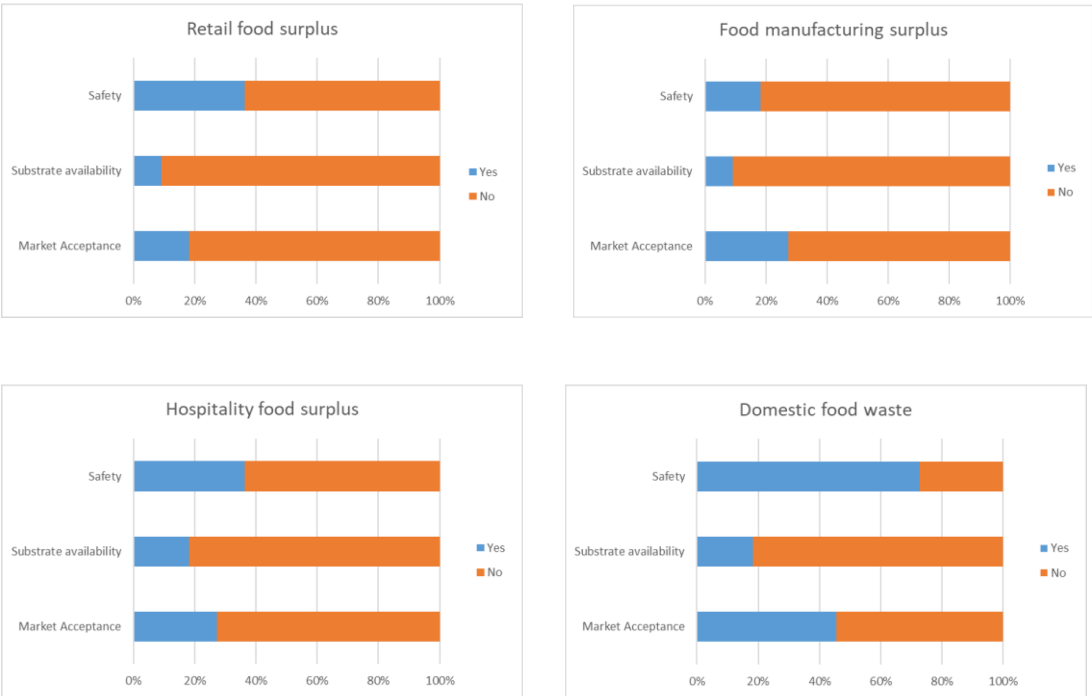


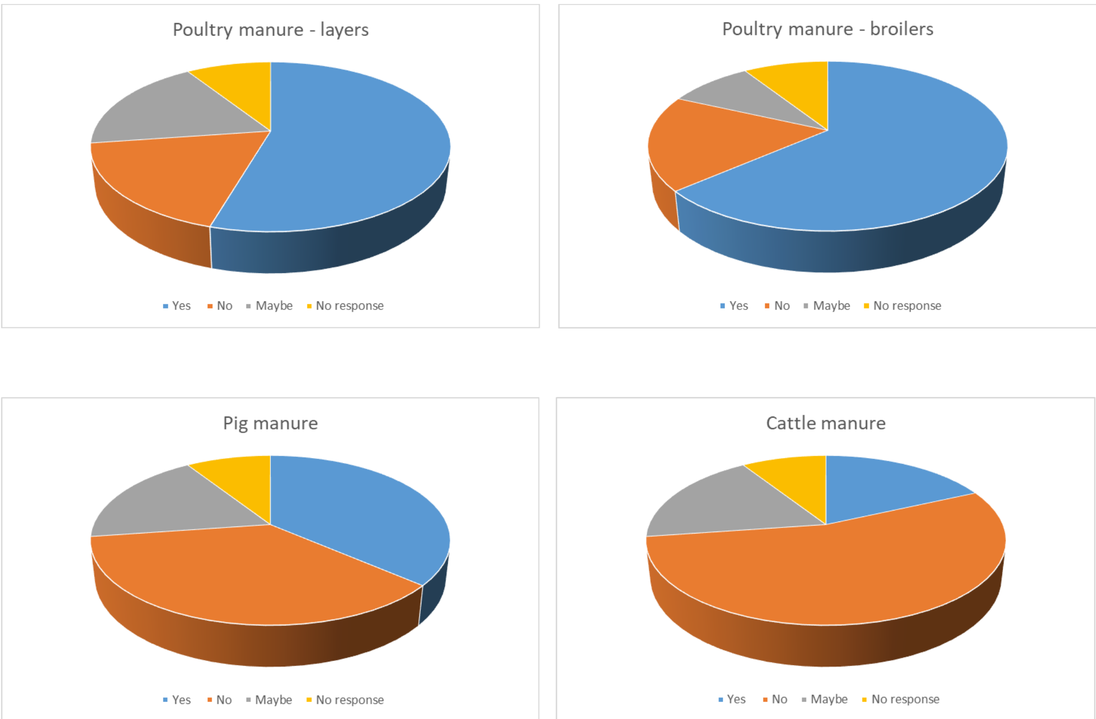
Figure 1. Summary of responses to questionnaire looking at food-based categories as rearing substrates for insects. A. % of respondents who would consider using this category as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain. B. % of respondents with concerns relating to safety, substrate availability or market acceptance.

Manure-based categories

There was a noticeable difference in the number of respondents indicating that they would consider the use of the manure-based categories as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain depending on the source of the manure. A greater number indicated that they would consider use of poultry manure from either broilers or layers (64% and 55% respectively) than for using pig manure (36%) or cattle manure (18%) (Figure 2A).

For all four manure types, availability was not considered a concern, but safety and market acceptance were of concern (Figure 2B).

A.



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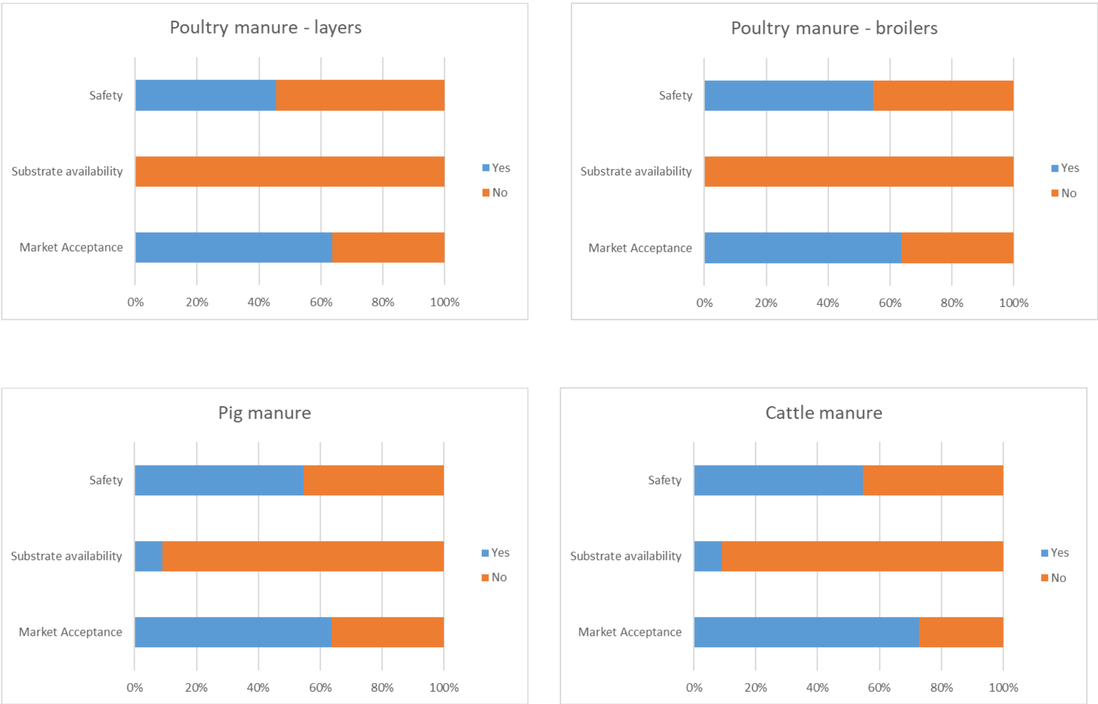


Figure 2. Summary of responses to questionnaire looking at manure-based categories as rearing substrates for insects. A. % of respondents who would consider using this category as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain. B. % of respondents with concerns relating to safety, substrate availability or market acceptance.

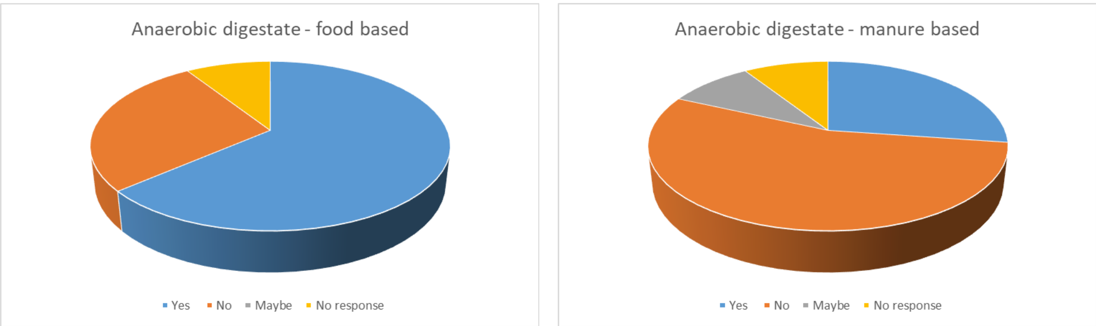
Comments indicated that the risk from microbial contaminants such as *Salmonella* was a concern for this category (Appendix A). However, there was interest in testing this category as it was considered that this could be a solution for more problematic waste streams, but that uses for the larvae other than in the food and feed chain may need to be considered (Appendix I).

Anaerobic digestate-based categories

There was a noticeable difference in the number of respondents indicating that they would consider the use of the anaerobic digestate-based categories as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain depending on the source of the anaerobic digestate. This difference reflected the differences previously seen with the food-based and manure-based categories with 64% of respondents indicating that they would consider use of food based anaerobic digestate and only 27% indicating that they would consider use of manure based anaerobic digestate (Figure 3A).

Availability of both sources of anaerobic digestate was not considered a concern but there were concerns on safety and market acceptance and a greater number of respondents had concerns for the manure-based anaerobic digestate (Figure 3B).

A.



B.

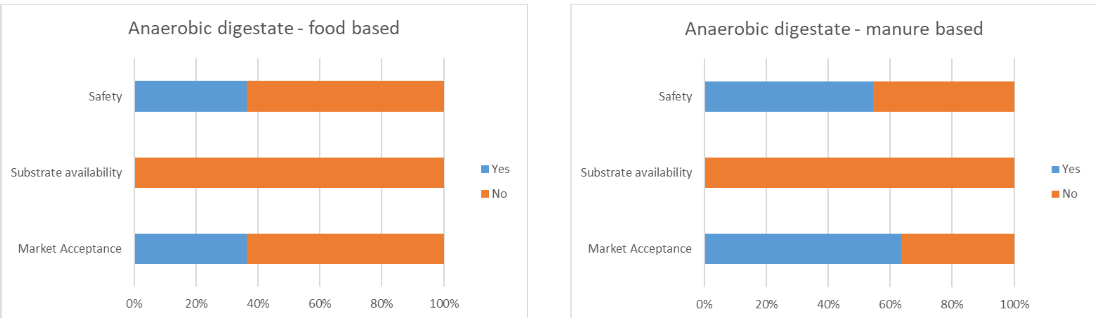


Figure 3. Summary of responses to questionnaire looking at anaerobic digestate-based categories as rearing substrates for insects. A. % of respondents who would consider using this category as a substrate to rear insects or inclusion of insects reared on these substrates in their supply chain. B. % of respondents with concerns relating to safety, substrate availability or market acceptance.

Additional comments from respondents are shown in Appendix A.

Based on the responses and following review and discussion with FSA risk assessment and policy staff, four categories were selected for testing in this project. These were:

- Supermarket surplus containing ABPs (Supermarket)
- Food processing surplus containing ABPs (Manufacturing)
- Kitchen waste from hospitality sector containing ABPs (Catering)
- Broiler poultry manure (Poultry manure)

2. Insect rearing trials

2.1 Sample supply and preparation

Currently permitted rearing substrates

Baseline samples of rearing substrate, larvae and frass were provided by Better Origin. Two types of rearing substrate designated as Core Diet and Core Diet +20% were used. The rearing substrates were comprised of:

- Core Diet - fruit & vegetables (apples, potatoes, tomatoes) and bakery waste (bread loaves)
- Core Diet +20% – As for Core Diet with the inclusion of 20% grain and grain by-products (wheat bran, spent brewers grains)

Feedstocks were shredded using a mechanical shredder (Voran), to <5 mm particle size, and blended. The feedstocks were inoculated with silage additive (Provita Advance Plus applied in accordance with the manufacturer's recommendation), to ensure lactofermentation. pH was monitored throughout use of the feedstock.

Sampling of currently non-permitted rearing substrates

Sampling was undertaken to ensure that the samples of rearing substrate were as *representative* as possible. Representative means that the average proportion of components and concentration of contaminants in the sample is close to the true equivalent values in the material that sample was taken from, that is, the material that the sample represents.

There is a broader kind of representativeness: the extent to which the specific materials we sampled were themselves representative of the broader population of that kind of material: for example, the extent to which the contaminants in the supermarket waste that we took samples were representative of supermarket waste from other supermarkets or at other times of year. While we took efforts to ensure that the samples we took were representative of the materials from which they were taken, the extent to which those materials were representative of the broader population was outside of the scope of this project and remains an unknown.

For the collection of all trial rearing substrates, visits were made to the suppliers to find out how and where the materials were produced and stored. Discussions were held with staff about the best way to collect the material to ensure it was as representative as possible. With the exception of the broiler poultry manure, the material for testing was collected on two separate occasions, to allow for variation over time.

The rearing substrates were homogenised prior to use as described below. Equal quantities of the rearing substrate at each feeding point were combined and samples taken to provide two bulk samples (A and B). One of these bulk samples was analysed once (A) and the second bulk sample was analysed twice (B1 and B2; Figure 4). Differences between the concentration of contaminants in A and B samples were assessed to give an indication of the representativeness of the sampling.

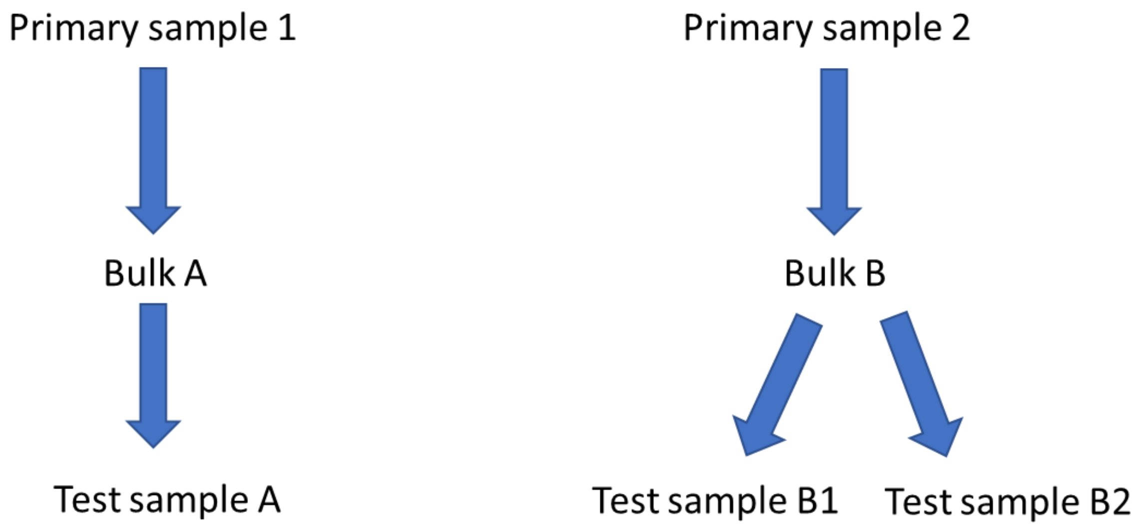


Figure 4. Illustration of sampling process for rearing substrate, larvae and frass.

Currently non-permitted rearing substrates

Manufacturing Surplus

Two 30 kg batches of manufacturing surplus containing animal-by-products were delivered to Fera from a local food manufacturer on trial day -2 and 5. Both batches consisted of 10 kg raw puff pastry, 10 kg cooked pastry products (primarily pork sausage rolls), and 10 kg of raw beef trimmings (primarily sinew and cartilaginous tissue) (Figure 5). This was distributed into two plastic containers, sealed, and left at ambient temperature (approximately 20°C) for 48 h to simulate a collection and distribution period. The material was then homogenised into a paste and transferred to a cold store (1-4°C) for use during the trial.

Figure 5. Example of manufacturing surplus used in insect rearing trial.

Supermarket surplus

Two 30 kg batches of supermarket surplus containing animal-by-products were transferred to Fera from a local supermarket on trial day -2 and 5. This consisted of assorted items that were past the use by or best before date and were set aside for collection by the supplier. The waste consisted of approx. 53% bread, 8% deli meats (e.g., ham and charcuterie), 8% coleslaw, 7% raw beef, 6% raw and cooked chicken products, 5% cheese, 3% olives and deli counter veg (e.g., pickles and sundried tomatoes), 3% raw pork, 3% ready meals (e.g., spaghetti bolognaise). 2% desserts (e.g., yoghurt and tiramisu) (w/v). This was distributed evenly into two containers, sealed, and left at ambient temperature (approximately 20°C) for 48 h to simulate a collection and distribution period. The material was then homogenised and transferred to a cold store (1-4°C) for use during the trial.

Figure 6. Example of supermarket surplus used in insect rearing trial.

Catering Waste

Two 30 kg batches of pre-consumer kitchen waste containing animal-by-products collected over approximately 24 h were transferred to Fera from a local restaurant on trial day -2 and 5. The waste consisted of assorted items classed as past the use by date or defined as unsuitable for retail within the restaurant. This waste consisted of approximately 50% cooked chicken, 10% coleslaw, 10% partially cooked chipped potatoes, 10% white bread rolls, and 20% (w/v) of other unidentified food items or ingredients typically found on the restaurant's menu such as sweetcorn, salad leaves and cheese (primarily halloumi) (Figure 7). This was distributed into two plastic containers, sealed, and left at ambient temperature (approximately 20°C) for 48 h to simulate a collection and distribution period. The food was then homogenised and transferred to a cold store (1-4°C) for use during the trial.

Figure 7. Example of catering waste used in insect rearing trial.

Poultry Manure

Broiler poultry manure (40 kg) from a shed at the end of a production cycle was collected from a local farm. Manure was selected from random locations in the poultry shed by Fera staff and transferred to site on trial day -5. The waste consisted of chicken manure, un-eaten feed, wood shavings, and sawdust. The manure required hydration to make it suitable for development of the larvae and this was achieved by adding 100 ml of water for every 70 g of substrate. The hydrated manure was stored in a cold store (1-4°C) and left to hydrate for 48 hours before the trial commenced.

2.2 Production of BSF larvae and frass

Production of BSF larvae and frass on currently permitted rearing substrates.

BSF larvae were reared by Better Origin from hatch to 5 days old (mass of a single larva approx.10 mg) on a seedstock comprising of an enriched Gainesville diet. Larvae were then transferred to 600 x 400 mm rearing trays containing Core Diet or Core Diet + 20%. Larvae were fed daily following a predetermined feed curve. Larvae were maintained at 28°C, 55-60% relative humidity (r.h.).

After 12 days of rearing, larvae were harvested through mechanical sieving. The larvae were washed with water to remove residual rearing substrate on the surface and were then culled by blanching in boiling water (approx. 100°C) and cooking until core temperature exceeded 75°C. The exact time will depend on the quantity of larvae and the size of the vessel The larvae were then drained, cooled at room temperature, bagged and frozen.

Data from the rearing of the larvae on the substrates provided was not available, but typically the biomass conversion ratio for the core diet is 6.6 and for the core diet +20% is 4.5 with average yield per tray of 1.1 kg and 1.7 kg for the core diet and core diet + 20% respectively.

Samples of the two rearing substrates and the larvae reared on these together with the resulting frass were sent to Fera Science Ltd. Samples were supplied frozen and were stored in a freezer at approximately -18°C prior to analysis.

Production of BSF larvae and frass on currently non-permitted wastes

Neonate larvae approximately 0-24 h old were used in all studies. Each trial was carried out using a nursery container for days 0 to 7, then transferring the larvae to a rearing tray system from day 7 to trial termination.

For each replicate the rearing substrate (500 g) was weighed into a 2.7 litre rearing container (nursery container) and neonate larvae (250 mg) were added. Lids with five 8 mm diameter holes were put on the containers and the containers were placed into a rearing room at 27°C, 70% rh. After 7 days the contents of the nursery container were transferred to a 600 mm by 400 mm rearing tray. Larvae were monitored and re-fed at set periods. Prior to each feed 50 larvae were removed, washed, and dried, and weighed to monitor growth. There were six replicate trays for each rearing substrate tested.

Trials were terminated when the first pre-pupae were identified, with larvae then separated from the frass. Total larvae mass and mass of remaining frass/feed substrate were determined.

Dry matter readings of the rearing substrate were determined before trial set up. Some substrates had moisture content adjusted to provide approximately 30% dry matter content or to improve the consistency of the substrate for the larvae to consume, whichever was deemed most appropriate for success of the trial. Due to the variance in nutritional quality between each substrate, adaptations were made to each trial and are highlighted below.

Trial 1 – Manufacturing surplus

Dry matter content was determined and was adjusted to approximately 45% dry matter prior to feeding. The first batch of manufacturing surplus was used for feed on trial day 0; the second batch was used for feeding on days 7 and 12. The trial ran for 14 days.

Trial 2 – Supermarket surplus

Dry matter content was adjusted to approximately 30% prior to feeding. The first batch of supermarket surplus was used for feed on trial day 0; the second batch was used for feeding on days 9 and 13. The trial ran for 15 days.

Trial 3 – Catering Waste

Dry matter content was adjusted to approximately 32% dry matter prior to feeding. The first batch of catering waste was used for feed on trial day 0; the second batch was used for feeding on days 7 and 10. The trial ran for 14 days.

Trial 4 – Poultry Manure

Dry matter content was adjusted to approximately 22% dry matter prior to feeding. The BSFL were fed on days 0, 7, and 10. Adaptations to the protocol were made to enable feeding and data collection to be performed in a Class II microbiological safety cabinet. Adaptations included starting the trial in twelve nursery containers with 125 mg of neonate larvae, then combining two nursery containers randomly on day 7 when transferring to a 600 mm by 400 mm rearing tray. The trial ran for 15 days.

Sampling

On trial termination samples were collected for use in safety testing. The objective of the sampling method was to obtain material that was representative of the whole bulk from which it was taken.

For sampling of larvae, twelve random handfuls were selected from each replicate and combined into a bulk sample. This was repeated to create a second bulk sample. A sub sample was taken from these bulks and sent for analysis. This was repeated for frass and the two bulk samples of feed. Samples for analysis of chemical contaminants were stored in a freezer for up to four weeks prior to analysis. For analysis, bulk A was analysed once and bulk B (B1+B2) was analysed twice (Figure 4).

Data analysis

The following parameters were calculated for each treatment:

Substrate reduction as a percentage was calculated on a wet weight basis as:

Substrate reduction (%) = (feed added – residue) ÷ feed added × 100

Biomass conversion ratio was calculated on a wet weight basis as:

Biomass conversion ratio = feed added ÷ larval mass

Bioconversion as a percentage was calculated on a wet weight basis as:

Bioconversion (%) = larval mass ÷ feed added × 100

Results

A sufficient quantity of larvae and frass for safety testing was obtained from all four rearing substrates. As expected, survival and mass of the BSF larvae was determined by the rearing substrate. Although the trials were set up to ensure development of the larvae, the objective for this study was to examine the safety of the larvae produced, and therefore optimisation of larval production was not examined in this project. Therefore, although general observations can be made regarding the different rearing substrates in this study it must be borne in mind that production of larvae can be improved for all substrates and comparisons between substrate types should be undertaken with caution. It must also be recognised that only a single source of rearing substrate was tested under these general headings and therefore the nature of the rearing substrate could change significantly between providers and throughout the year.

For some rearing substrate types e.g., food manufacturing and poultry manure, separation of the larvae from the frass at the end of the trial was difficult and although as many larvae as possible were separated the proportion of larvae remaining in the substrate may have been higher for some substrates than others leading to an underestimate of survival and the mass of larvae obtained.

In these trials, food manufacturing surplus provided the lowest mass of larvae and the highest biomass conversion ratio (Table 1). The nature of the food manufacturing surplus tested was very fatty and this will have impacted the development of the larvae. The larvae developed best on the supermarket surplus, where the highest mass of larvae and the lowest biomass bioconversion ratio was observed (Table 1).

There was some variation in the development of the larvae between replicates and for food manufacturing and supermarket surplus one replicate failed to produce larvae. The reason for this is not known.

Waste reduction was high (49.4% – 91.0% w/w) for all rearing substrates except poultry manure (29.7%), where it is possible that a higher proportion of uneaten substrate remained.

Table 1. Key parameters for BSF larvae reared on food manufacturing, catering and supermarket waste and poultry manure. Figures are mean ± standard error. All figures are on a wet weight basis. N=6 except food manufacturing and supermarket where N=5.

	Mass of 50 larvae (g)	Indicative mass of individual larva (mg)	Total mass of larvae (g)	Estimated number of larvae	Biomass conversion ratio	Waste reduction (%)
Food manufacturing	5.1 ± 0.65	97	259.7 ± 71.3	2607 ± 717	24.6 ± 8.5	54.0 ± 4.9
Catering	10.1 ± 0.6	202	720.5 ± 144.0	3749.3 ± 905.3	10.3 ± 3.9	49.4 ± 2.9
Supermarket	7.6 ± 0.6	151	794 ± 92.3	5603 ± 1139	4.1 ± 0.6	91.0 ± 0.7
Poultry manure	7.2 ± 0.7	144	484.5 ± 161.8	3896 ± 1585	17.4 ± 4.9	29.7 ± 3.2

3. Safety testing

Materials and methods

Sample pre-processing / preparation

Core diets

Sub-samples for microbiological analyses were taken aseptically, after defrosting of the samples (rearing substrate, larvae, frass) from representative core diet and core diet+ 20% trials, to ensure they were representative of the original samples. The remainder of each sample was frozen before freeze-drying further sub-samples for a minimum of 48 h. After drying the samples were homogenised and aliquoted into sub-samples for the chemical analyses.

Sample pre-processing / preparation

Test diets

Sub-samples for microbiological analyses were taken aseptically for the three sample types (rearing substrate, larvae, frass). The remainder of each sample was frozen before freeze-drying further sub-samples for a minimum of 48 h. After drying the samples were homogenised and aliquoted into sub-samples for the chemical analyses.

Safety analysis

Metals

Deionized (18.2 MΩ cm) water, metal analysis grade reagents and acid cleaned plasticware were used throughout. Aliquots of sample were weighed into allotted digestion vessels and a mixture (4:1) of nitric acid and hydrochloric acid added. The vessels were capped, and the contents digested under high temperature and pressure using a single reaction chamber microwave digester system (Ultrawave, Milestone). Reagent blanks, certified reference materials and a spiked sample were also used in the procedure. The resulting solutions were transferred to pre-marked acid-cleaned plastic test tubes and diluted with deionised water. The digest solutions, together with a set of standards covering the expected concentration range, were internally standardised with indium in dilute nitric acid (1% v/v). Multi-element measurements were made using an Agilent 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS) with collision cell. The concentrations of elements in the samples were measured within the range 0.001-25,000 mg/kg.

Veterinary Medicines

Two sub-samples of each sample were extracted using either 1% oxalic acid or 1% acetic acid in acetonitrile. After homogenising, sodium sulphate was added and the sample shaken and centrifuged at 4000-4500 rpm for 10 mins. The supernatant was then applied to dispersive C18 and / or NH_2 solid phase extraction material. After further shaking and subsequent centrifugation, an aliquot of the supernatant was analysed by reverse phase ultra high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) (Agilent 6490 TQ).

Pesticides

Samples were hydrated with HPLC grade water and extracted in acetonitrile, in the presence of sodium citrate, sodium chloride and magnesium sulphate. The extract was then divided with one portion mixed with dispersive SPE material (PSA and C18) before a solvent swap (into ethyl acetate) and subsequent analysis by gas chromatography with mass spectrometric detection (GC-MS, Agilent 5973 Inert MSD). The second portion was directly analysed using liquid chromatography with mass spectrometric detection (UPLC-MS/MS) in selected reaction monitoring mode (Agilent 6490 TQ). The presence of residues was confirmed using the same technique in multiple reaction monitoring mode. The concentration of pesticide residues was measured with limits of detection of 10, 20 or 50 µg/kg.

Mycotoxins

The sample was weighed into a plastic centrifuge tube. The extraction solvent used was a mixture of acetonitrile:water:acetic acid (79 : 20 : 1). Tubes were vortex mixed, then extracted for 2 hours on an orbital shaker. After extraction, tubes were centrifuged at 4000 rpm for 20 minutes at 4°C. An aliquot of the supernatant was transferred to a glass vial and diluted with a mixture of acetonitrile:water:acetic acid (20 : 79 : 1). The vials were stored overnight in a fridge at 4-8°C. Sample extracts were filtered by syringe filter (0.22 µm, nylon) and collected in glass autosampler vials for analysis.

UPLC-MS/MS analysis was carried out using a Waters UPLC system with a XEVO TQ-S mass spectrometer. Two analytical runs, one using neutral mobile phase conditions and one using acidic conditions, were required to ensure optimum chromatographic performance and ionisation of analytes. The method can detect several groups of mycotoxins including aflatoxins, fumonisins, trichothecenes, *Alternaria* toxins, ergot alkaloids, zearalenone and derivatives, enniatins, as well as many other *Fusarium* and *Penicillium* mycotoxins. In addition, masked forms of some mycotoxins i.e. metabolites of the parent mycotoxin, can also be detected.

PAHs

An aliquot of each homogenised sample was fortified with appropriate ^{13}C internal standards and subjected to saponification followed by liquid-liquid extraction. Desired analytes were extracted from the matrix using a DMF/cyclohexane partition followed by elution through a silica gel column. Analysis was by GC-MS using a Thermo Trace Ultra GC/ISQ with a Select PAH 30m column with a 0.25 mm diameter and a 0.15 µm column film.

Nitrates and nitrites

Aliquots of each sample were extracted using water and clarified using acetonitrile. Sample extracts were chromatographed using an AS11-HC column (Thermo Scientific) with a guard column. The mobile phase was a sodium hydroxide solution with a flow rate of 1 ml/min at ambient temperature. The injection volume was 100 ul and detection was by UV. Aliquots of a reference material, with an assigned value for nitrate only, were analysed with the samples.

PFAS

Aliquots of the test samples were spiked with isotope standards (internal standards) and extracted using basic methanol. The resulting solvent extracts were solvent exchanged into water and passed through WAX SPE columns. WAX columns allow for the retention of both short and longer chain PFAS analytes due to the ionic exchange and reverse phase properties. Non-specific interferences were retained on the column whilst PFAS analytes were eluted using ammonia in methanol. Samples were concentrated and reconstituted in methanol, analysed by HPLC-MS/MS, and quantified against calibration standards of known concentrations of the PFAS (0-5 ug/kg).

Toxin screen

Each sample was extracted in triplicate with 1% acetic acid in acetonitrile by homogenisation. Anhydrous sodium sulphate was added and the mixture shaken. After centrifugation (4500 rpm, 5 min, 5ºC), the supernatant was poured into a tube containing Bondesil C18 sorbent (500 mg), shaken and centrifuged (4500 rpm, 5 min, 5°C). Two aliquots were evaporated to dryness under a stream of nitrogen at 40 – 45°C. Aliquot 1 was resuspended in 1:1 methanol:0.2% aqueous acetic acid by dissolving the residue in 400 µL methanol and 100 µL internal standard mix (1 µg/mL) in methanol, vortex-mixing and adding 500 µL 0.2% aqueous acetic acid and mixing. Aliquot 2 was resuspended in 0.2% aqueous acetic acid (900 µL) and 100 µL internal standard mix (1 µg/mL) in methanol. Aliquot 1 was centrifuged (2000 rpm, 5min, 5^{o}C) and then passed through a PTFE filter (0.2 µm). Aliquot 2 was centrifuged (14000 rpm, 2min, 5°C). Single (internal standard added) and double (100 µL methanol added) blanks were prepared in the same manner.

Portions of the extracts were analysed using an Agilent 1200 series liquid chromatograph coupled with an Agilent 6320 TOF. Chromatographic separation was achieved on a Zorbax SB-Aq (50 x 2.1mm, 1.8 µm) held at 60°C. The mobile phases comprised of water containing 0.2% acetic acid and methanol containing 0.2% acetic acid (B). The gradient started at 2% B and changed to 98% B at 13 minutes before returning to 2% B at 19 minutes. The flow rate was 0.6 mL/min and the injection volume was 2 µL. The MS was operated in positive and negative ESI with monitoring from 100 – 1600 m/z.

Sample replicate raw data files were profiled against procedural blanks using a combination of MassHunter Qualitative software (Agilent) and Mass Profiler Professional (MPP, Agilent) to produce a list of ion features present in the samples but not in the controls. This feature list was mined by comparison to an in-house database of 1250 compounds consisting of biological toxins (e.g. fyco-, myco-, phyto-). All toxins in the database are given in Appendix B for information.

Results were filtered using the following criteria:

- Not present in the procedural blanks.
- Present in all three sample replicates.
- Response in MPP software > 50,000. (Represents a summation of all software-assigned ions).
- Mass accuracy of match >-5 and <5 ppm.
- Adduct ion match. Compounds were rejected where the spectral data indicated that the identified adduct ion was incorrect.
- Spectral data. Compounds were rejected if the presence of atoms other than those in the compound formula are indicated (i.e. halogen), the ion charge is incorrect, or the ion corresponds to an isotopic ion of a lower mass ion.

The finalised ion list was then compared to the MetLin database to assess the likelihood of a potential compound being due to a naturally occurring metabolite in the sample.

Data was filtered on the following criteria:

- Compound rejected if there were more than 10 MetLin database matches.

Data was also reviewed against the likelihood of both the ion formed and the retention time and also against any other available information (such as for example geographical distribution of the organism producing the toxin). Finally, any compound identification with a formula match score <80% was removed.

Microbiological analysis

The microbiological analysis included organisms that are regulated for in animal feed containing animal products i.e. *Salmonella* spp. and Enterobacteriacae and organisms that are indicative of hygiene methods or requested by the FSA.

The microbiological content of the sample was determined following methods outlined in the following ISO standard methods:

- ISO 4833 (2013), "Enumeration of Micro-Organisms - Colony Count Technique At 30°C (Pour Plate)"
- ISO 21528, part 2 (2004), "Enumeration of Enterobacteriaceae"
- ISO 4832 (2006), "Enumeration of Coliforms - Colony Count Technique"
- ISO 6579, part 1 (2017), "Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp."
- ISO 10272 part 2 (2017) and amendment 1 (2023), "Microbiology of the food chain – horizontal method for the detection and enumeration of Campylobacter" for poultry manure only
- ISO 6888 part 1 (2021), "Microbiology of the food chain – horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species)" for catering kitchen waste and supermarket surplus only.

- ISO 16649 part 2 (2001), "Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli*"
- Enumeration of ESBL *E. coli* – In-house method based on ISO 16649 part 2 (2001) using ESBL selective agar plates
- Enumeration of Enterococci – In-house method using Slanetz & Bartley agar and the spread plate technique. Plates incubated at 37°C for 48 h. (ISO method currently under development). For frass only.

Briefly, for the colony count technique and enumeration assays the sample (10 g) was added to diluent (90 ml) and homogenised in a stomacher-style blender for 2 mins. Further dilutions (1:10) were prepared and vortexed to mix. Each dilution was used for duplicate agar plates. Agar plates were incubated as required for the method and colony numbers were counted on completion of the incubation period.

For the detection of Salmonella spp. sample (25 g) was added to pre-enrichment broth (BPW; 225 ml) and homogenised for 2 minutes in a stomacher-style blender. The sample was incubated as stated in the Standard method. Aliquots from the incubated test portion were subcultured into selective enrichment broth (RVS and MKTTn) and incubated. The selective enrichment broths were streaked onto selective agar (XLD and BGA) and incubated. When incubation was complete, the plates were examined for the presence of colonies which display a typical morphology for *Salmonella* spp. Duplicate or single plates were undertaken on samples (replicates A, B1 and B2) the average $\log_{10}$ concentration for a replicate was estimated as the average of each $\log_{10}$ plate observation, then the average $\log_{10}$ for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

An indication of within replicate variation was given by $B1 - B2$ and of between replicate variation by $A - ((B1 + B2)/2)$.

Results that were reported as <10 cfu/g were treated as being at the limit of detection. Results reported as being above a value were treated as being at that value.

Non-targeted virus screen

Samples were frozen at -70°C and stored prior to extraction. Each sample (2.5 g) was extracted using the CTAB based method described in Adams *et al*. (2009).The extracted RNA was further purified using the RNAeasy kit (Qiagen, UK) with optional on column DNAse treatment following the manufacturer's instructions. Illumina compatible indexed DNA sequencing libraries were constructed using the truSeq Stranded RNA library kit (Illumina, UK) including the ribosome depletion steps. The libraries were indexed using the compatible IDT unique dual indexes (Illumina UK). Further details of the library preparation can be found in the instructions for the kits provided by Illumina. Equimolar quantities of the libraries were checked on a Tapestation (Agilent, UK) and pooled. The pooled library was sequenced on a 500 cycle SP NovaSeq flowcell at Newcastle University. Data was provided as demultiplexed fastq files. The sequenced data was processed using the Angua pipeline as described in Fowkes *et al*. (2021).

Assessment of the extent to which the results are representative of the substrate, larvae and frass

Analytical results were evaluated to provide a measure of how representative the samples for each contaminant were for each rearing substrate and to indicate the likely reason for any lack of representativeness (e.g. too few sampling times; too few primary samples; lack of homogeneity in bulk material) and how this might affect the study outcomes.

Results

The following sections highlight analytes found in larvae or, in the case of metals, those for which there are regulatory limits for presence in animal feed. The full results for each group of chemicals are provided in Appendices B-I.

Note all results, with the exception of those for microbiology, are presented on a dry weight basis.

Metals

In the UK and the EU there are regulatory limits for arsenic, cadmium, mercury and lead in animal feed (Directive 2002/32/EC) (Table 2). In the EU, there are also regulatory limits for the presence of chromium (VI), cadmium, mercury and lead in organic fertilisers (EU 2019/1009) (Table 2).

Table 2. Regulatory limits for metals in animal feed and organic fertilisers as specified in EU 2002/32/EC and EU 2019/1009.

	Arsenic (mg/kg)	Chromium (mg/kg)	Cadmium (mg/kg)	Mercury (mg/kg)	Lead (mg/kg)
RL feed 1	4	N/A	0.5	0.1	10
RL feed 2	2	N/A	2	0.1	10
RL feed 3	2	N/A	0.5	0.1	5
RL fertiliser	N/A	16.6*	1.5	1	120

*Note: equivalent to 2 mg/kg of chromium (VI)
RL feed 1 = regulatory limit *"complementary feed"*, EU 2002/32/EC
RL feed 2 = regulatory limit *"feed materials of animal origin"*, EU 2002/32/EC
RL feed 3 = regulatory limit "complete feed", EU2002/32/EC
RL fertiliser = regulatory limit for organic fertiliser, EU 2019/1009

Low levels of arsenic were found in larvae reared on the baseline diets and on all four of the tested rearing substrates, but all larvae samples were below the regulatory limit (Figure 8). There was no evidence of bioaccumulation in the larvae (Appendix 2)

Cadmium was present in larvae reared on the baseline samples and on the four currently non-permitted rearing substrates tested (Figure 8). Levels were below the regulatory limits for feed materials of animal origin (2 mg/kg). The highest level was found in larvae reared on poultry manure (0.610 mg/kg). There was evidence of bioaccumulation in larvae reared on all rearing substrates (Appendix B). Bioaccumulation of cadmium by BSF larvae is known to occur when using both spiked and naturally occurring levels in rearing substrates (Charlton *et al*., 2015; Purschke *et al*., 2017; Gao *et al*., 2017; Addeo *et al*., 2024).

Low levels of lead were found in larvae reared on the baseline diets and on all four of the tested rearing substrates (Figure 8), but all larvae samples were below the regulatory limit for feed materials of animal origin. There was a low level of bioaccumulation in the larvae reared on the core diet +20%, catering surplus, manufacturing surplus, poultry manure and supermarket surplus (Appendix B). Some studies have also shown bioaccumulation of lead by BSF larvae (Addeo *et al*., 2024), whilst other studies have reported no bioaccumulation (Proc *et al*., 2020).

Mercury was not detected in any of the samples tested.

In addition to the heavy metals that have regulatory limits for their presence in feed and food, the levels of other metals are also of importance, both as micronutrients for the larvae and also for their presence in animal feed for farmed animals and pets. There was evidence for bioaccumulation of some other metals e.g. calcium. phosphorus, selenium (Appendix B). It may be necessary to assess the levels of some of these other essential metals to ensure that correct nutritional profiles are provided and that any nutritional limits are not exceeded. For example, calcium and phosphorus are essential elements for nutrition and are required in, for example, poultry feed for bone and muscle growth and eggshell formation together with other functions. However, too much calcium can reduce growth rates in broiler and layer pullets, whilst an oversupply of phosphorus can have an environmental impact when manure is applied to soils.

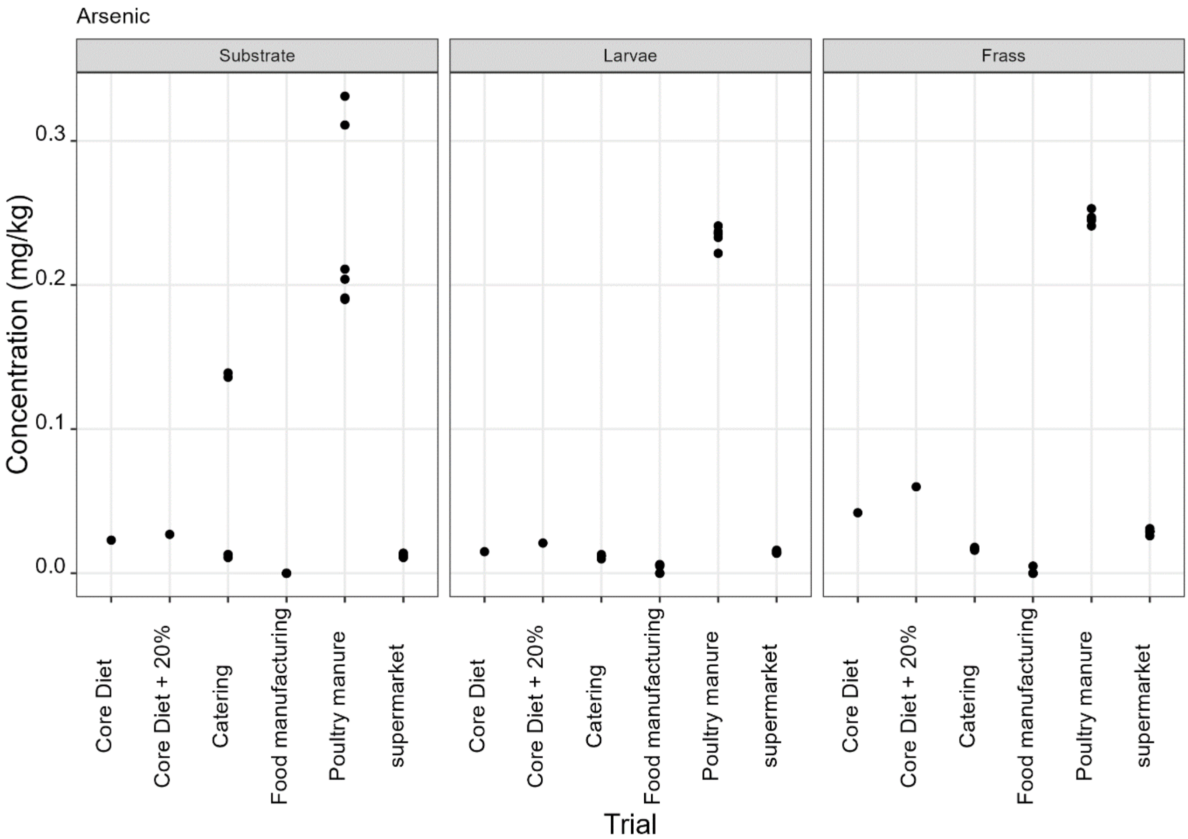
Regulation (EU) 2019/1009 specifies the following criteria for solid organic fertilisers: Contaminants in an organic fertiliser must not exceed the following limit values:

- cadmium (Cd): 1.5 mg/kg dry matter,
- hexavalent chromium (Cr VI): 2 mg/kg dry matter,
- mercury (Hg): 1 mg/kg dry matter,
- nickel (Ni): 50 mg/kg dry matter,
- lead (Pb): 120 mg/kg dry matter, and
- inorganic arsenic (As): 40 mg/kg dry matter.

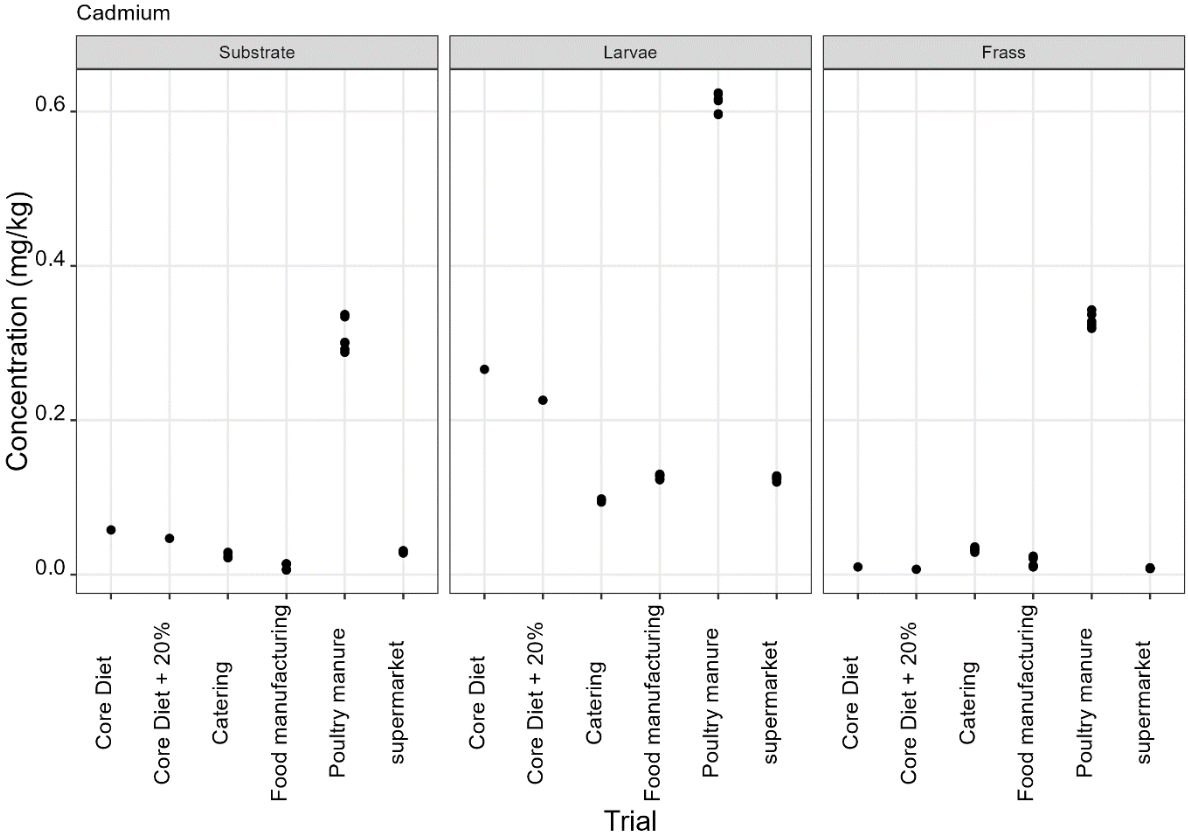
The copper (Cu) content in an organic fertiliser must not exceed 300 mg/kg dry matter and the zinc (Zn) content in an organic fertiliser must not exceed 800 mg/kg dry matter.

These levels were not exceeded in any of the frass samples tested (Appendix B).

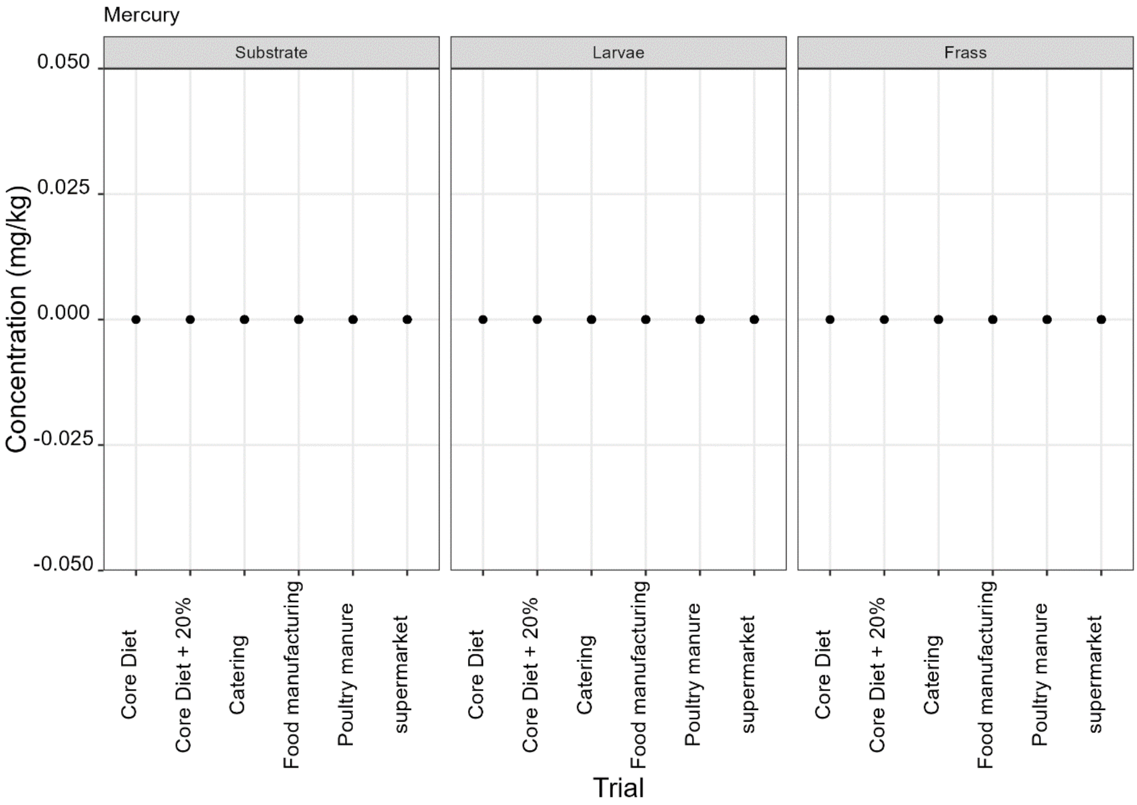
A.



B.


Cadmium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

C.


Mercury
Substrate
Larvae
Frass
Concentration (mg/kg)
0.050
0.025
0.000
-0.025
-0.050
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

D.

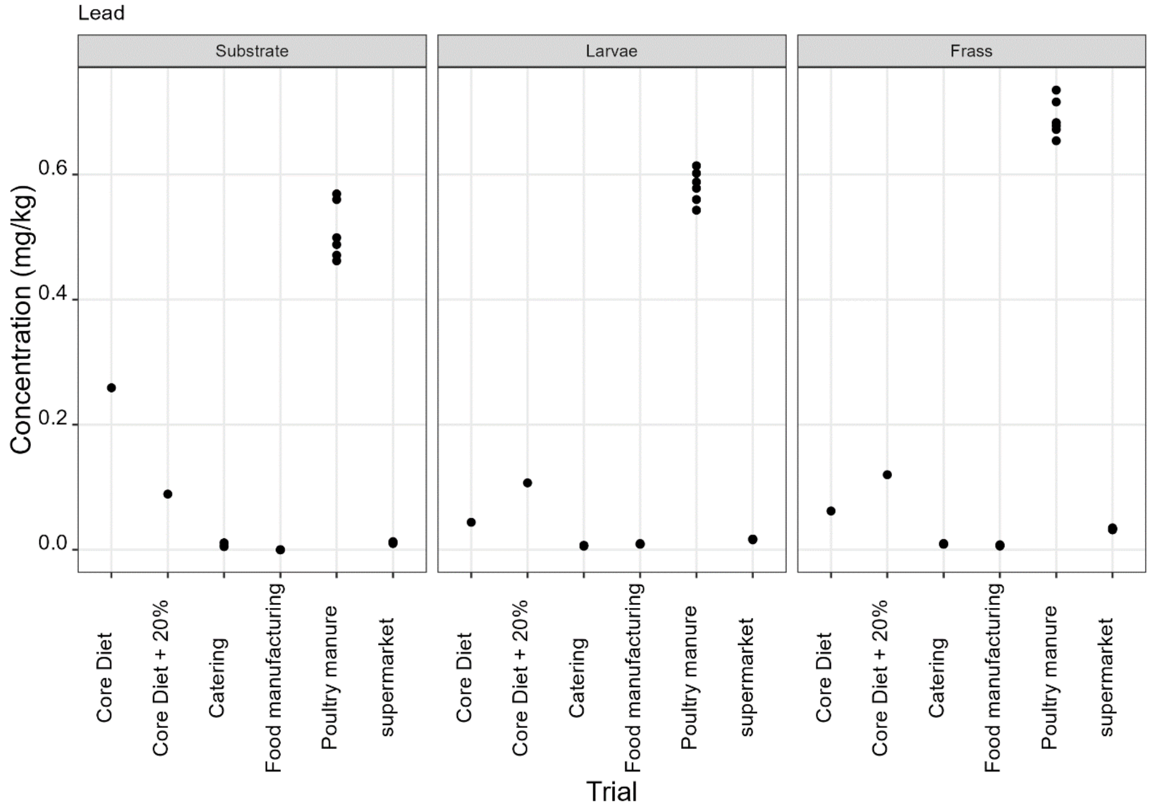


Figure 8. Concentrations of arsenic (A), cadmium (B), mercury (C), and lead (D) found in different rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections except for core diet and core diet +20% where there was a single injection.

Veterinary Medicines

The veterinary medicines method screens for 115 compounds. Traces of eight compounds were detected in at least one extract from one sample type (Figure 9). Full results are shown in Appendix C.

Nicarbazin is a coccidiostat and was detected in the poultry manure rearing substrate, the larvae that were produced and the resulting frass (Figure 9). Levels in the larvae were much lower than those found in the rearing substrate or the frass (means 5775, 363.8 and 2725 µg/kg respectively). Low levels of nicarazin were also found in the catering waste (mean of 12.25 µg/kg) and the frass produced from the catering waste (mean of 36.25 µg/kg), but was not detected in the larvae reared on the catering waste.

The regulatory limit for the presence of nicarbazin in feed materials relative to a feed with a moisture content of 12% is 1.25 mg/kg. Therefore, although the level of nicarbazin in the larvae reared on the poultry manure would not exceed the regulatory limit for a feed material, the level present in the poultry manure rearing substrate does exceed the regulatory limit if larvae are classed as a farmed animal and this substrate were to be provided as feed.

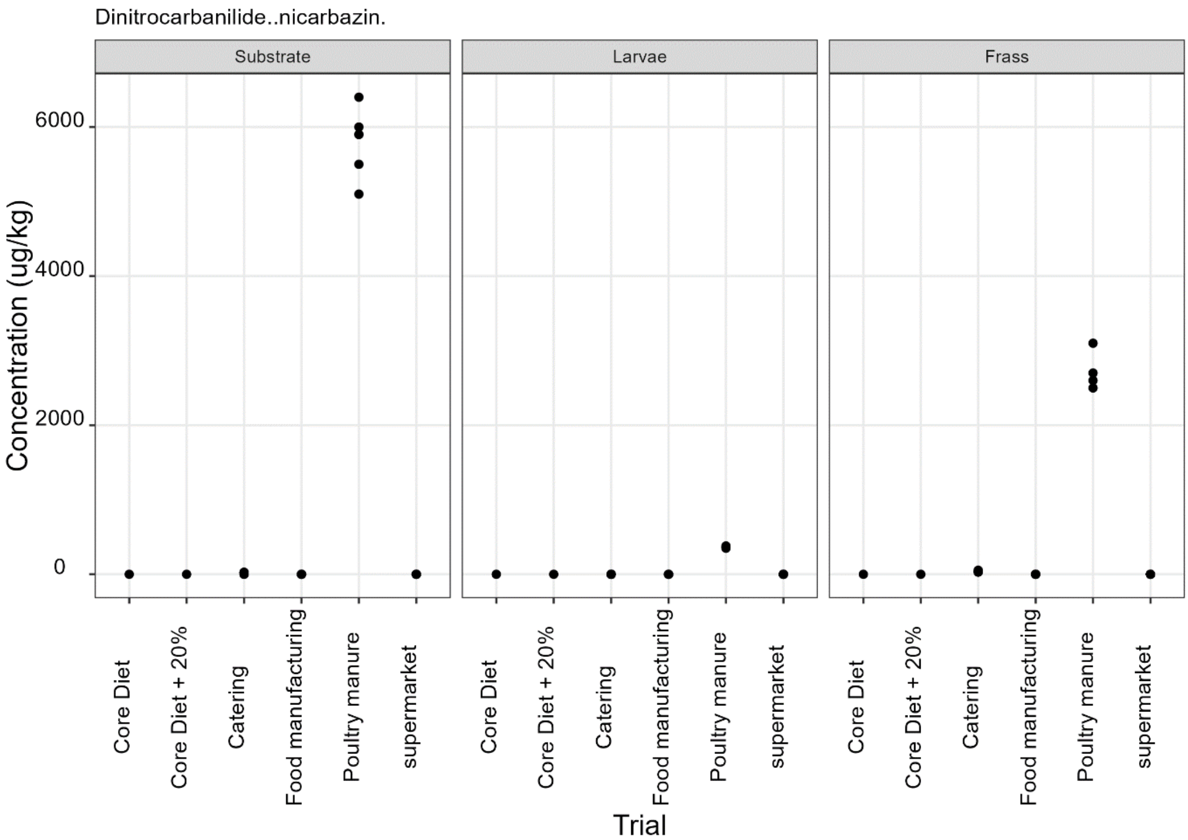
Flubendazole is an anthelmintic used as a wormer in poultry. It was not detected in the rearing substrates or the frass but was detected in larvae reared on the manufacturing surplus (mean 20.5 µg/kg) (Figure 9). It is possible that as the larvae would have greater homogeneity than either the substrate or the frass, this increased the likelihood of detecting this compound. Alternatively, the larvae may have bioaccumulated the compound from the substrate to a level where it could be detected. Further research would be needed to determine whether bioaccumulation of this compound does occur. There is no regulatory limit for this compound in feed ingredients.

Lasalocid is an antibiotic and coccidiostat used as a feed additive. Lasalocid was detected in larvae reared on manufacturing surplus (mean 86.9 µg/kg) (Figure 9). The regulatory limit for lasalocid in feed materials is 1.25 mg/kg and therefore the regulatory limit was not exceeded in these samples.

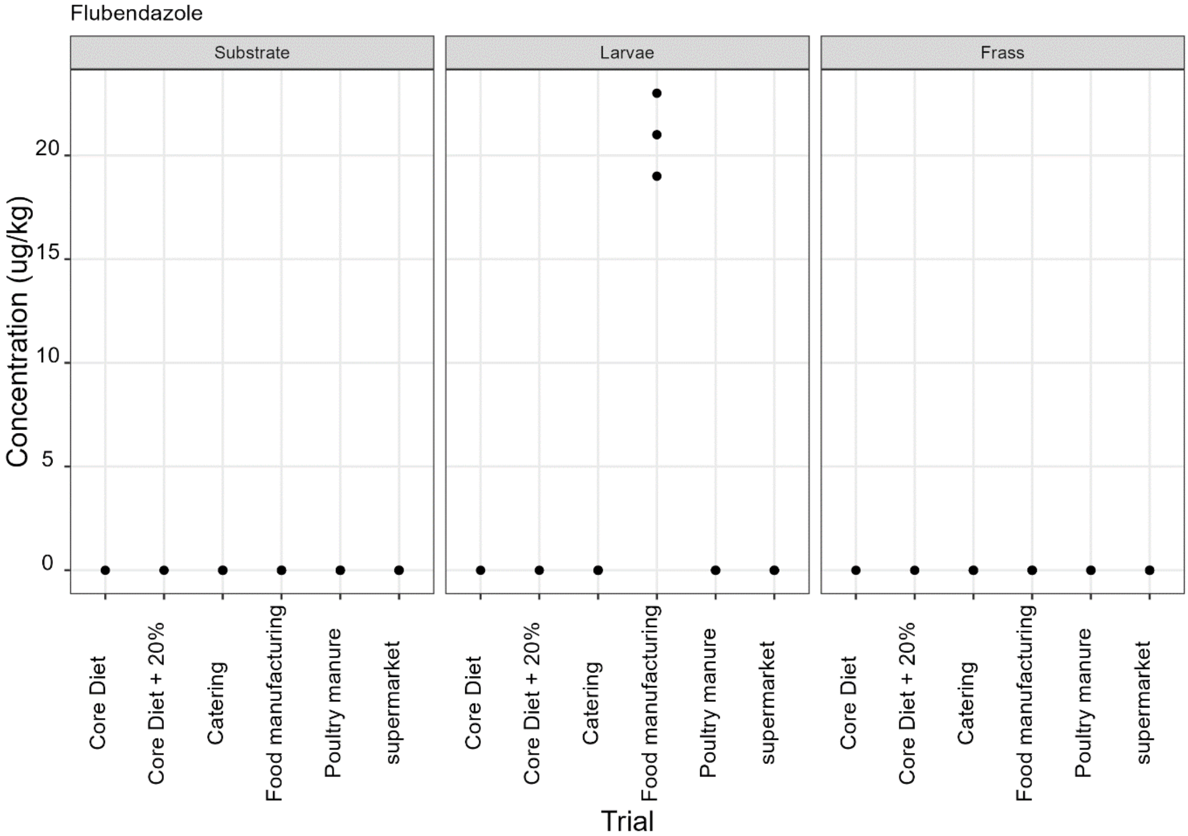
Narasin and salinomycin were detected at very low levels in larvae reared on poultry manure (means of 20.25 µg/kg and 14.75 µg/kg respectively). These levels were much lower than those detected in the poultry manure rearing substrate (means of 3562 µg/kg and 3875 µg/kg for narasin and salinomycin respectively) or in the frass (means of 385 µg/kg and 195 µg/kg respectively) (Figure 9). Both compounds have a regulatory limit of 0.7 mg/kg in feed materials. The level of these compounds in the larvae would not exceed the regulatory limit for a feed material, however, the level present in the rearing substrate does exceed the regulatory limit if larvae are classed as a farmed animal and this substrate were to be provided as feed.

Thiabendazole, triclabendazole sulphoxide and 5-hydroxythiabendazole are anthelmintics used to treat parasitic worms. Thiabendazole and triclabendazole sulphoxide were detected in the supermarket surplus rearing substrate (means of 74.4 µg/kg and 20.1 µg/kg respectively) and frass (means of 112.5 µg/kg and 12.38 µg/kg respectively) but were not detected in the larvae reared on supermarket surplus (Figure 9). 5-hydroxythiabendazole was detected in the frass from supermarket surplus (mean of 70.9 µg/kg) but was not detected in the rearing substrate (Figure 9). It is considered that the frass may be more homogenous than the rearing substrate due to the activity of the larvae and this may increase the likelihood of detection of this compound at low levels. There are no regulatory limits for these compounds in animal feed.

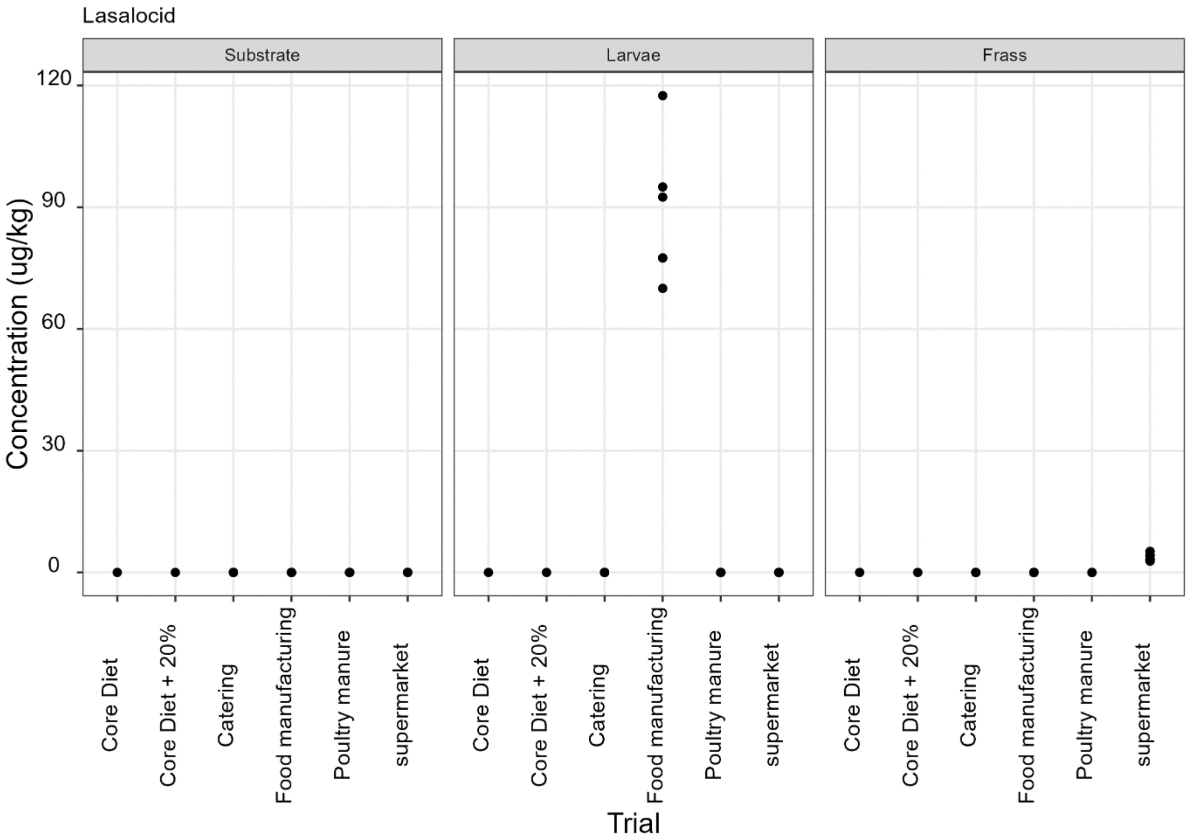
A.



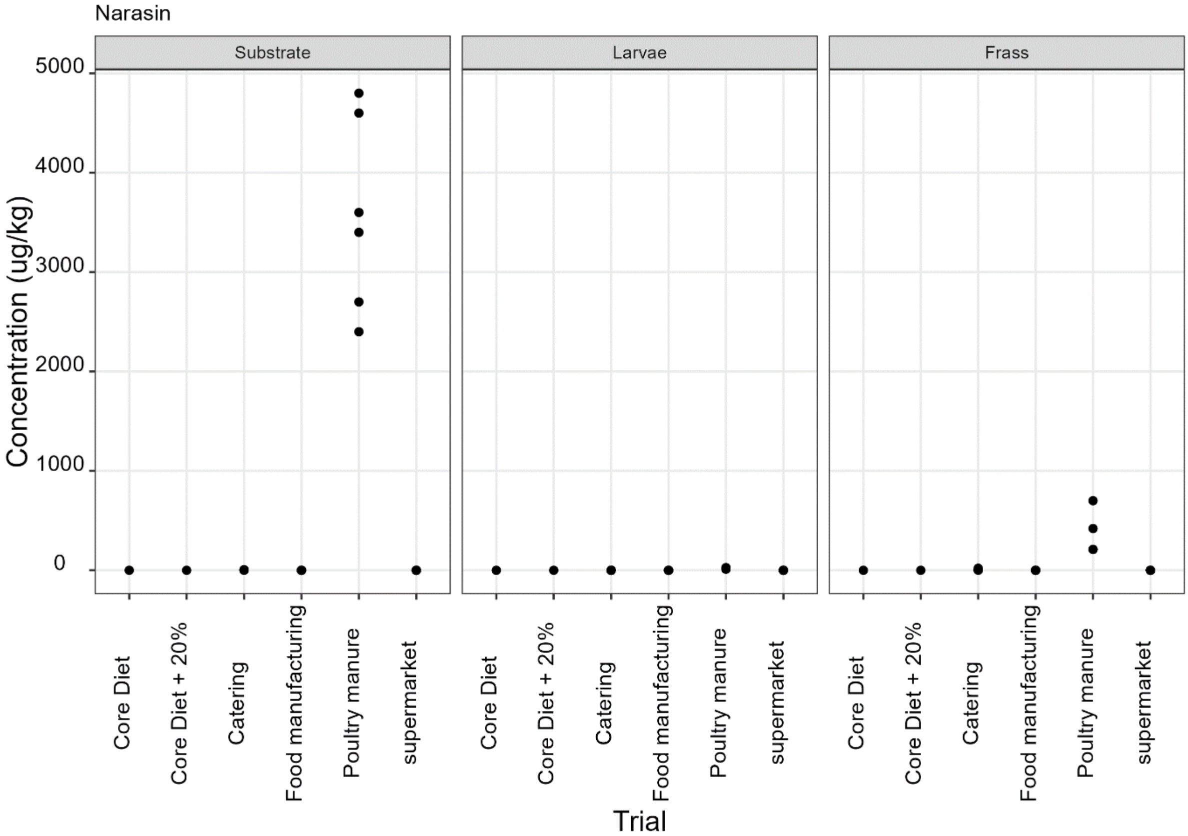
B.


Flubendazole
Substrate
Larvae
Frass
Concentration (ug/kg)
0
5
10
15
20
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

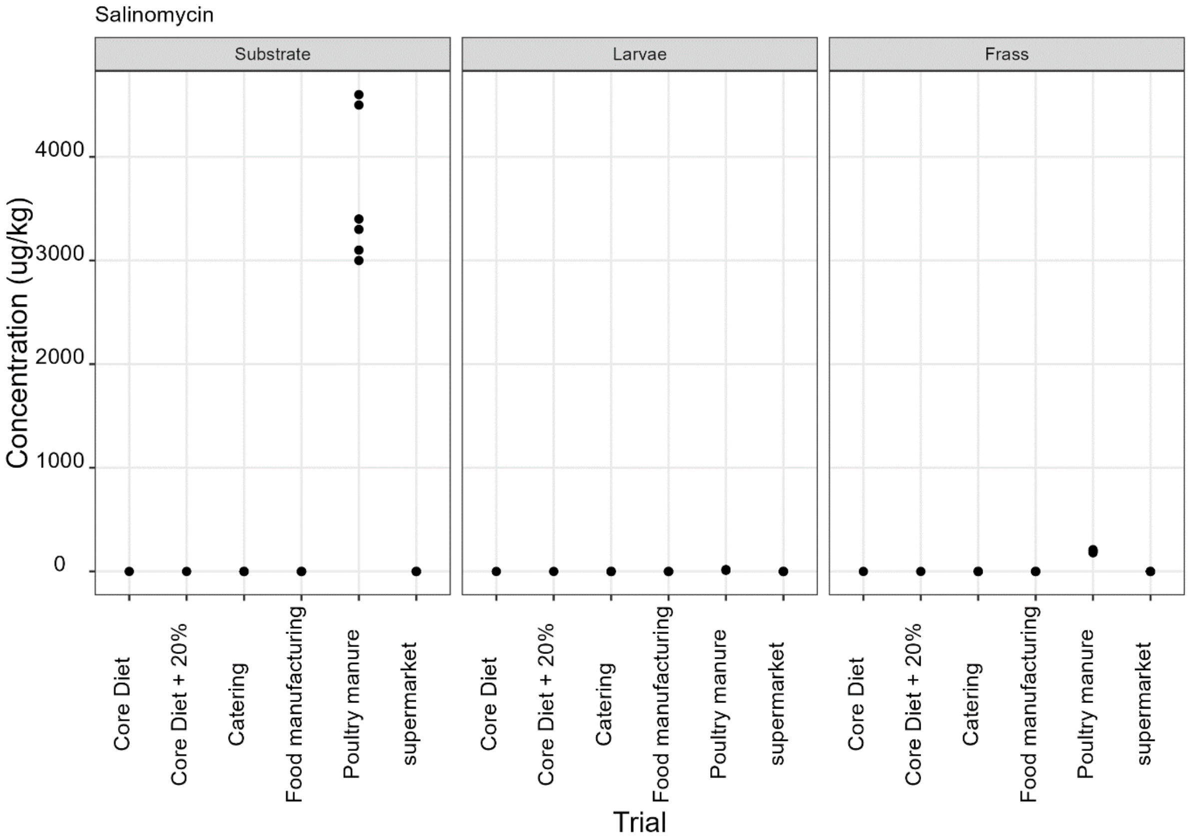
C.


Lasalocid
Substrate
Larvae
Frass
Concentration (ug/kg)
0
30
60
90
120
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

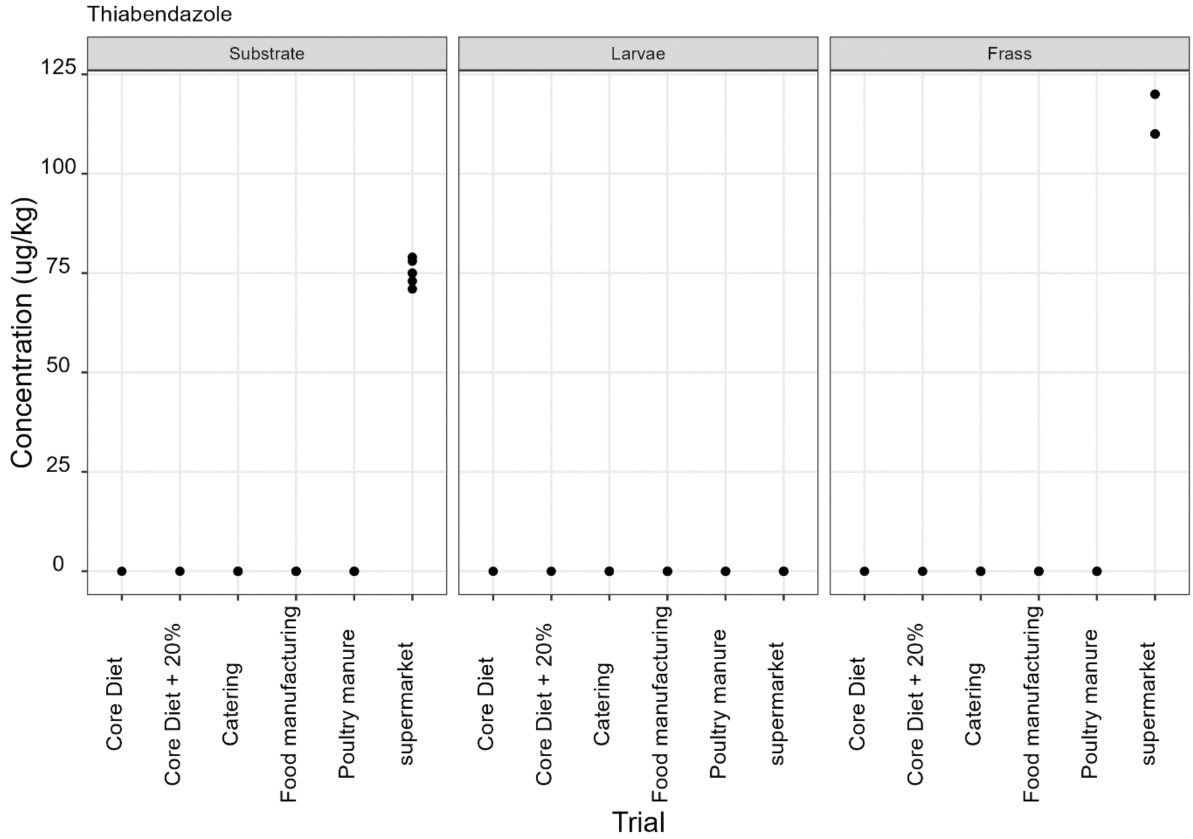
D.


Narasin
Substrate
Larvae
Frass
Concentration (ug/kg)
5000
4000
3000
2000
1000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

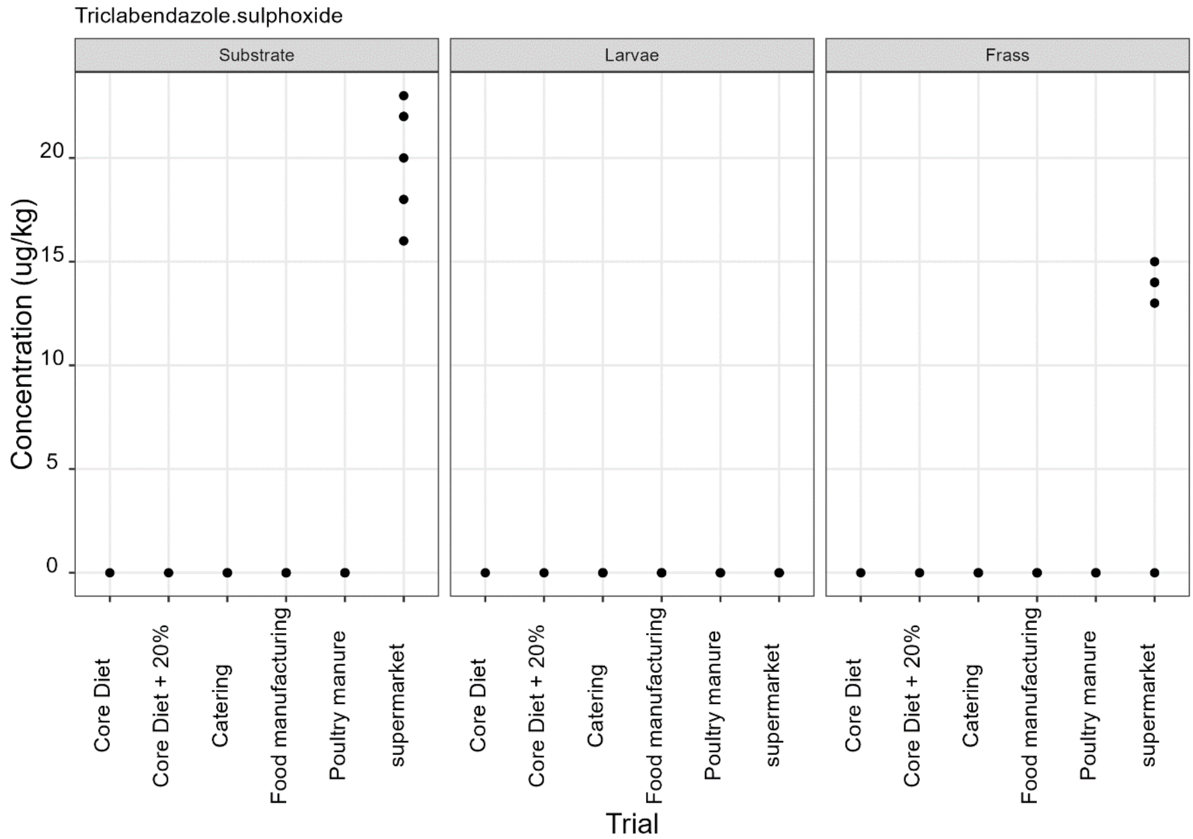
E.


Salinomycin
Substrate
Larvae
Frass
Concentration (ug/kg)
4000
3000
2000
1000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

F.


Thiabendazole
Substrate
Larvae
Frass
Concentration (ug/kg)
125
100
75
50
25
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

G.


Triclabendazole.sulphoxide
Substrate
Larvae
Frass
Concentration (ug/kg)
20
15
10
5
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

H.

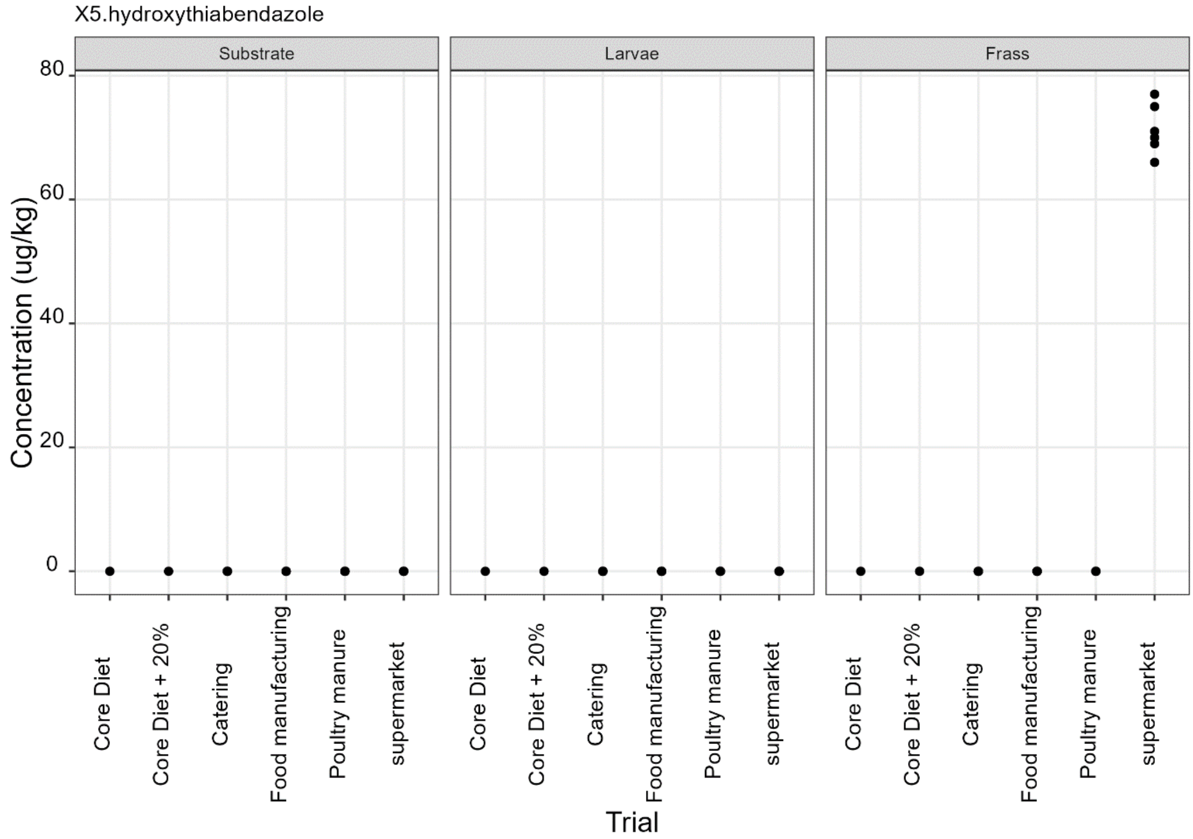


Figure 9 (A–H). Concentrations of veterinary medicines found in different rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections except for core diet and core diet +20% where there was a single injection.

Pesticides

The pesticides analysis screens for 427 compounds. Eleven of these compounds were detected in larvae and the majority of these were reared on either poultry manure or supermarket surplus (Figure 10). With one exception, the pesticide found in the larvae was also present in the rearing substrate. The exception was the presence of DDAC in larvae reared on core diet where DDAC was detected at 0.09 µg/kg in a single injection. Full results are provided in Appendix D.

Compounds that were detected in the larvae were:

- BAC 12, BAC 14 and DDAC are quaternary ammonium compounds widely used as biocides for the cleaning and sanitation of surfaces in food production and for surface cleaning of milking equipment and milk storage tanks.

- 2-phenylphenol is a biocide used as a surface disinfectant.
- Diphenylamine, fludioxonil, imazalil, and pyrimethanil are fungicides.
- Haloxyfop and 2,4-D are herbicides.

None of these compounds have regulatory limits in animal feed ingredients.

It was noted that the levels of DDAC in larvae reared on core diet and poultry manure (means of 0.09 µg/kg and 0.9 µg/kg) were greater than in the rearing substrate (not detected and mean of 0.175 µg/kg in core diet and poultry manure respectively). However, this was not observed for larvae reared on other types of rearing substrate (catering, manufacturing and supermarket surplus).

A greater level of haloxyfop in larvae reared on poultry manure (mean of 0.03 µg/kg) compared to the poultry manure rearing substrate (mean of 0.01 µg/kg) was also noted. This may be an indication of bioaccumulation, but further research would be needed to ascertain if this is the case.

There has been less research on the uptake of pesticides by BSF larvae than for heavy metals and mycotoxins but studies have shown that the selected pesticides assessed do not bioaccumulate in BSF larvae (chlorpyrifos, chlorpyrifos-methyl, pirimiphos methyl (Purschke *et al*., 2017); chlorpyrifos, propoxur, cypermethrin, imidacloprid, spinosad, tebufenozide (Meijer *et al*., 2021)). However, in a study using almond hulls as the rearing substrate it was demonstrated that bifenthrin bioaccumulated in BSF larvae, whilst there was no bioaccumulation of other pyrethroids present in the almond hulls (Li and Bischel., 2022).

There were an additional nine compounds that were detected in one or more of the rearing substrates that were not detected in the larvae and two compounds that were found only in the frass of one of the rearing substrates (Appendix D).

Maximum levels for pesticides are specified in the EU Directive on Undesirable Substances and Products (Directive 2002/32/EC) and in the EU Regulation on Pesticide Maximum Residue Limits (MRLs) (Regulation (EC) No 396/2005).

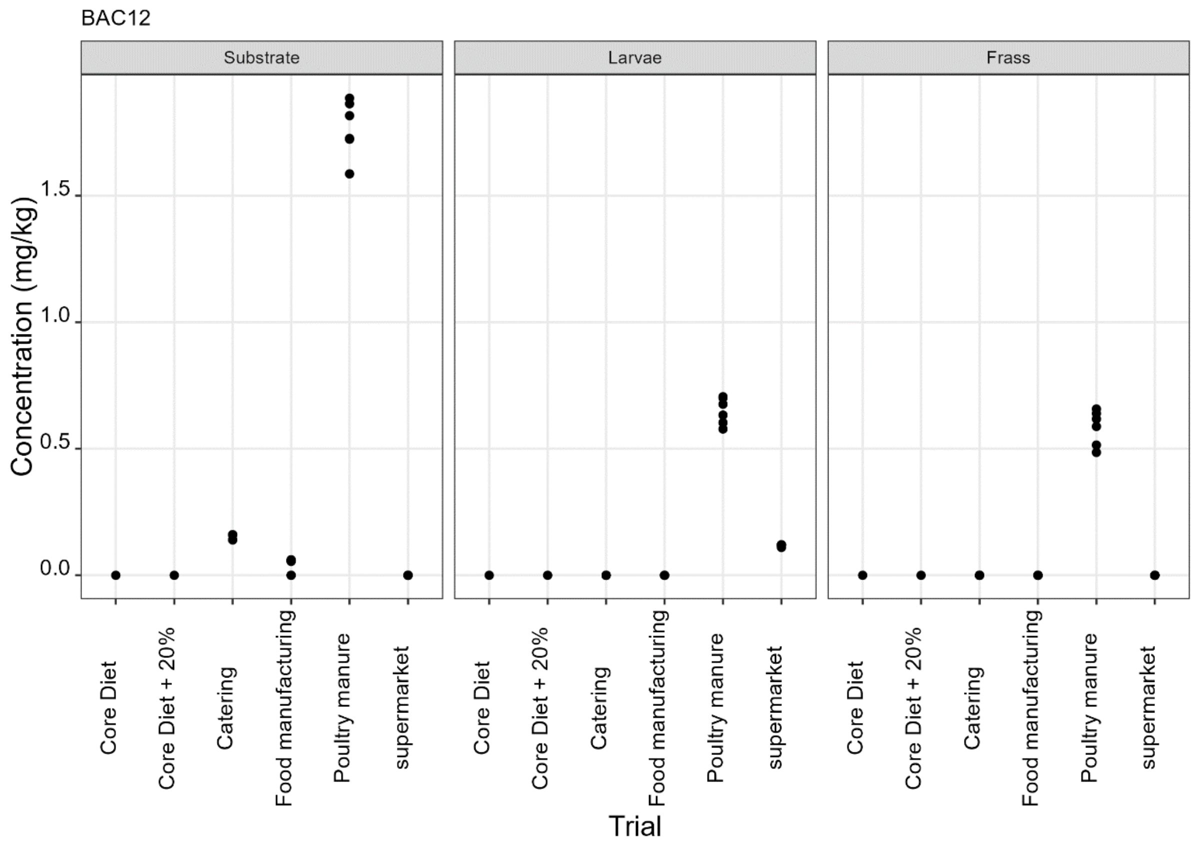
The levels of the pesticides found in larvae did not exceed the MRLs for these compounds for products exclusively used for animal feed production, but some did exceed the MRLs for terrestrial invertebrate animals (Table 3). This may have implications if the larvae were to be used as food.

Table 3. Pesticide residues in larvae that exceeded the MRL for terrestrial invertebrate animals.

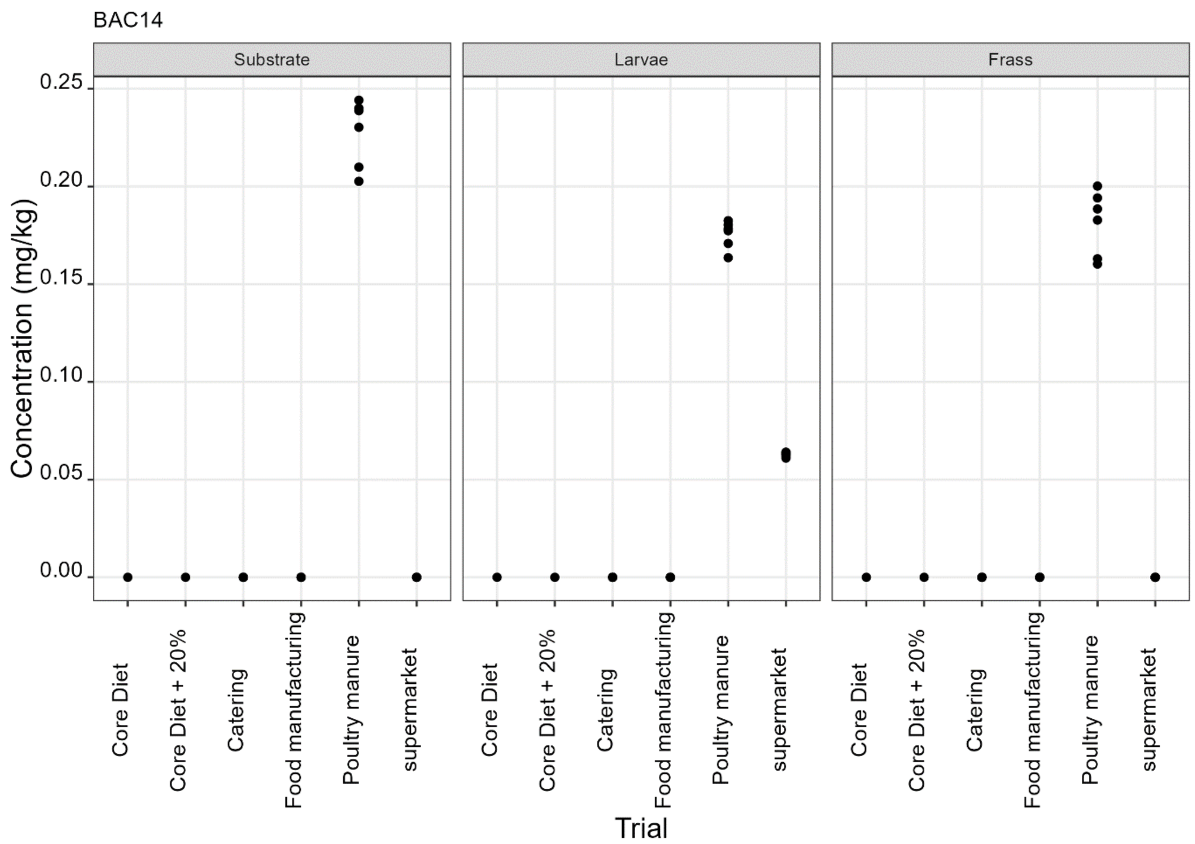
Pesticide	Rearing substrate	Mean level detected (mg/kg)	Maximum level detected (mg/kg)	MRL (mg/kg)
Fludioxonil	Supermarket surplus	0.064	0.080	0.01[a]
Imazalil	Supermarket surplus	0.024	0.026	0.01[b]
Haloxyfop	Poultry manure	0.035	0.040	0.01[c]
Thiabendazole	Supermarket surplus	0.037	0.038	0.01[d]
2-phenylphenol	Poultry manure	0.037	0.079	0.01[e]
2-phenylphenol	Supermarket surplus	0.11	0.13	0.01[e]

[a] Regulation (EU) 2022/1264, [b] Regulation (EU) 2020/856, [c] Regulation (EU) 2017/1016, [d] Regulation (EU) 2023/377, [e] Regulation. (EU) 2018/78

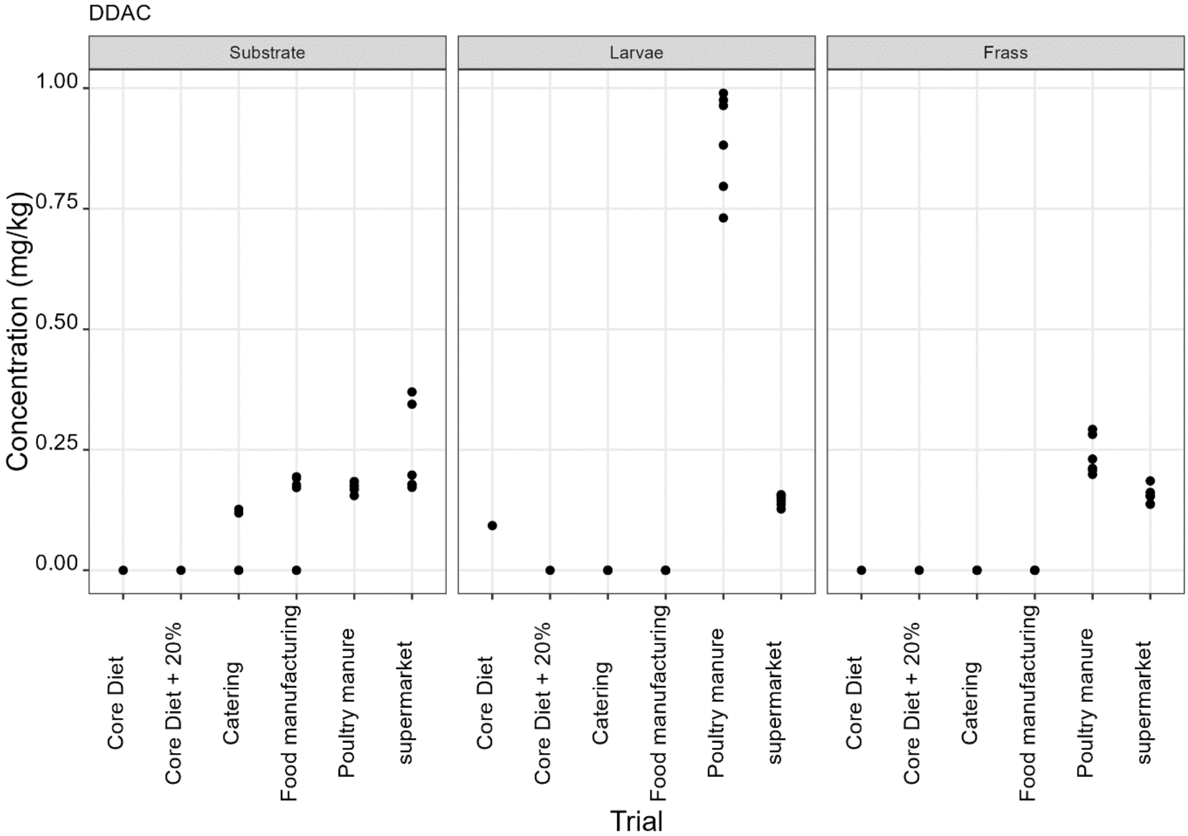
A.


BAC12
Substrate
Larvae
Frass
Concentration (mg/kg)
0.0
0.5
1.0
1.5
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

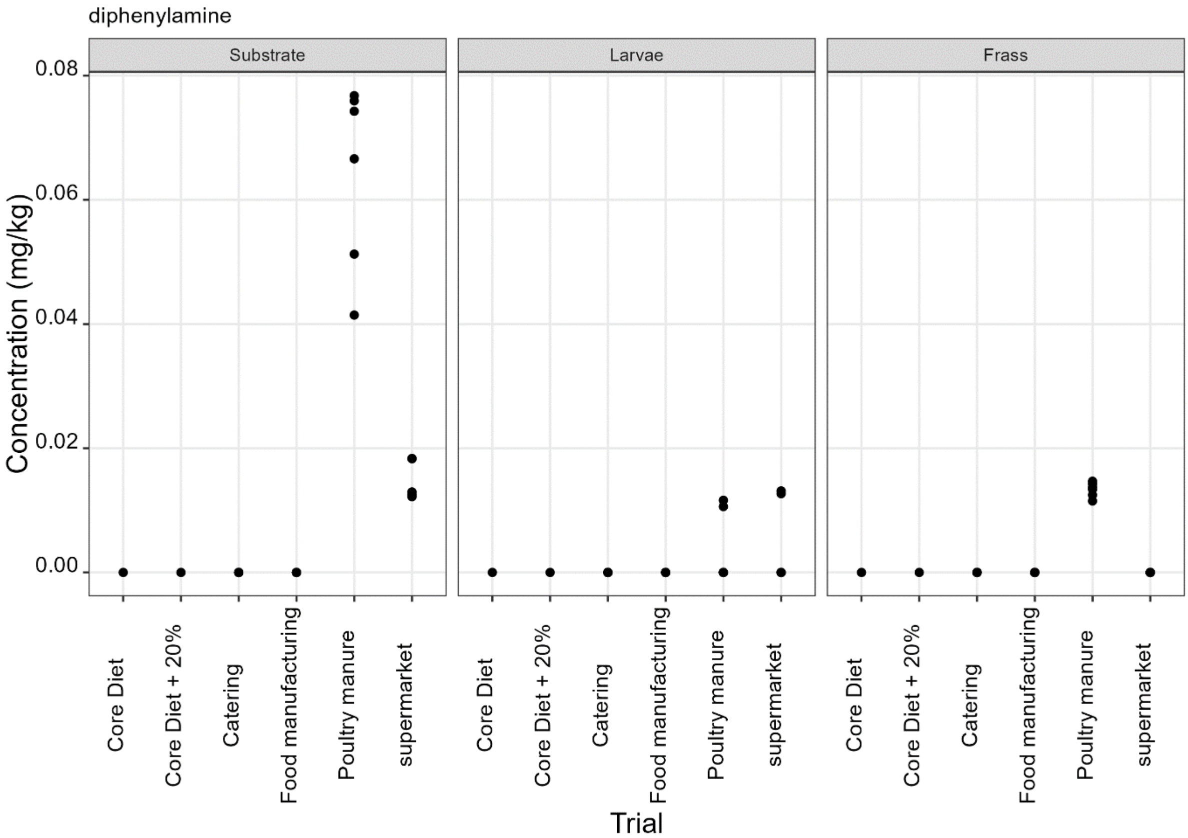
B.


BAC14
Substrate
Larvae
Frass
Concentration (mg/kg)
0.00
0.05
0.10
0.15
0.20
0.25
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

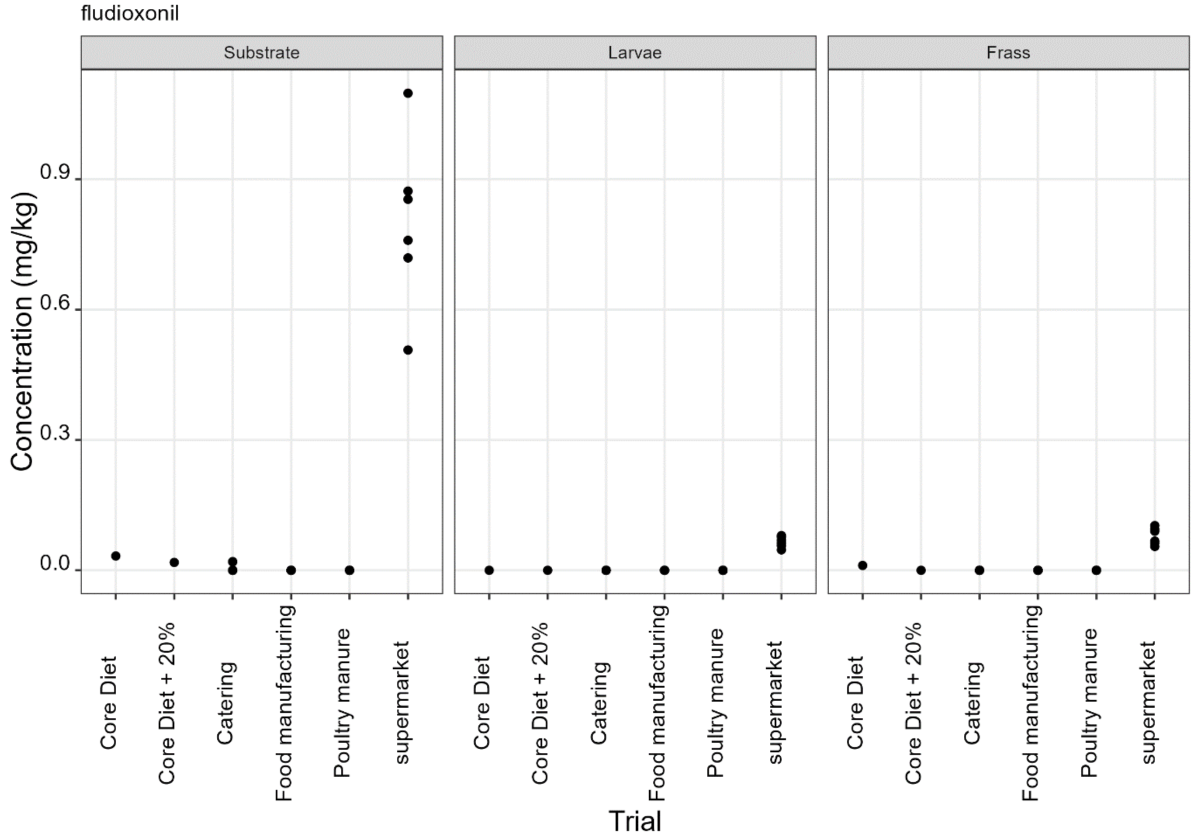
C.


DDAC
Substrate
Larvae
Frass
Concentration (mg/kg)
1.00
0.75
0.50
0.25
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

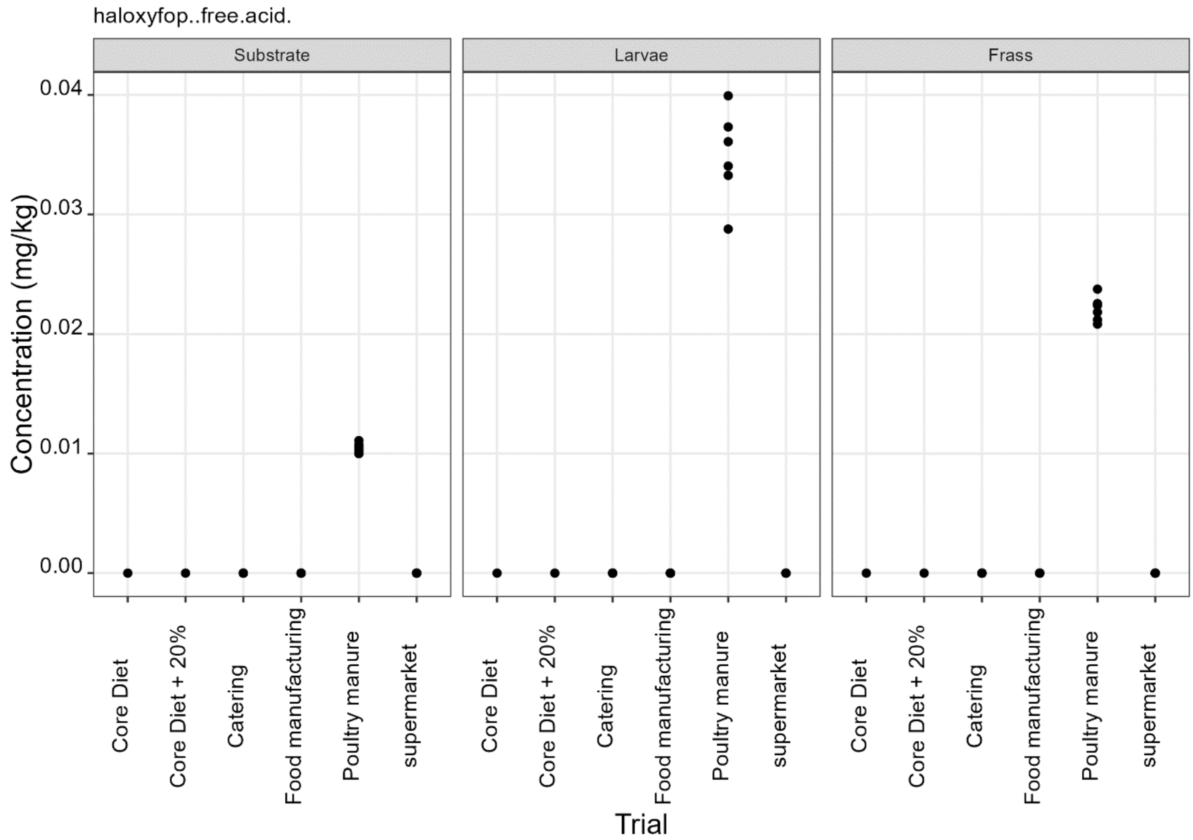
D.


diphenylamine
Substrate
Larvae
Frass
Concentration (mg/kg)
0.08
0.06
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

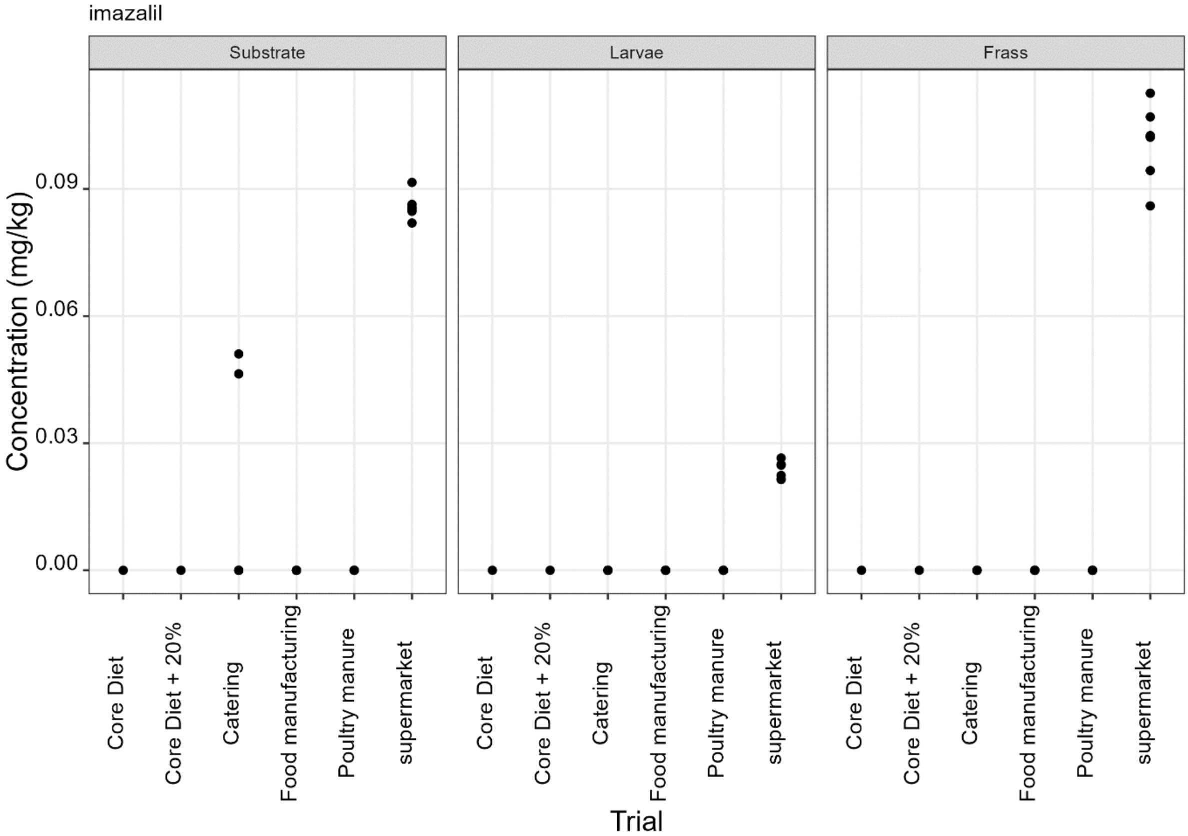
E.


fludioxonil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.9
0.6
0.3
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

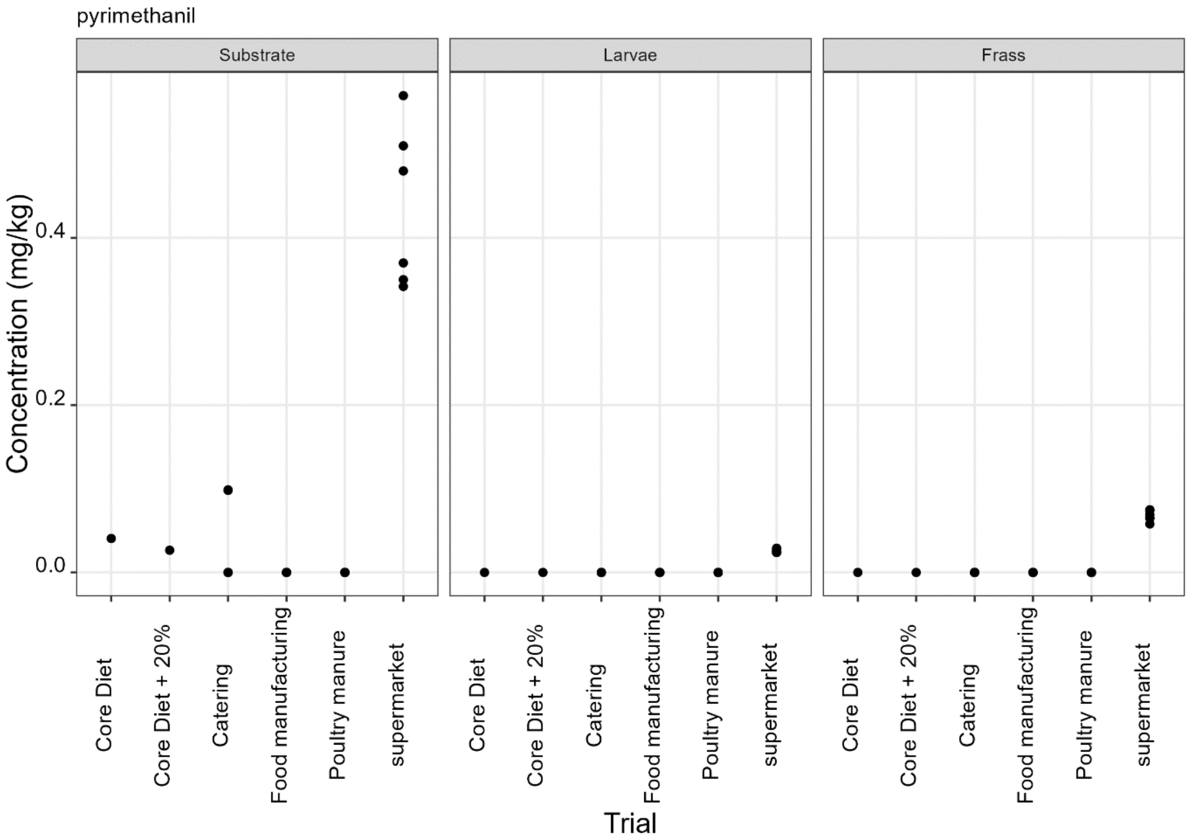
F.


haloxyfop..free.acid.
Substrate
Larvae
Frass
Concentration (mg/kg)
0.04
0.03
0.02
0.01
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

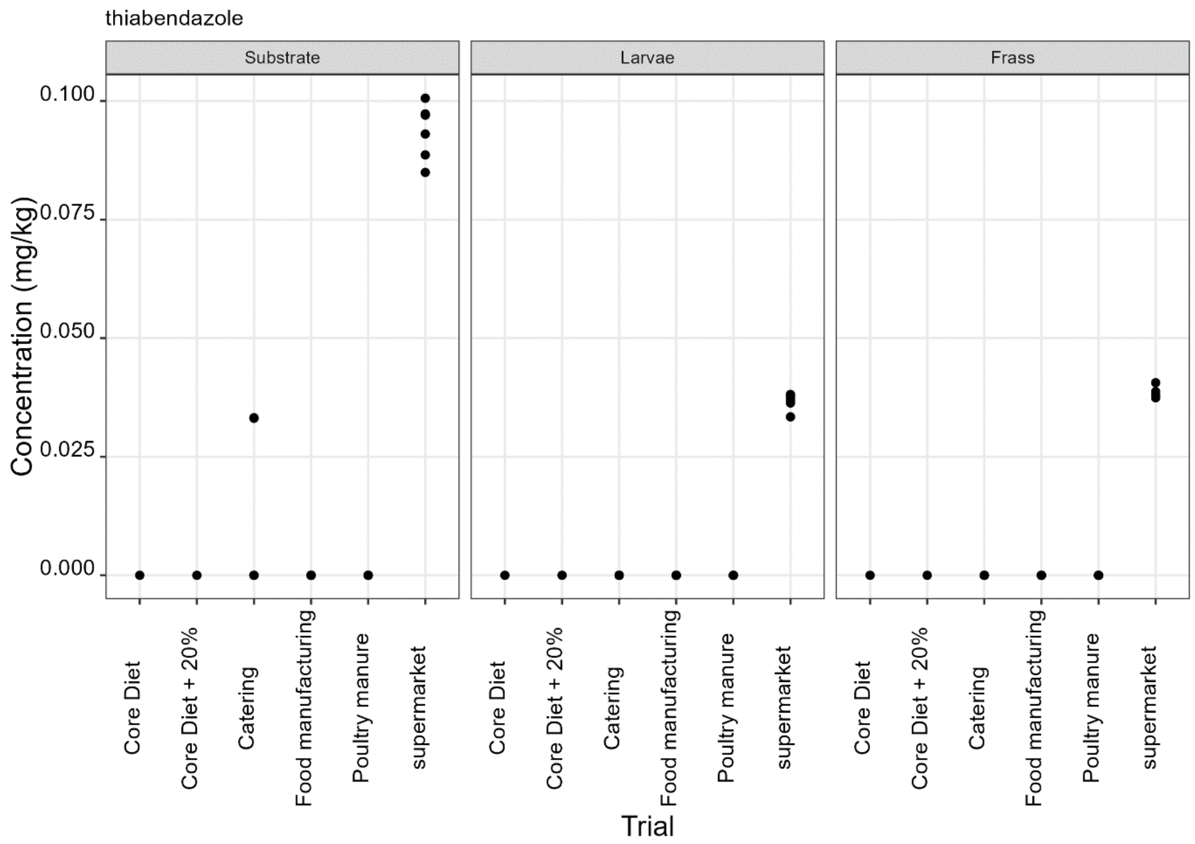
G.


imazalil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.00
0.03
0.06
0.09
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

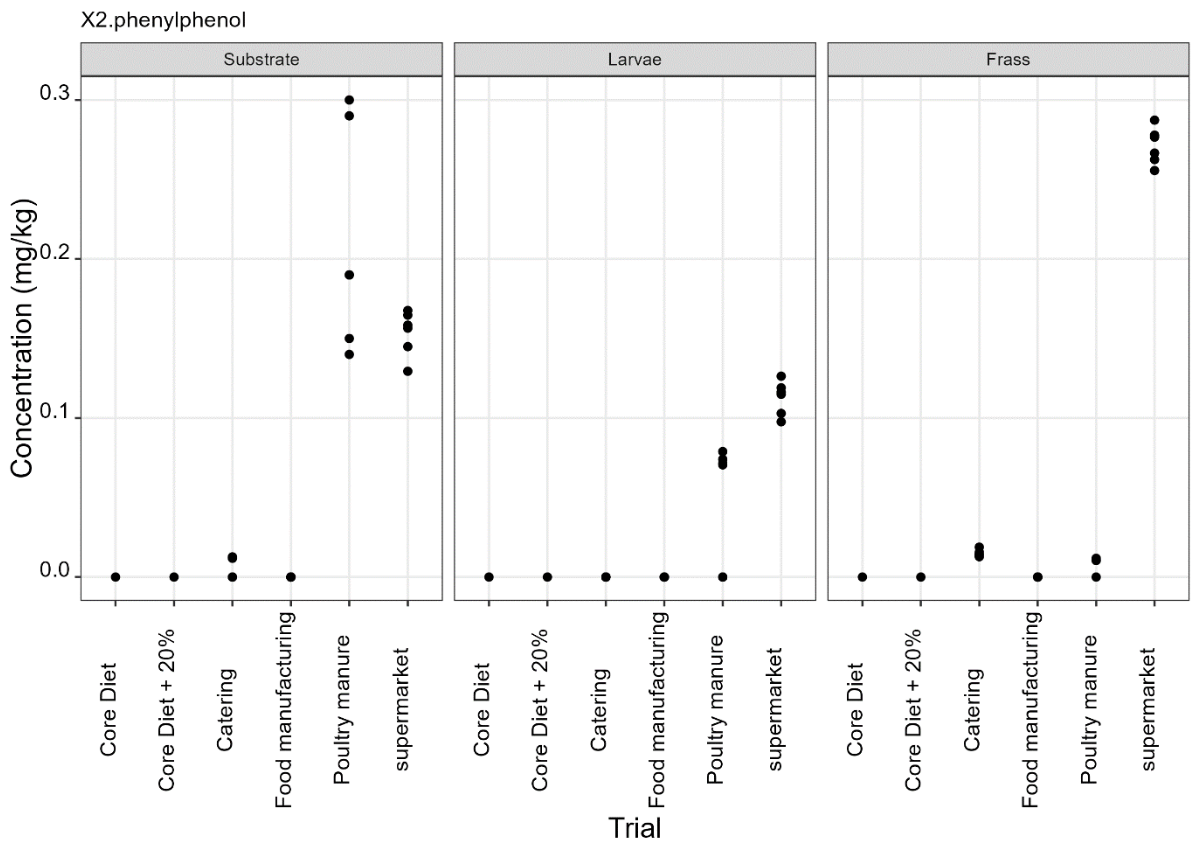
H.


pyrimethanil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.0
0.2
0.4
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

I.


thiabendazole
Substrate
Larvae
Frass
Concentration (mg/kg)
0.100
0.075
0.050
0.025
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

J.


X2.phenylphenol
Substrate
Larvae
Frass
Concentration (mg/kg)
0.3
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

K.

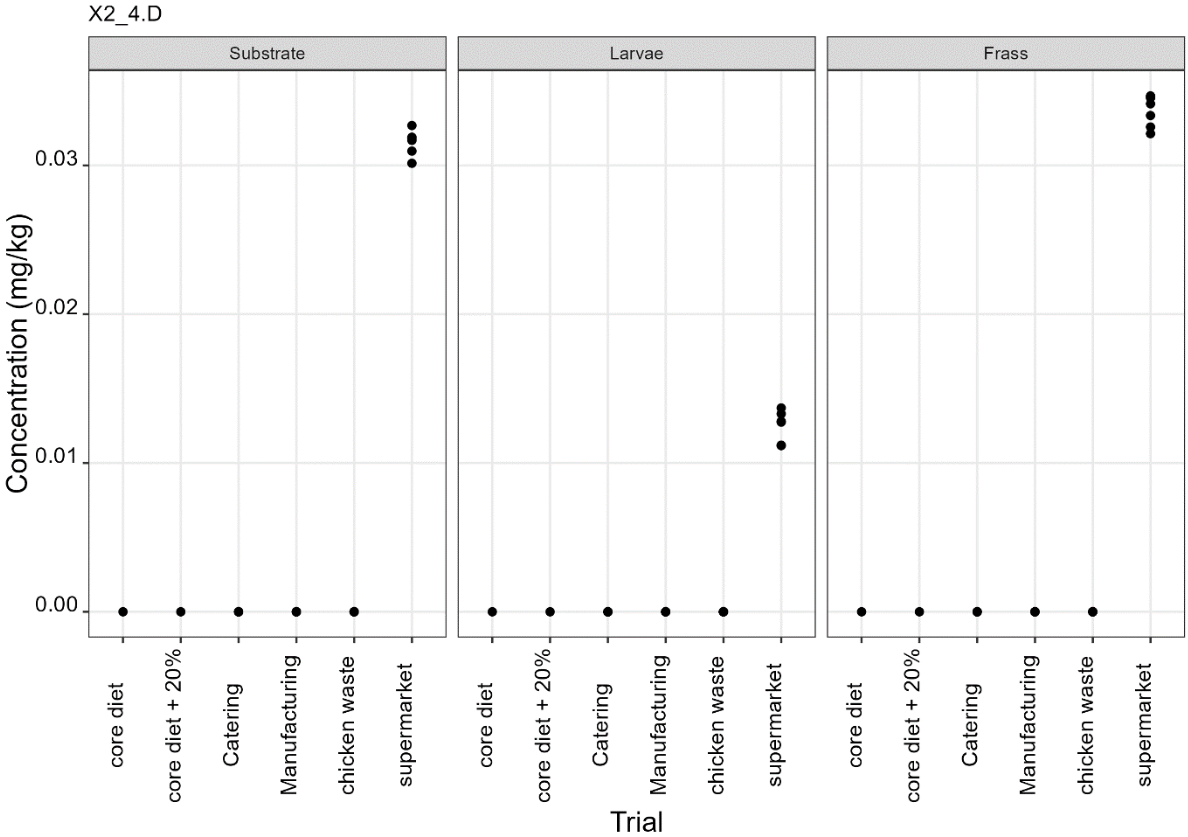


Figure 10 (A–K). Concentrations of pesticides found in different rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections except for core diet and core diet +20% where there was a single injection.

Mycotoxins

The mycotoxin analysis screens for 72 compounds. Results for all compounds are provided in Appendix E. Two compounds were detected in larvae: roquefortine C in larvae reared on the core diet (mean of 5.2 µg/kg) and wortmannin in larvae reared on manufacturing surplus (mean of 86.6 µg/kg) (Figure 11). There are no regulatory limits for these compounds in animal feed.

Roquefortine C is a relatively common mycotoxin produced by a number of *Penicillium* species. It has neurotoxic properties at high concentrations and has been found in levels between 0.05 and 1.47 mg/kg in blue cheeses and at these levels the authors considered that this was not hazardous for consumers (Finoli *et al*., 2001).

Wortmannin is a metabolite produced by *Fusarium oxysporum*, *Penicillium funiculosum* and *P. wortmannii*. It is an inhibitor of phosphoinositide 3-kinase and can disrupt signalling pathways and has high mammalian cytotoxicity.

Both roquefortine C and wortmannin were detected in the larvae but not in the rearing substrate. Although this could be an indication of bioaccumulation, the variation between samples (see later section) could also be a factor in this result. Further studies would be needed to ascertain whether there is any bioaccumulation of these compounds. Generally, it is considered that for the mycotoxins that have been investigated there is no bioaccumulation, but there are a relatively low number of mycotoxins that have been studied (Lievens *et al*., 2021).

A further seven mycotoxins were detected in either the rearing substrate or the frass, but were not detected in the larvae. These were:

- Beauvericin in poultry manure substrate and frass
- Cyclopiazonic acid in catering surplus substrate
- Enniatin A1 in poultry manure substrate
- Enniatin B in core diet + 20%, poultry manure and supermarket surplus substrates and supermarket surplus frass
- Enniatin B1 in core diet + 20%, poultry manure and supermarket surplus substrates and poultry manure and supermarket surplus frass
- Moniliformin in manufacturing and supermarket surplus rearing substrates
- Penicillic acid in supermarket surplus rearing substrate and frass

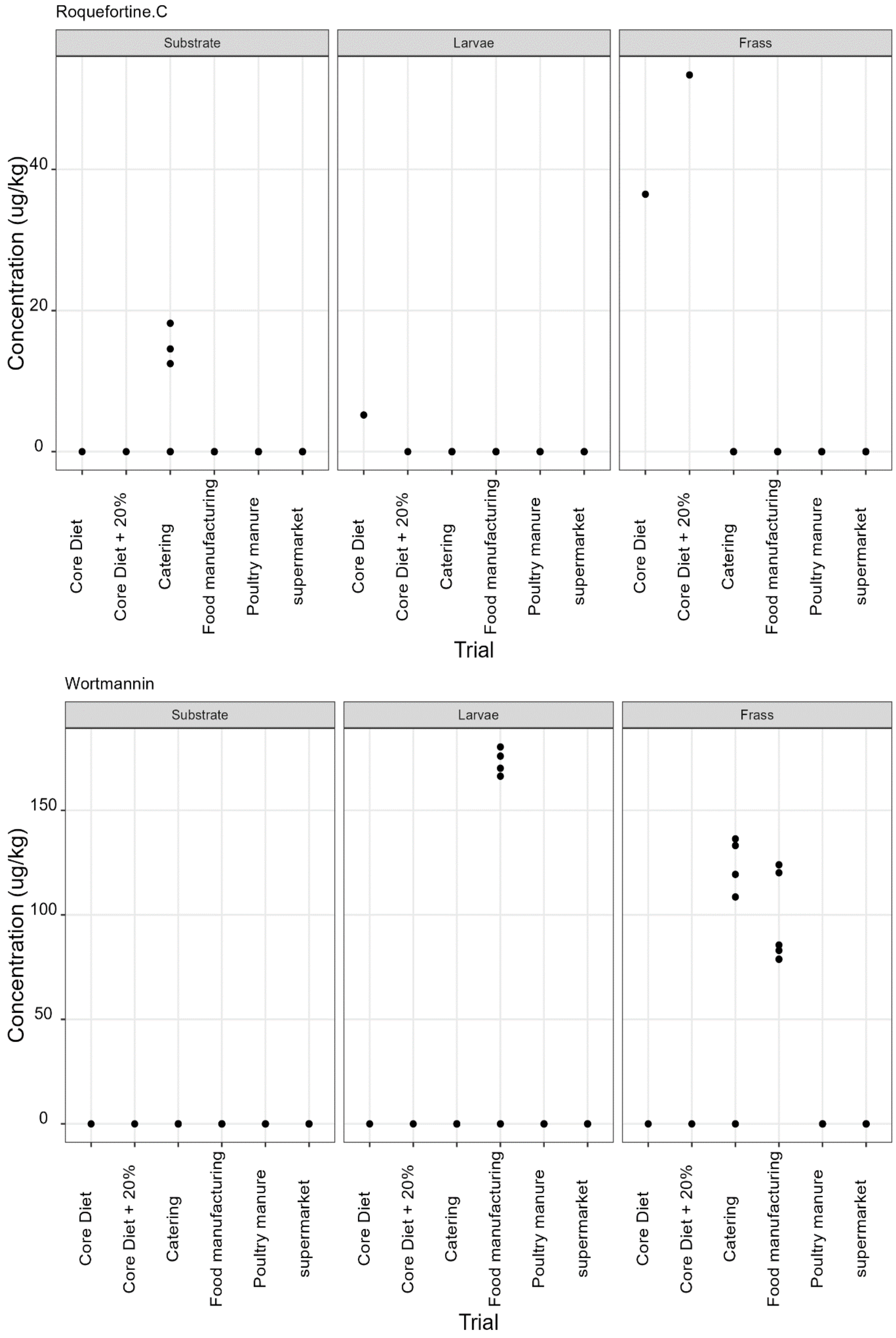


Figure 11. Concentrations of roquefortine C and wortmannin found in different rearing substrates, the larvae reared on the rearing substrates and the resultant

frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections except for core diet and core diet +20% where there was a single injection.

PAHs

PAHs can be found in food that has been smoked or cooked using grilling, frying or charbroiling. On the basis of likely introduction of PAHs to the rearing substrates used, the presence of PAHs was therefore only assessed for the catering waste. Of the 30 compounds included in the analysis eight PAH compounds were found in larvae in at least one sample (Figure 12). These compounds were also detected in the rearing substrate and the frass. Full results are provided in Appendix F.

The most important PAHs are considered to be Benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene and collectively their sum is known as PAH4. The PAH4 sum lower and upper for the rearing substrate, larvae and frass are shown in Tables 4 - 6.

Table 4. PAH4 sum lower and upper (µg/kg) for samples of rearing substrate.

Sample description	Rearing substrate A	Rearing substrate B1	Rearing substrate B2
Compound	µg/kg	µg/kg	µg/kg
PAH4 SUM Lower	0.62	0.56	0.55
PAH4 SUM Upper	0.62	0.56	0.55

PAH4 made up of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (BaP).

Table 5. PAH4 sum lower and upper (µg/kg) for samples of larvae.

Sample description	Larvae A	Larvae B1	Larvae B2
Compound	µg/kg	µg/kg	µg/kg
PAH4 SUM Lower	0	0	0
PAH4 SUM Upper	0.28	0.23	1.01

PAH4 made up of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (BaP).

Table 6. PAH4 sum lower and upper (µg/kg) for samples of frass.

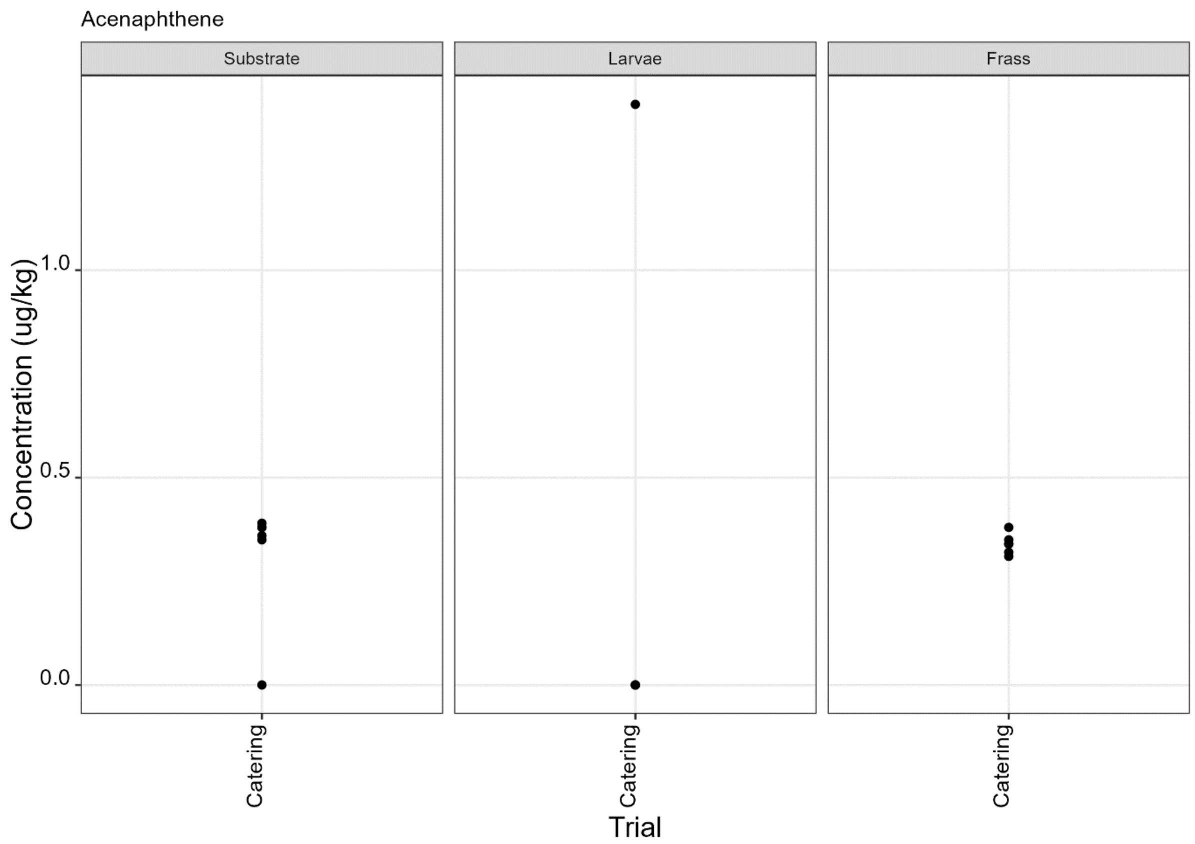
Sample description	Frass A	Frass B1	Frass B2
Compound	µg/kg	µg/kg	µg/kg
PAH4 SUM Lower	0.66	0.66	0.61
PAH4 SUM Upper	0.66	0.66	0.61

PAH4 made up of benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene (BaP).

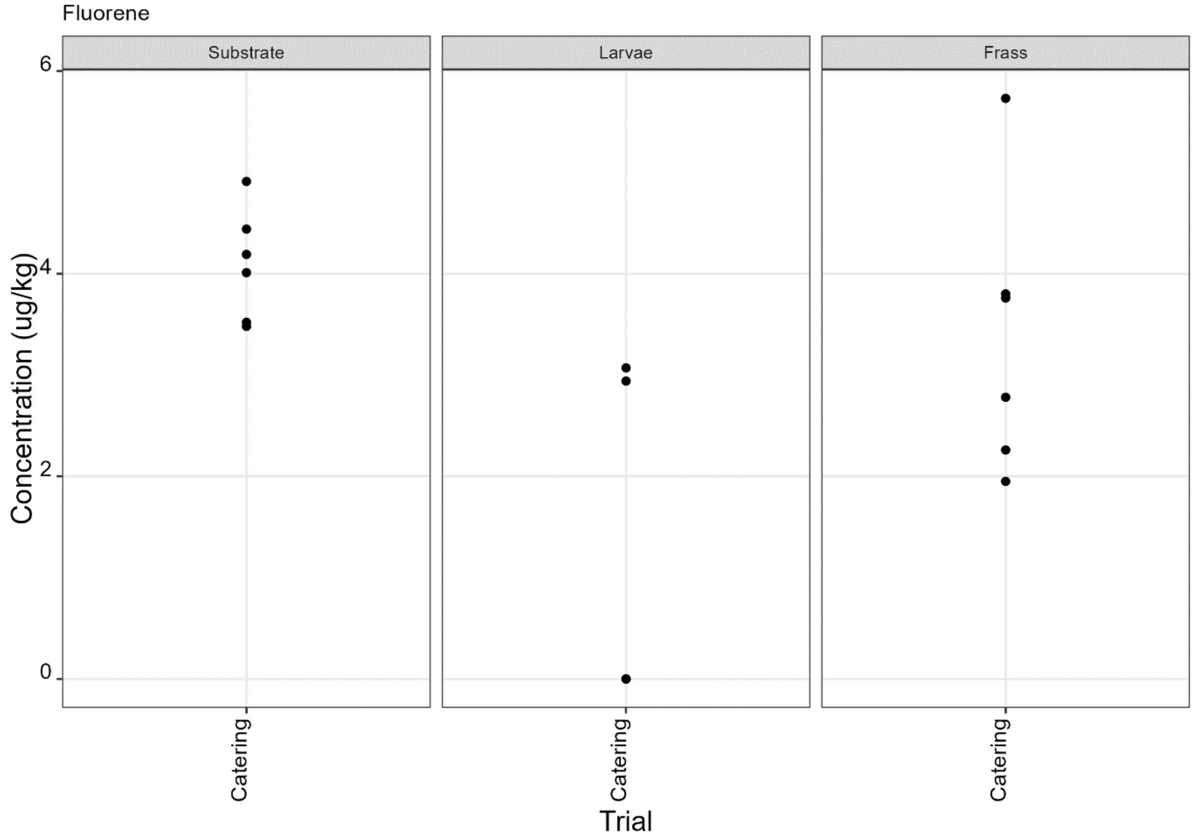
There are no maximum limits specified for PAHs in animal feed. There are limits for the PAH4 sum concentration in some foods, for example, baby food has a limit of < 1 µg/kg (Retained EU regulation No. 835/2011).

There was high variation between samples for some compounds and therefore the likelihood of bioaccumulation for some of these compounds was difficult to assess.

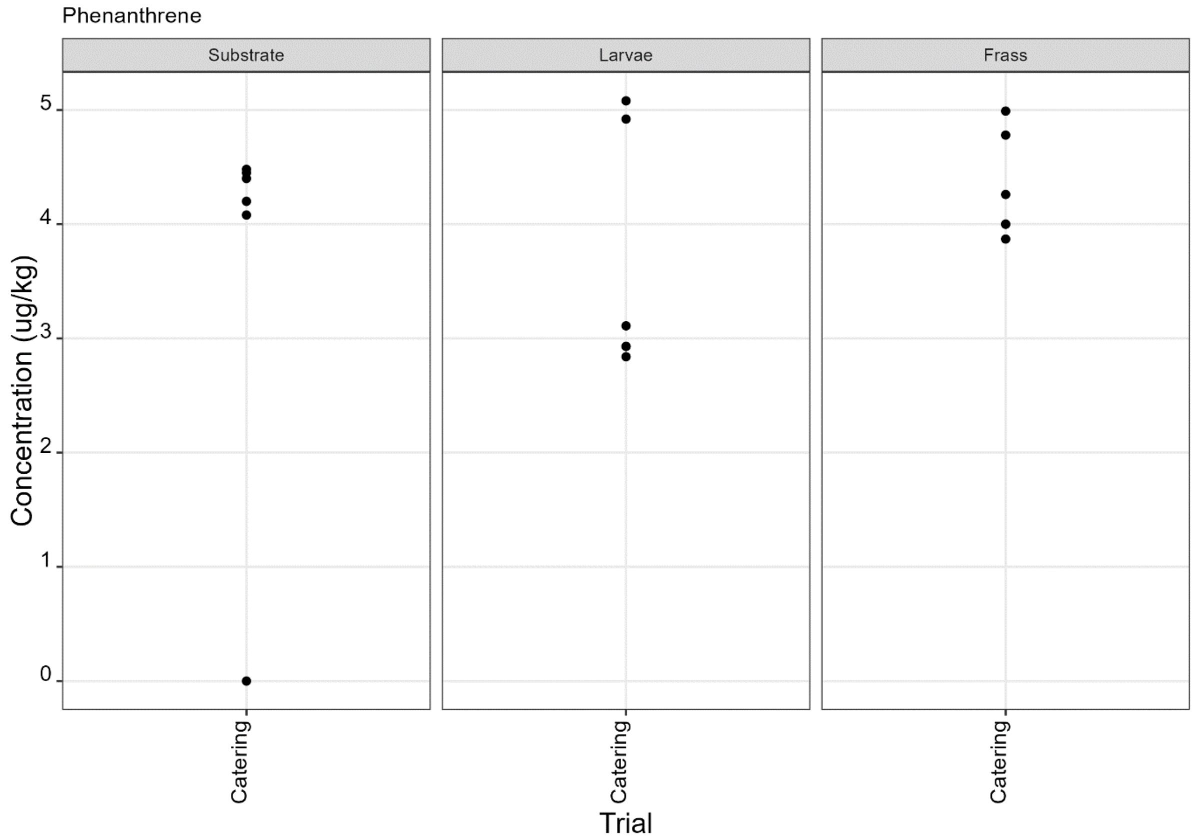
A.



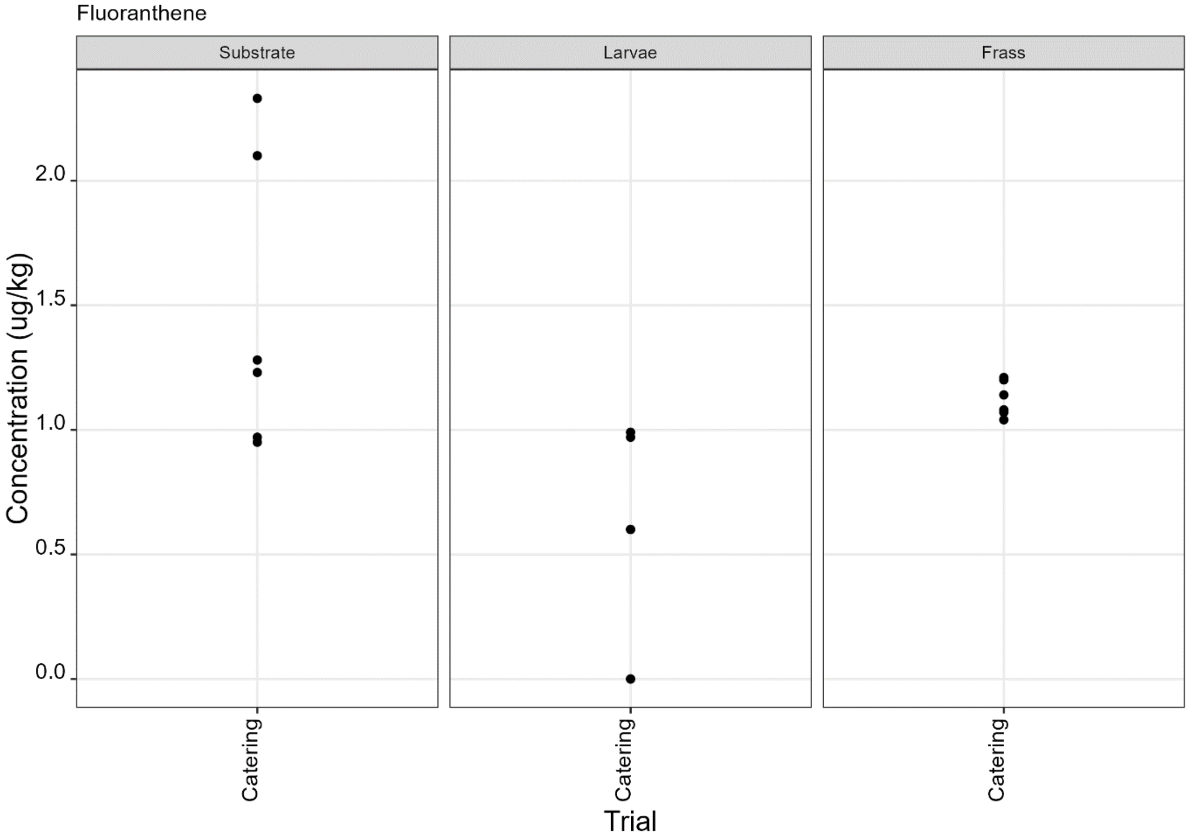
B.


Fluorene
Substrate
Larvae
Frass
6
4
2
0
Concentration (ug/kg)
Catering
Catering
Catering
Trial

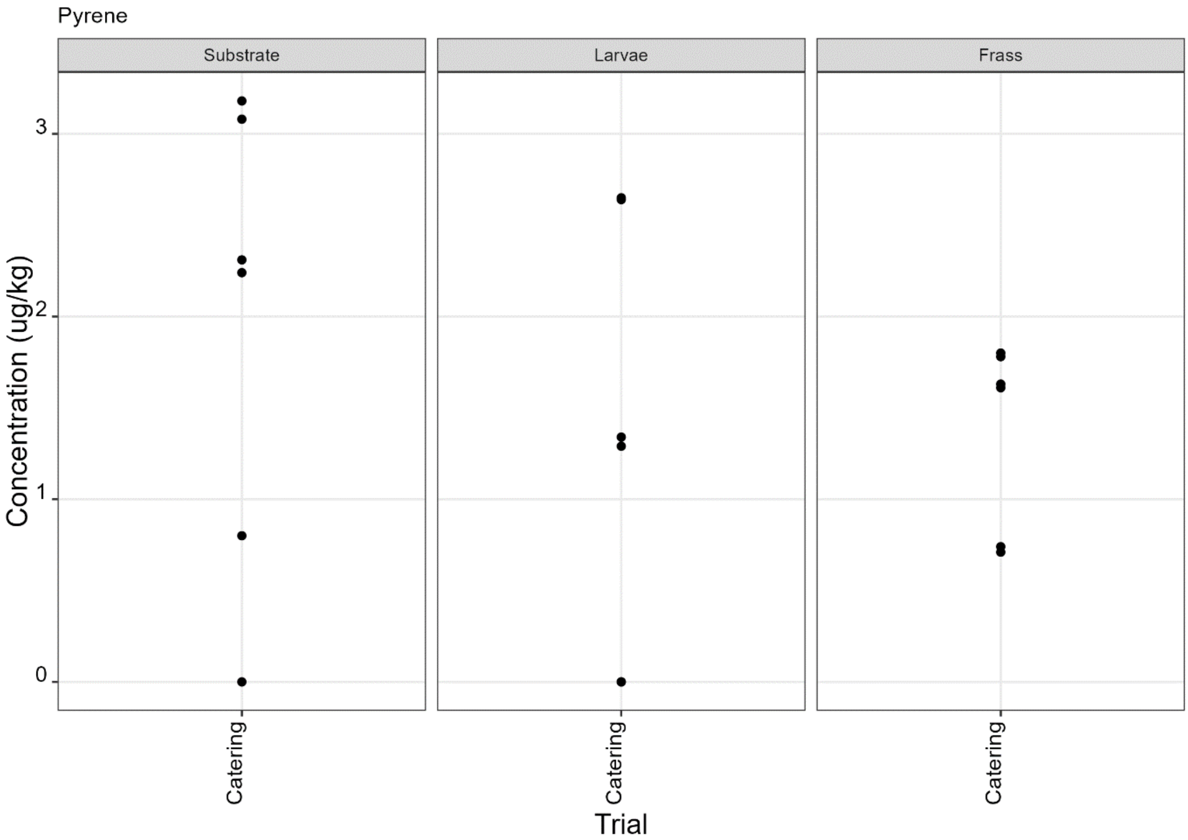
C.


Phenanthrene
Substrate
Larvae
Frass
5
4
3
2
1
0
Concentration (ug/kg)
Catering
Catering
Catering
Trial

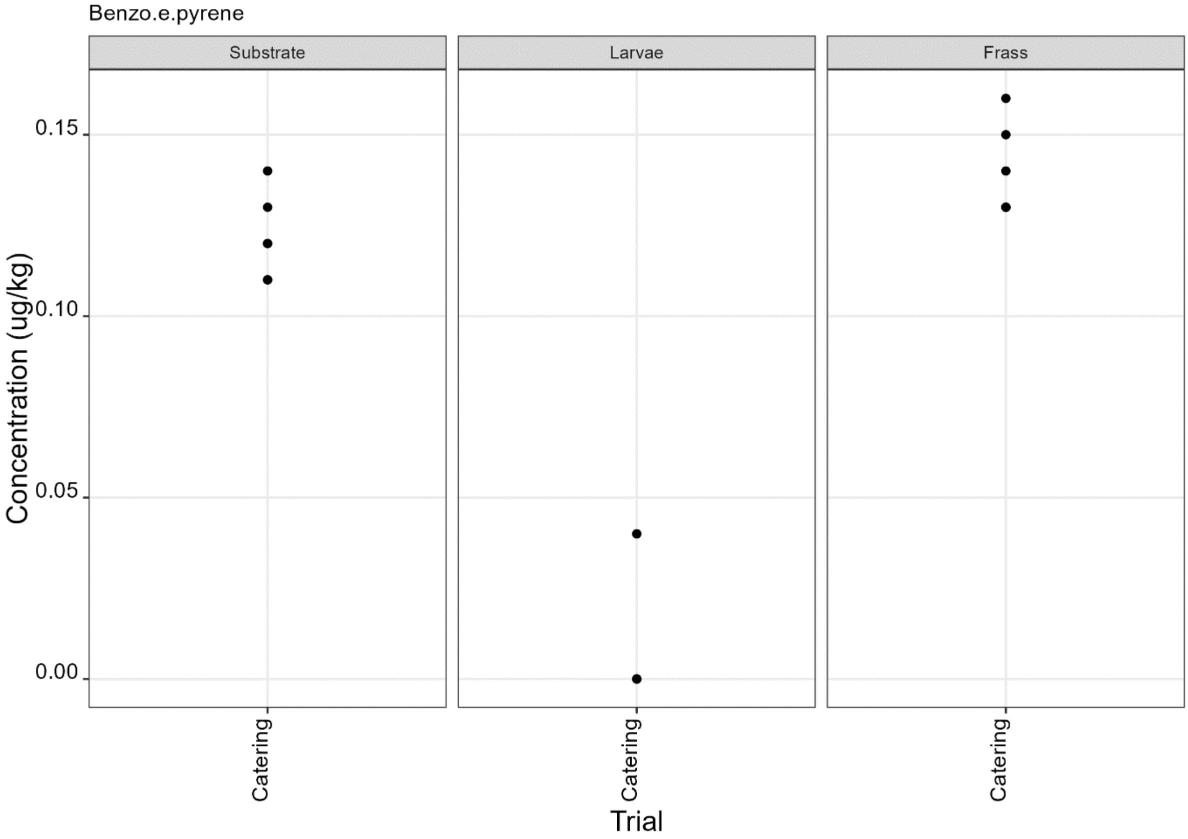
D.


Fluoranthene
Substrate
Larvae
Frass
Concentration (ug/kg)
2.0
1.5
1.0
0.5
0.0
Catering
Catering
Catering
Trial

E.

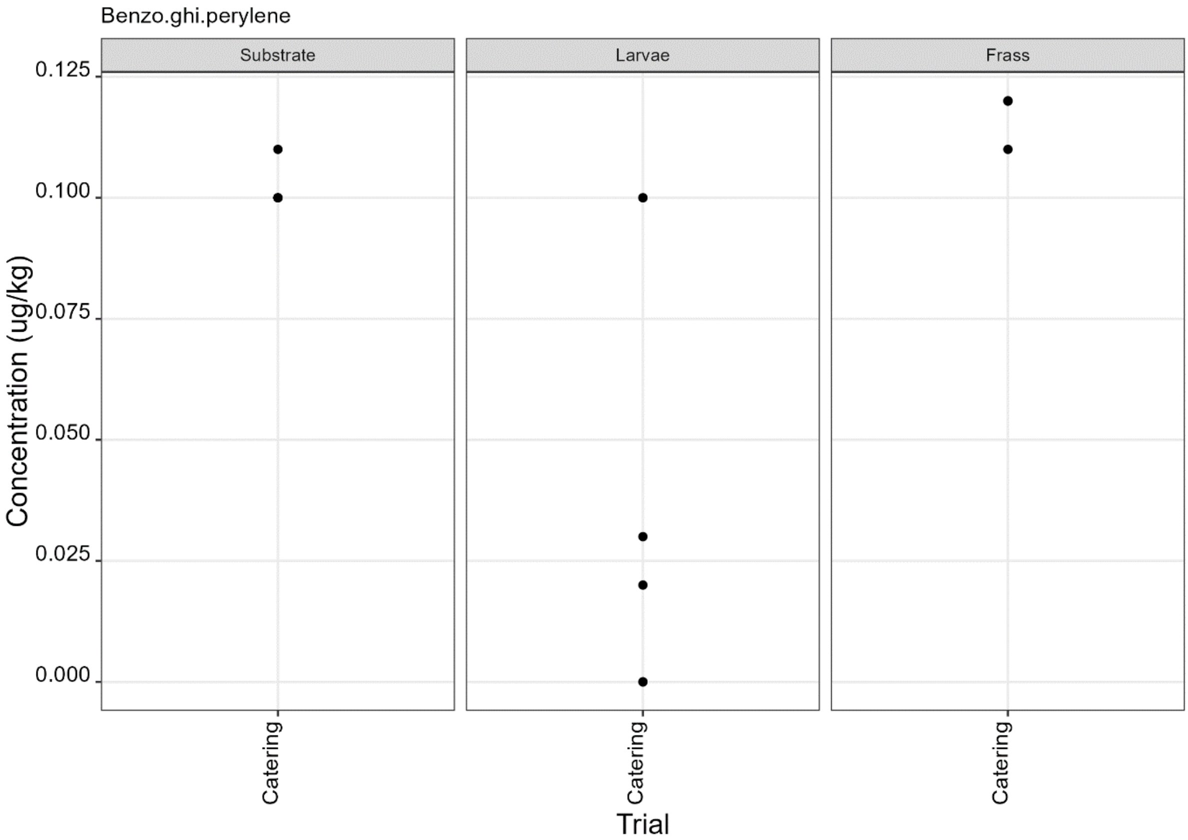

Pyrene
Substrate
Larvae
Frass
Concentration (ug/kg)
3
2
1
0
Catering
Catering
Catering
Trial

F.



Benzo.e.pyrene
Substrate
Larvae
Frass
0.15
0.10
0.05
0.00
Concentration (ug/kg)
Catering
Catering
Catering
Trial

G.



Benzo.ghi.perylene
Substrate
Larvae
Frass
0.125
0.100
0.075
0.050
0.025
0.000
Concentration (ug/kg)
Catering
Catering
Catering
Trial

H.

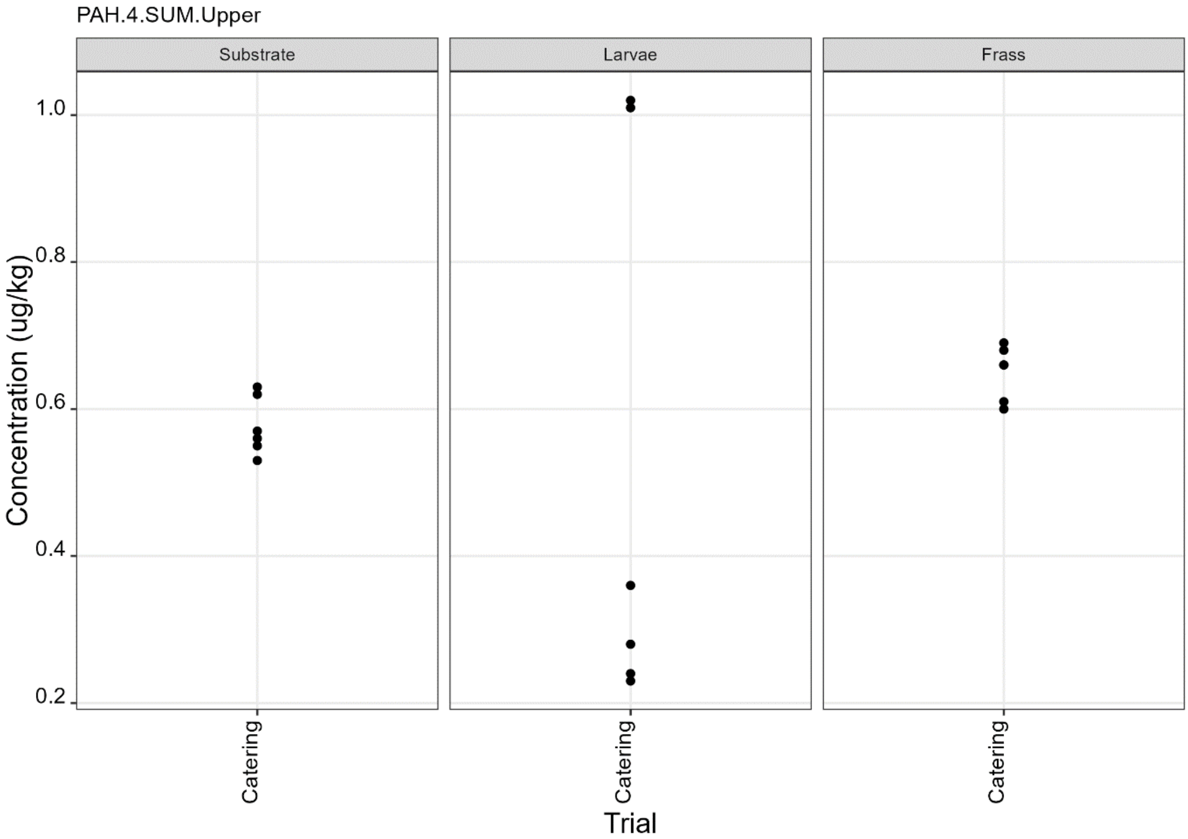
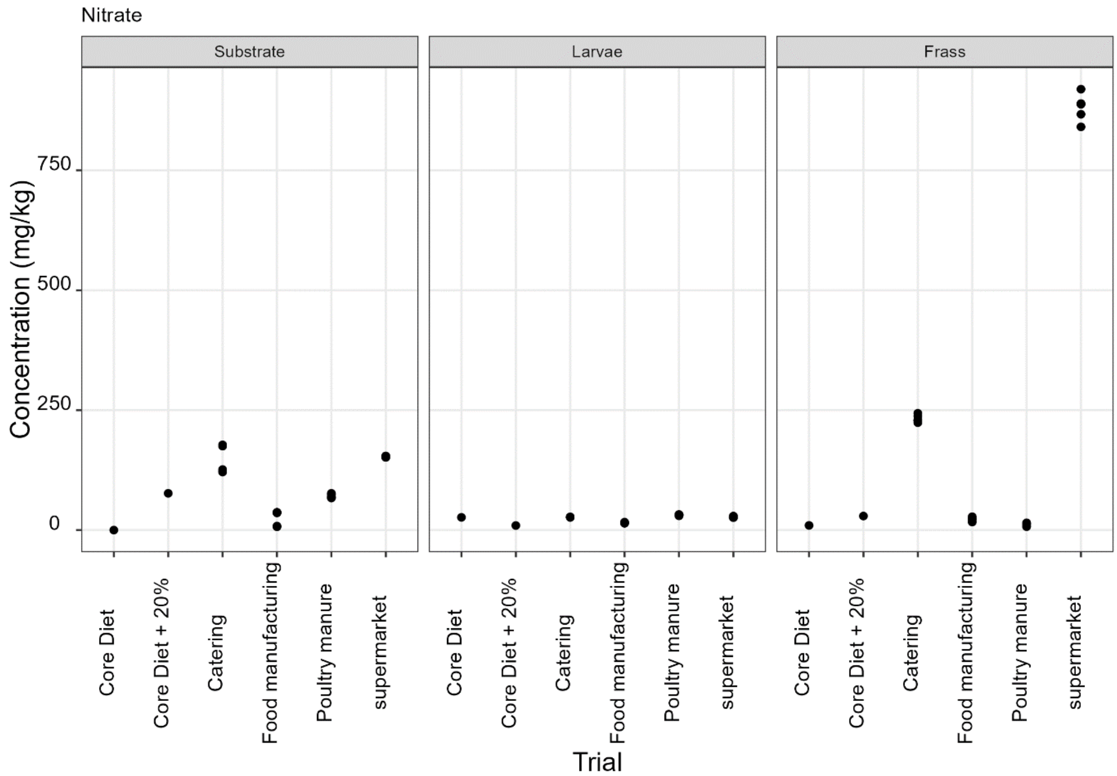


Figure 12 (A–H). Concentrations of PAH compounds found in catering waste, the larvae reared on catering waste and the resultant frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections.

Nitrates and nitrites

The levels of nitrates and nitrites found in the rearing substrates, larvae and frass are shown in Figure 13 and the full results are provided in Appendix G. There is a regulatory limit of 15 mg/kg for nitrite in feed materials (EU Directive 2002/32/EC). This level was not exceeded in the larvae from any of the rearing substrates and there was no evidence of bioaccumulation in the larvae (Appendix G).



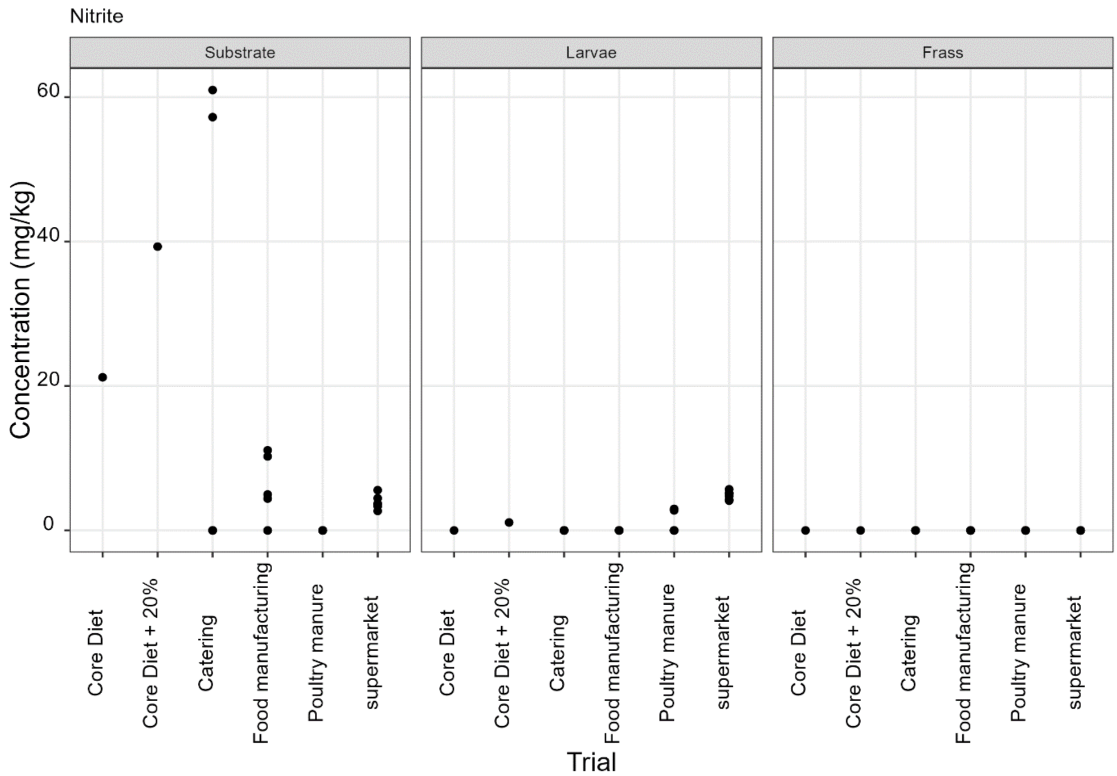


Figure 13. Concentrations of nitrate and nitrite found in rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Circles represent results from three samples (A, B1 and B2) with duplicate injections except for core diet and core diet +20% where there was a single injection.

PFAS

PFAS compounds were detected in the rearing substrates, larvae and frass. It was found that some extracts were very complex resulting in low and inconsistent recoveries for some of the analytes. Chromatographic retention time shift was seen for rearing substrate and sometimes the larvae samples. Three of the analytes (PFDoA, PFTrDA and PFTeDA were not possible to integrate due to retention time shift. Some compounds were found in the frass but not in the rearing substrate or the larvae. This may be due to the complexity of the samples or due to greater homogeneity of the frass compared with the rearing substrate due to the activity of the larvae.

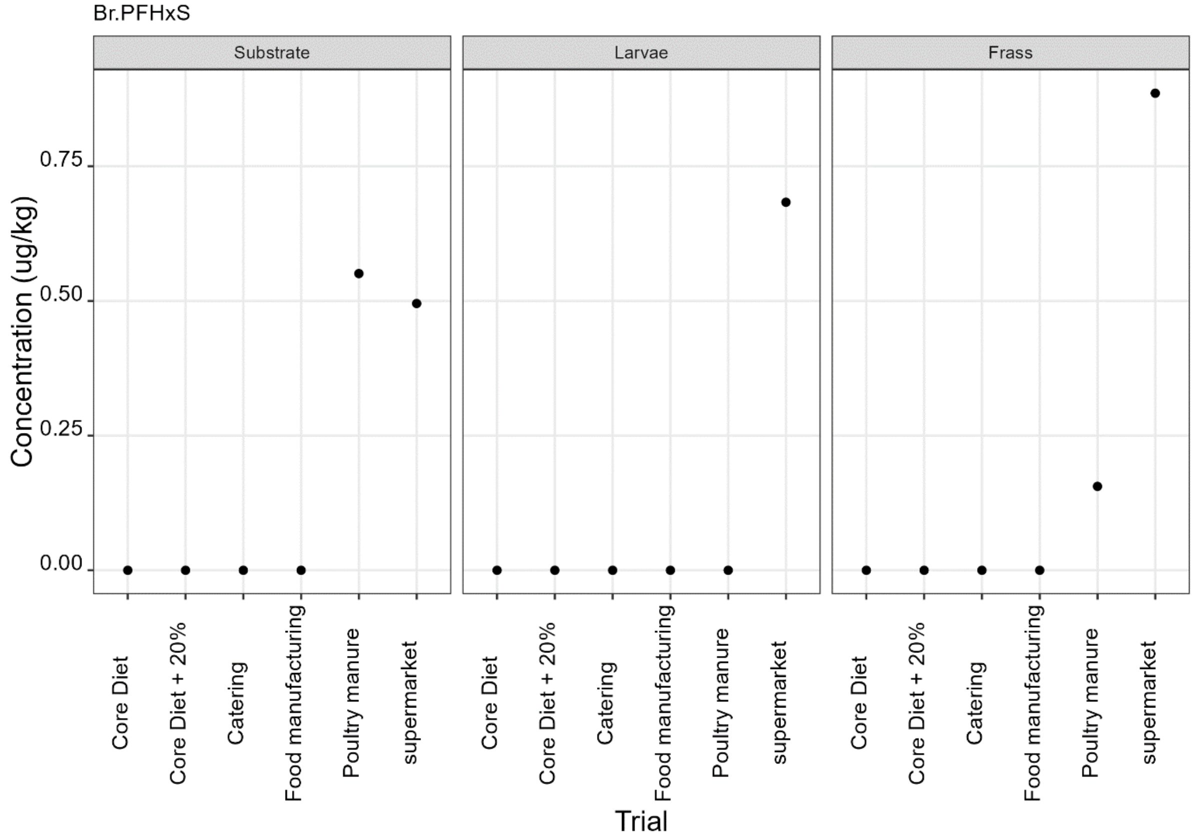
Compounds found in larvae are shown below (Figure 14) and the full results are provided in Appendix H.

Currently there are no regulatory limits for PFAS in animal feed but in the EU Commission Regulation 2023/915 specifies maximum limits for PFAS in animal products. This regulation sets maximum limits for PFOS, PFOA, PFNA and PFHxS individually and for their sum in meat, fish products, crustaceans and bivalve molluscs and eggs. fishery products, meat and eggs. These levels range from 0.2 to 50 µg/kg depending on the food and the compound. In this study the highest level of any PFAS compound found in larvae was for PFOA in larvae reared on poultry manure with a mean of 7.4 µg/kg. All other compounds had levels in larvae of below 0.7 µg/kg.

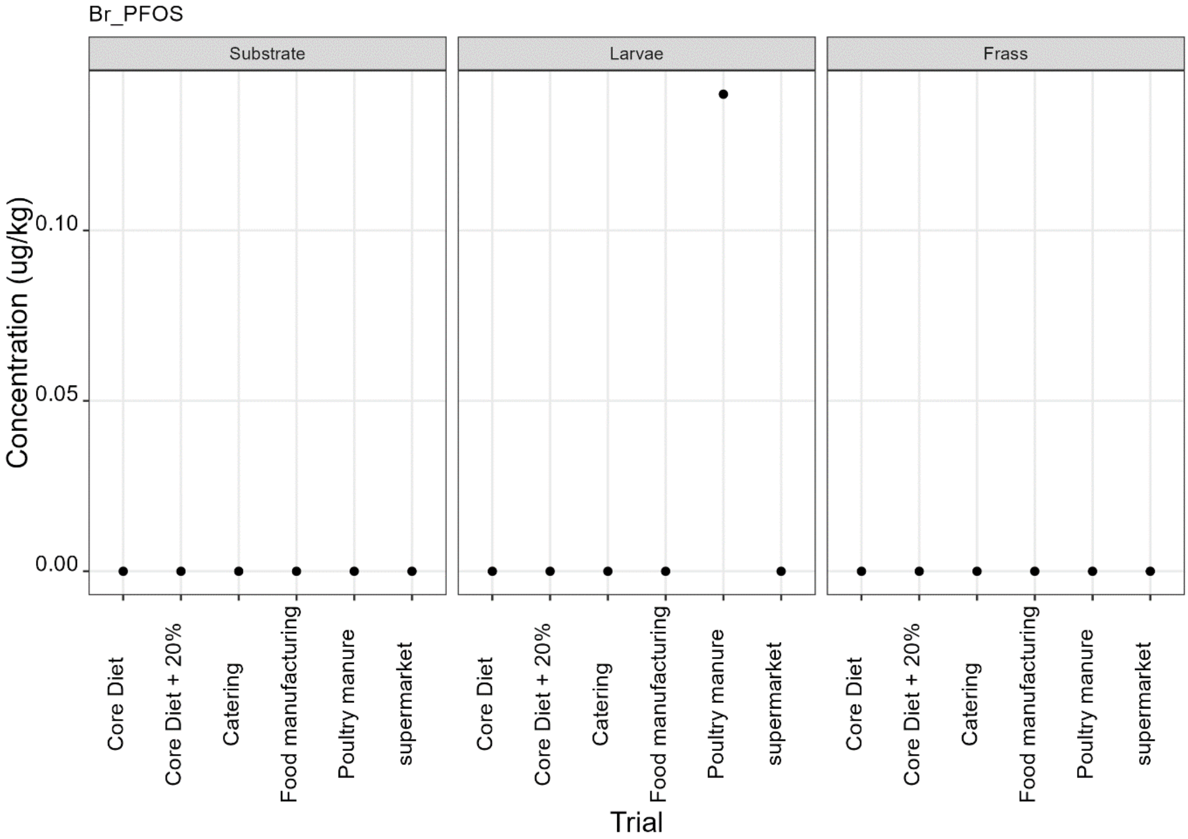
In this study, only a single replicate was analysed for each substrate and source and this should be considered when looking at the data. However, for some compounds levels found in the larvae were slightly higher than in the substrate e.g. HFPO.DA in larvae from manufacturing waste (substrate 0.14 µg/kg; larvae 0.20 µg/kg) and BrPHFX in larvae from supermarket surplus (substrate 0.49; larvae 0.68) (Appendix H).

Li and Bischel (2022) reported that PFBA, PFOA, PFBS and PFOS did not bioaccumulate in BSF larvae. Their study used almond hulls spiked with PFBA, PFOA, PFBS, and L-PFOS. BSF larvae were added to the spiked feed when 5-days-old and were reared on the spiked substrate for 14 days (Li and Bischel., 2022). Further studies on a wider range of these chemicals are required to ascertain whether bioaccumulation in larvae can occur.

A.

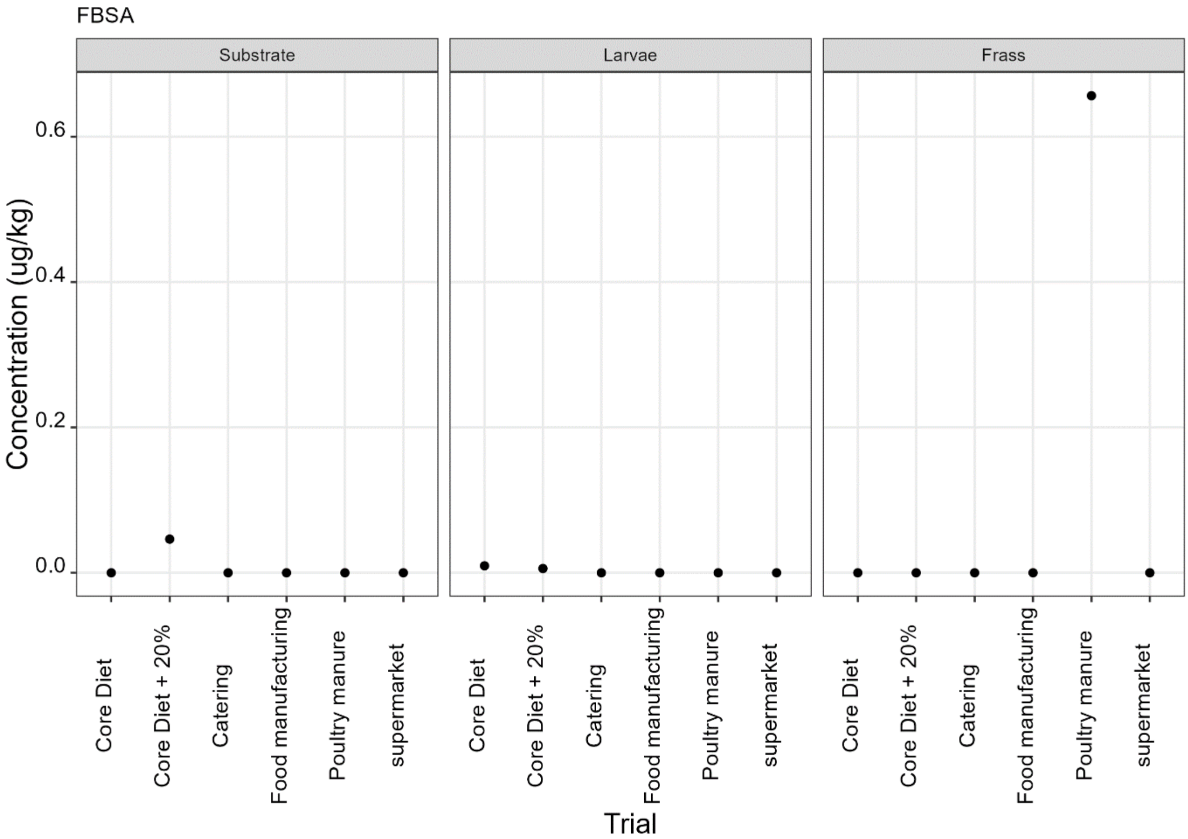


B.



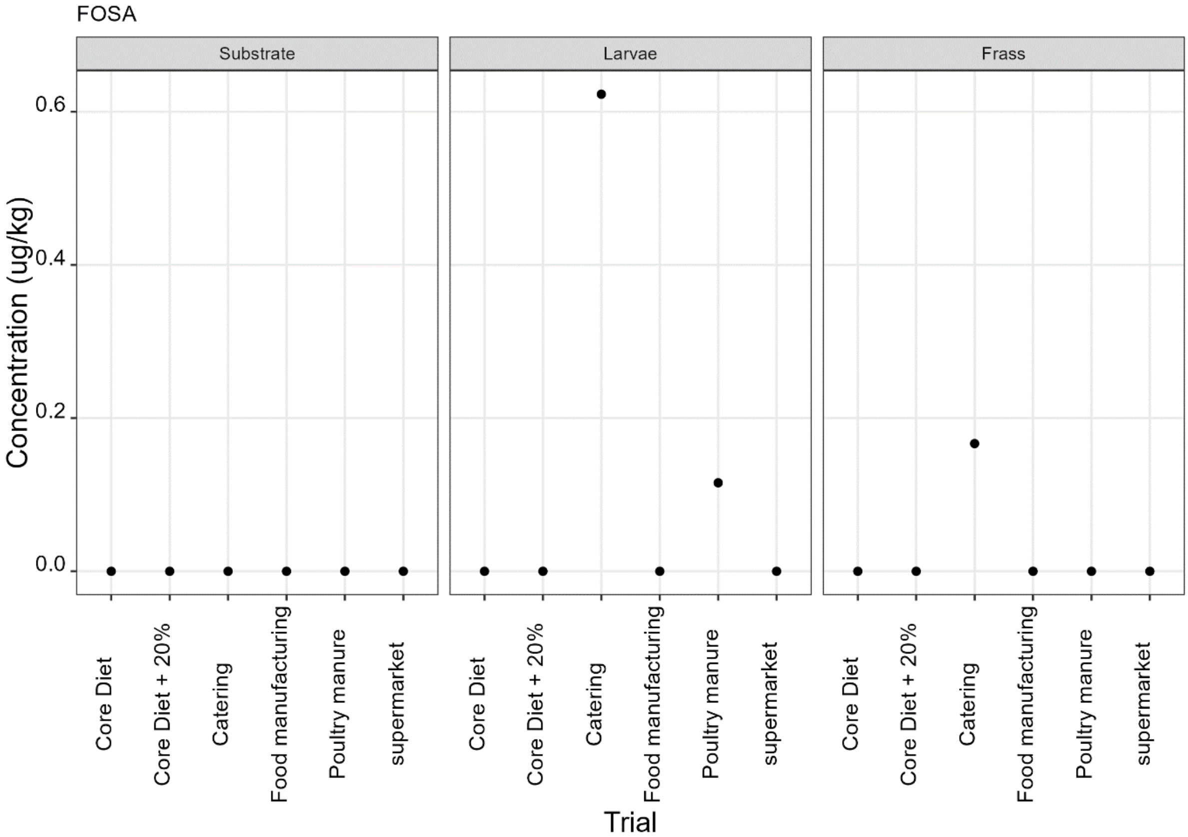
Br_PFOS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

C.

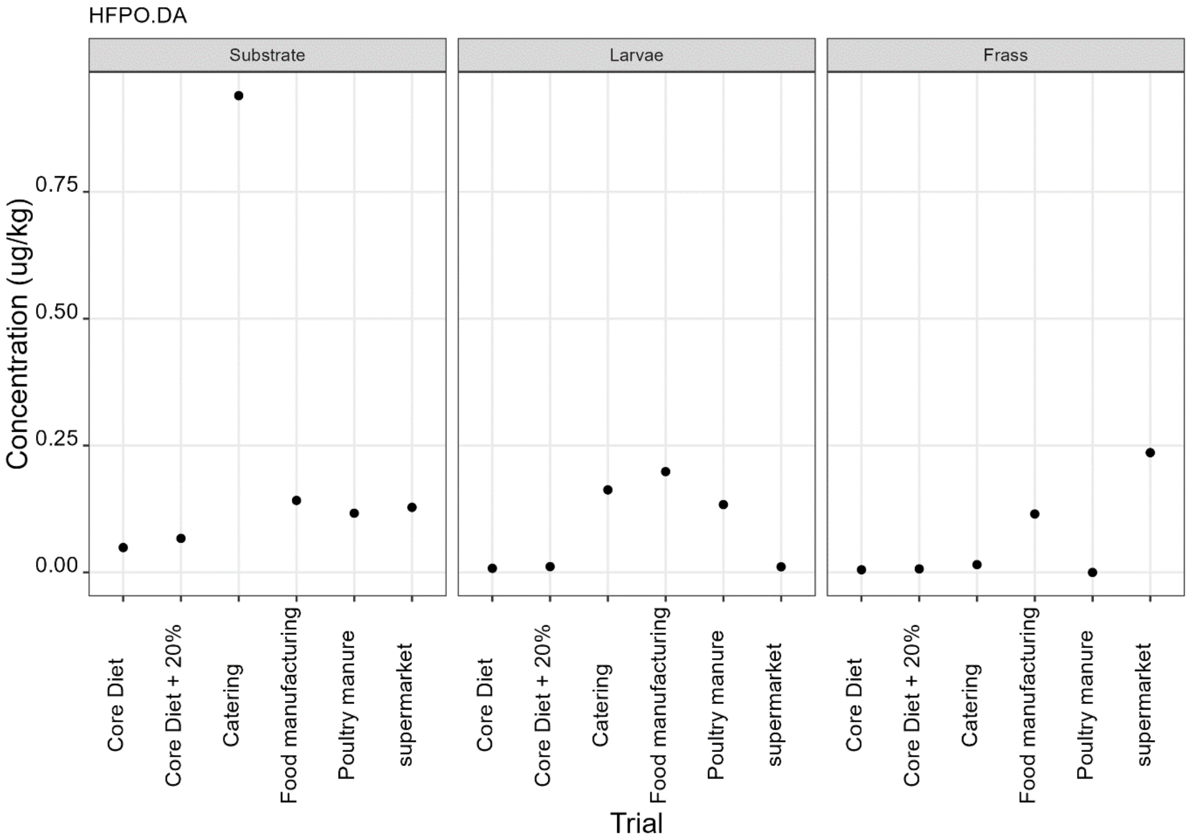


FBSA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

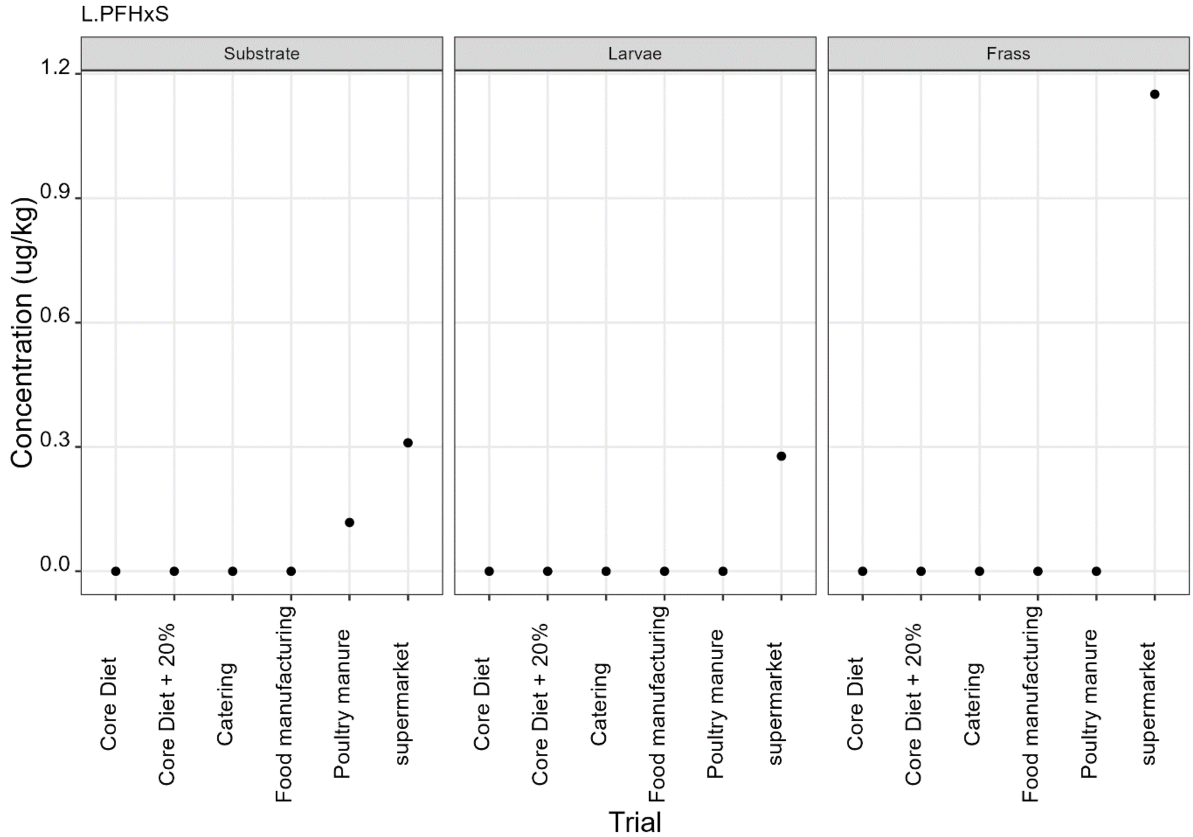
D.


FOSA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

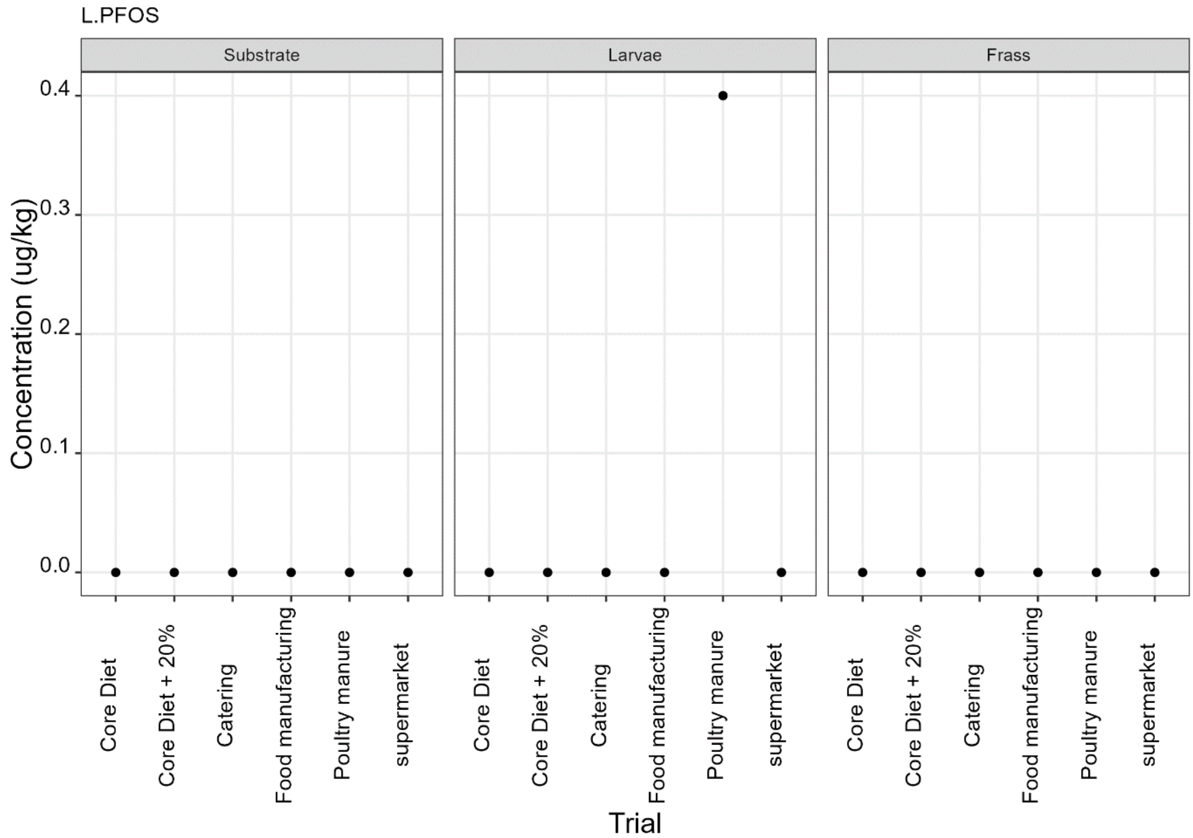
E.


HFPO.DA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.75
0.50
0.25
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

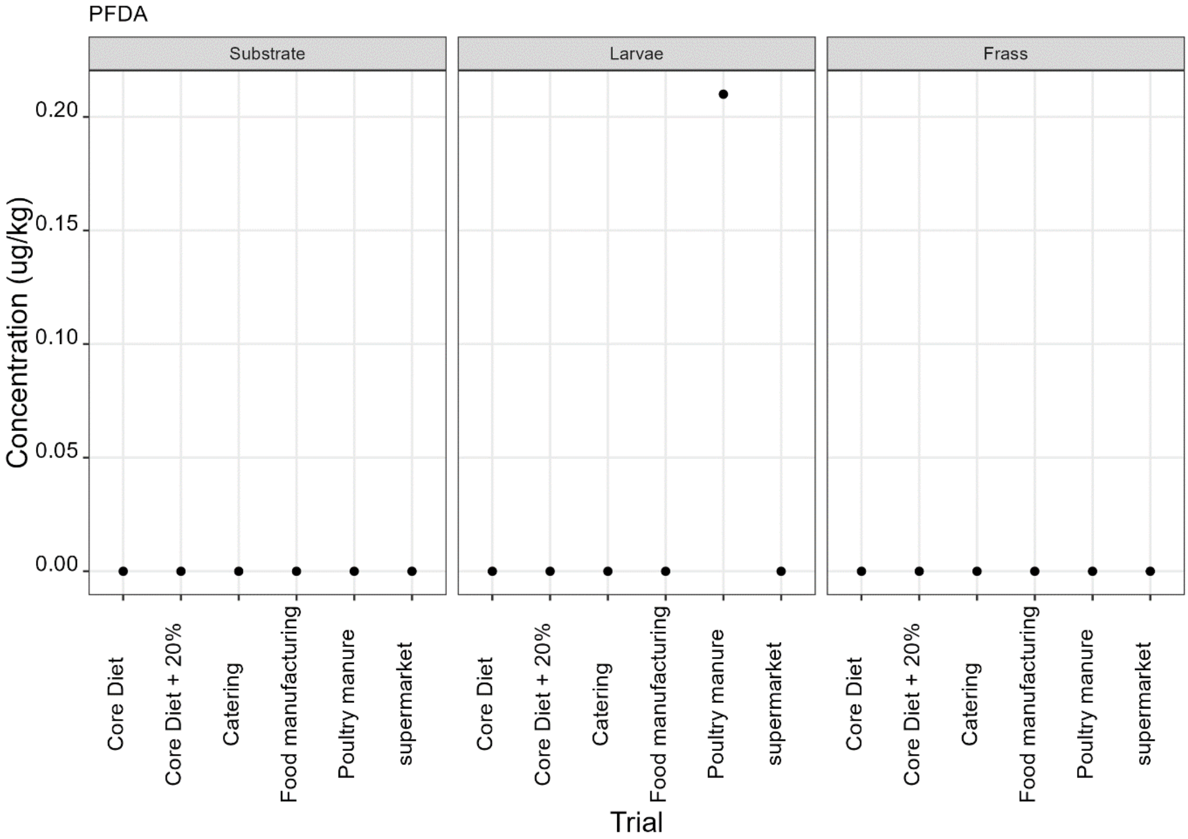
F.


L.PFHxS
Substrate
Larvae
Frass
Concentration (ug/kg)
1.2
0.9
0.6
0.3
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

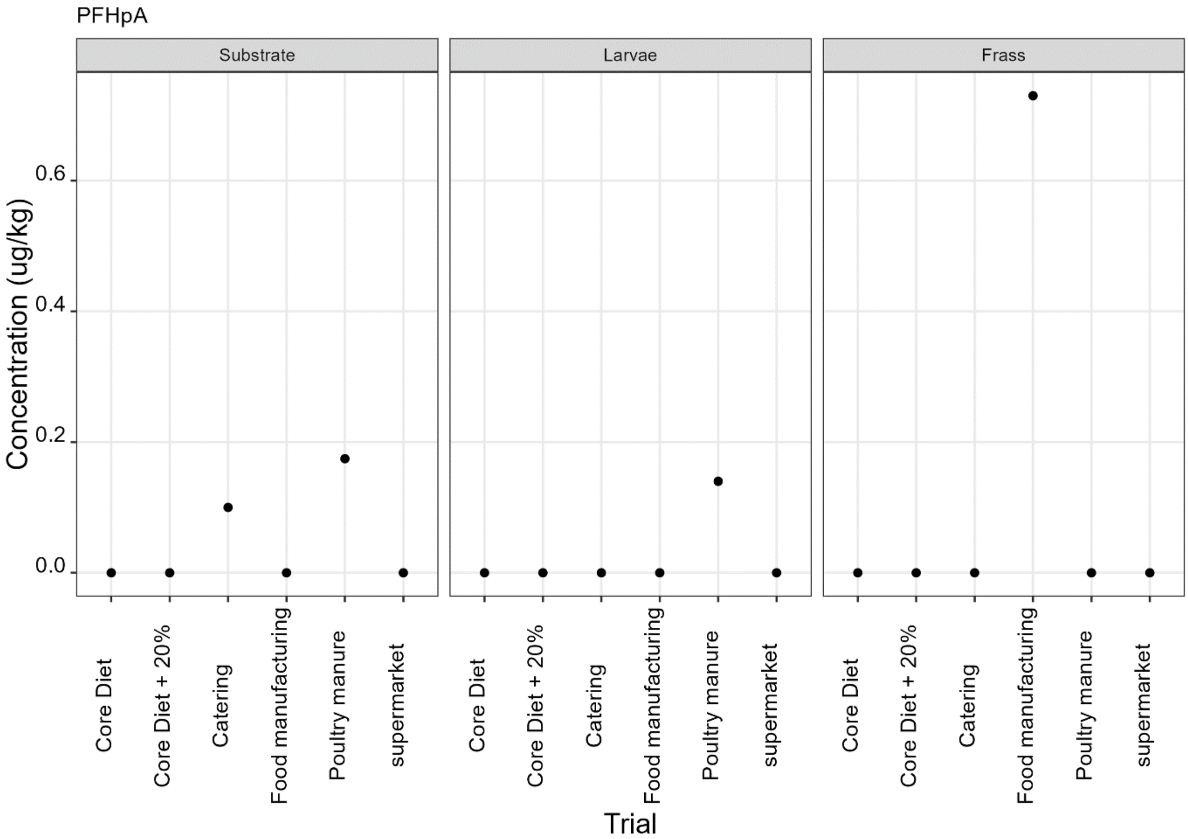
G.


L.PFOS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.4
0.3
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

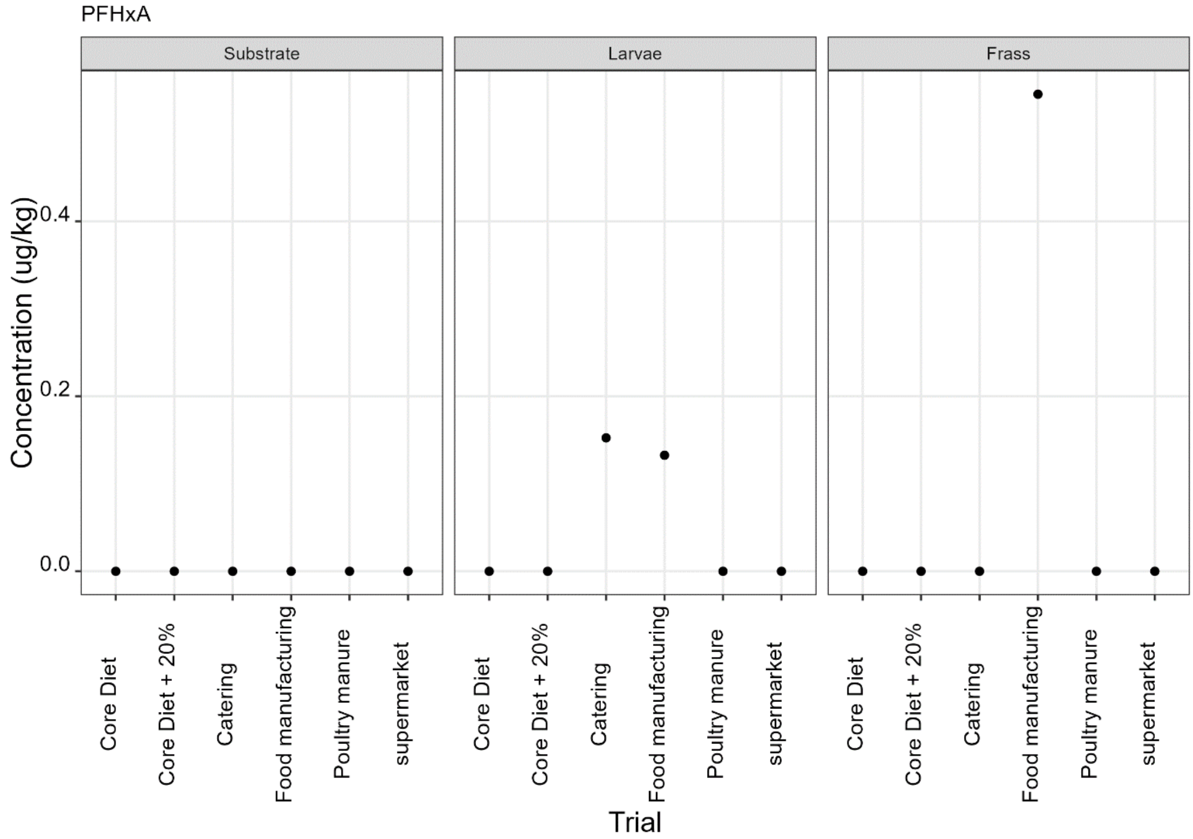
H.


PFDA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.20
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

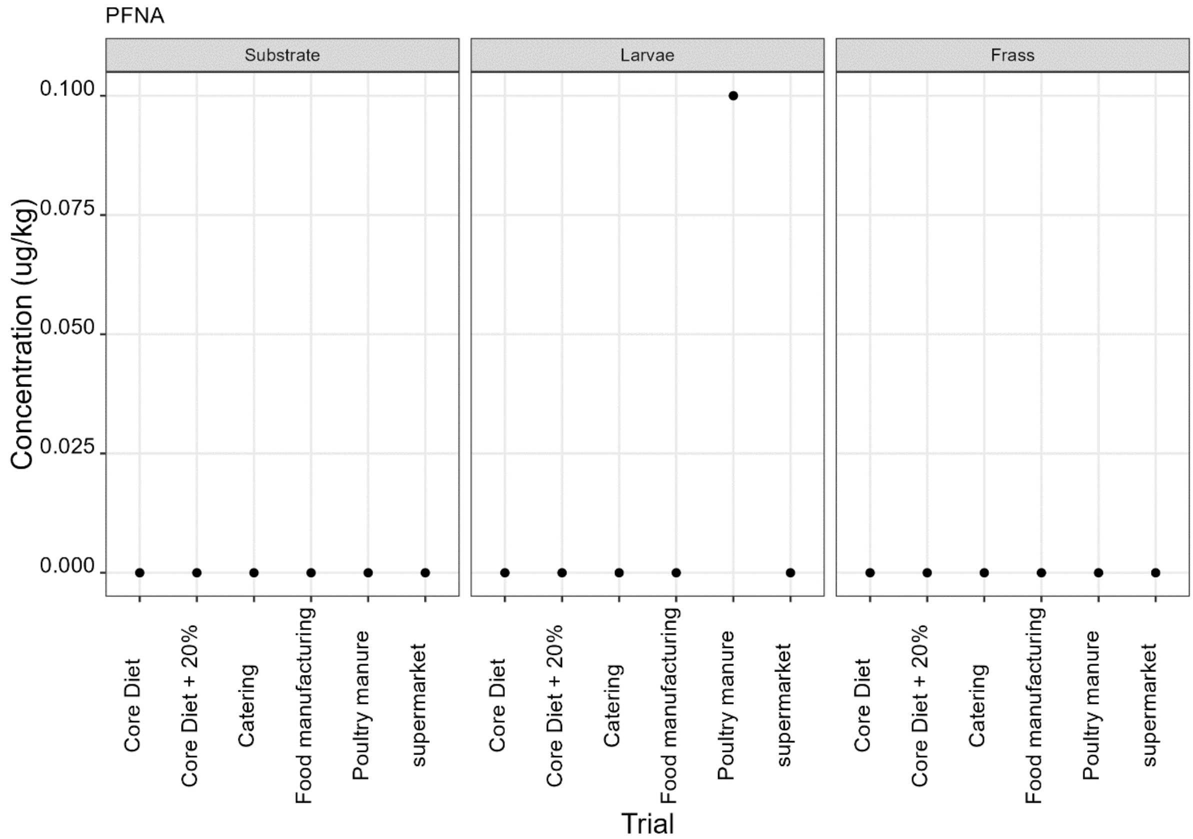
I.


PFHpA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

J.


PFHxA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

K.


PFNA
Substrate
Larvae
Frass
Concentration (ug/kg)
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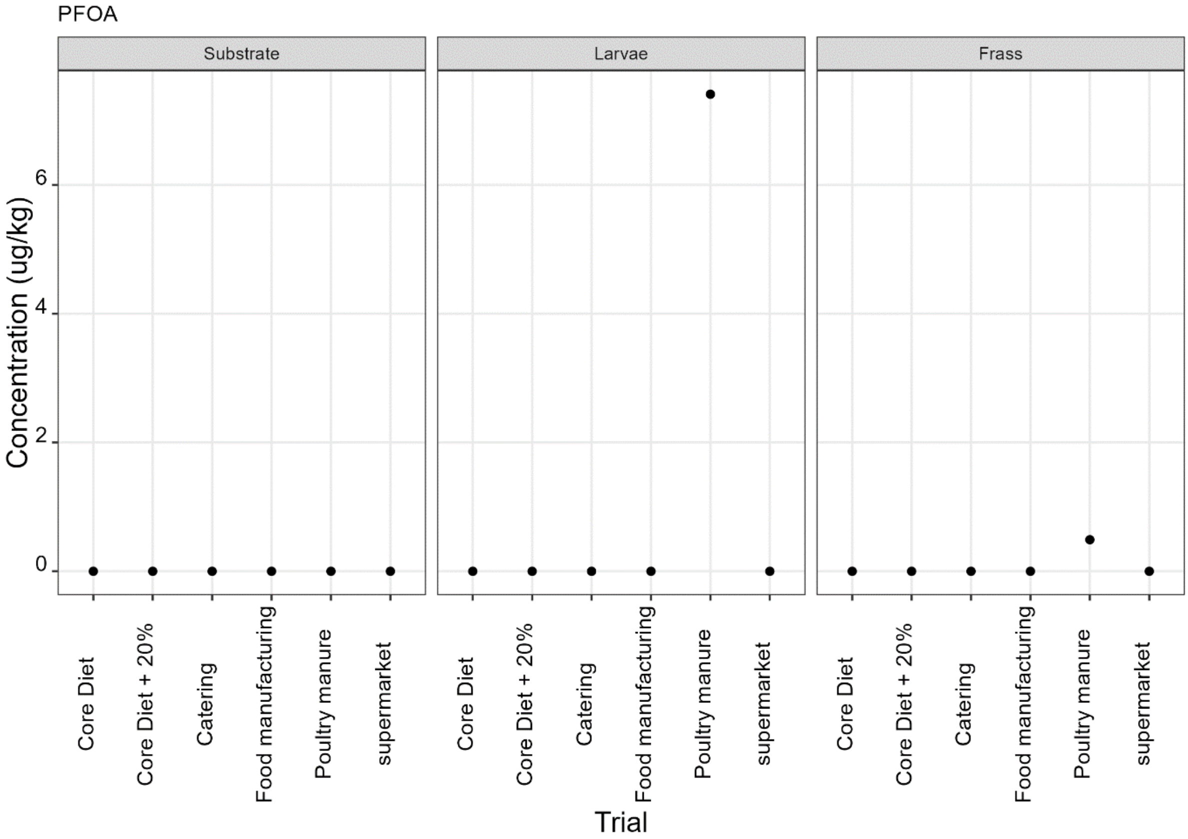
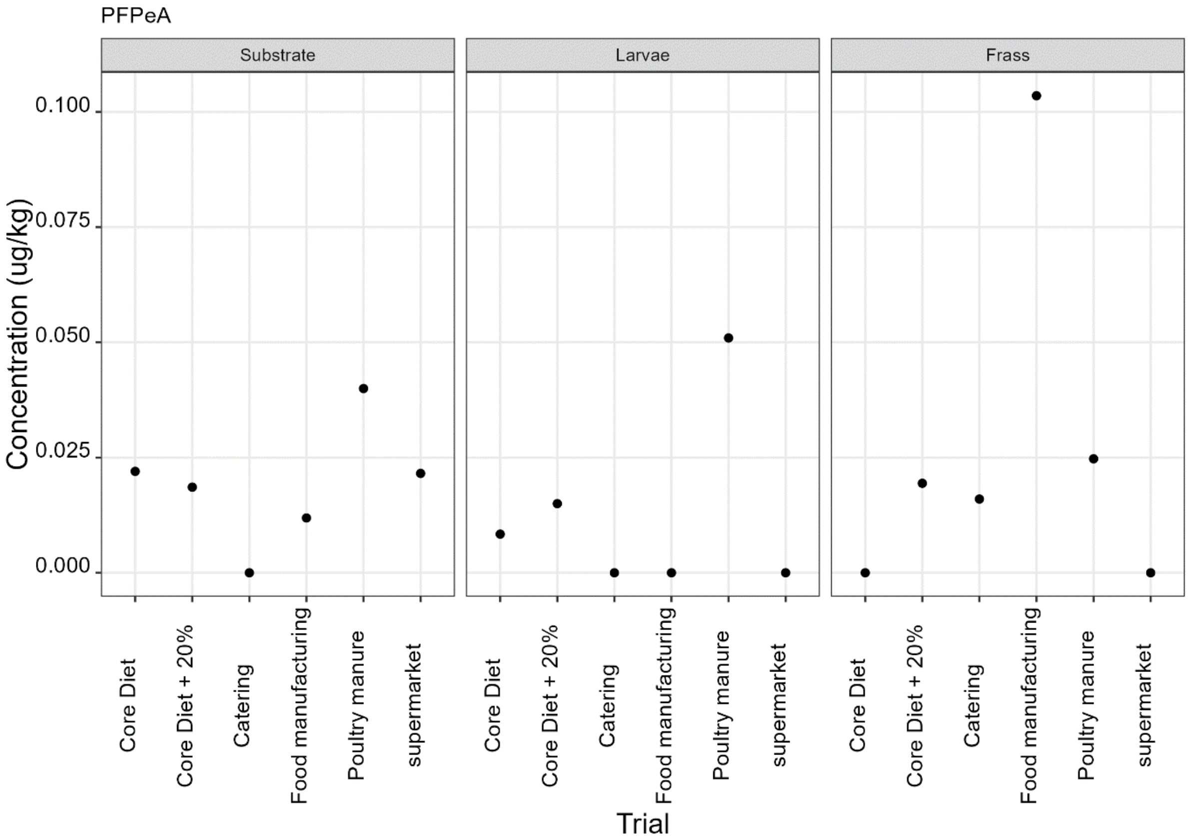
0.075
0.050
0.025
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

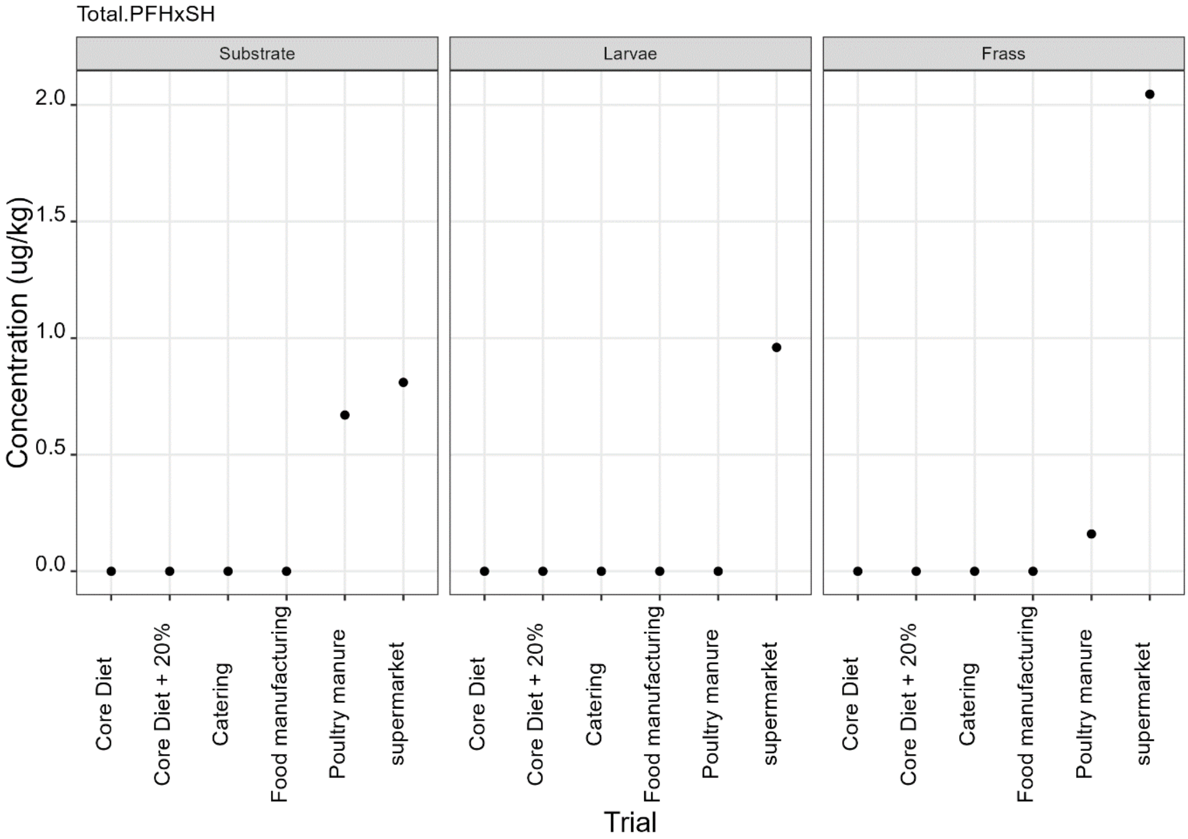
L.


PFOA
Substrate
Larvae
Frass
Concentration (ug/kg)
0
2
4
6
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

M.


PFPeA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.000
0.025
0.050
0.075
0.100
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

N.


Total.PFHxSH
Substrate
Larvae
Frass
Concentration (ug/kg)
2.0
1.5
1.0
0.5
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

O.

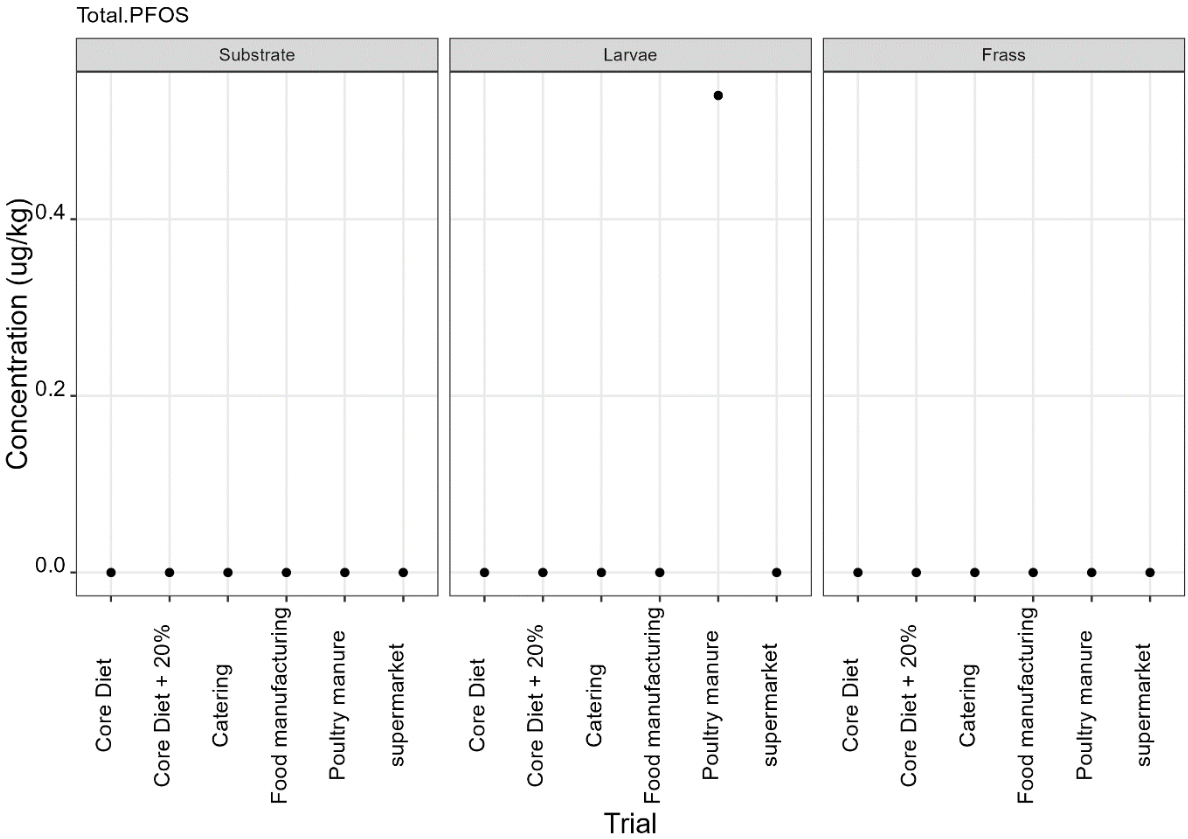

Total.PFOS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

Figure 14 (A–O). Concentrations of PFAS compounds found in different rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Circles represent results from one sample (C) with a single injection.

Toxin screen

Data Criteria

Following profiling and database matching of the samples against procedural blanks, the data was scored against the criteria summarised below (Table 7). A maximum score of 10 is possible. Normally only compounds scoring greater than or equivalent to 5 are assessed as being of potential interest or reported as tentative identities (IDs).

Table 7. Criteria and scoring of data from the toxin screen.

Criterion number	Criterion	Category	Score
1	Comparison with controls	Not in control	1
		Present at >5:1 ratio sample:control	0
		Present at <5:1 ratio sample:control	-1
2	Database or formula match score	>90	1
		80 – 90	0
		<80	-1
3	Mass accuracy/ppm	<5	1
		5 – 10	0
		>10	-1

Criterion number	Criterion	Category	Score
4	Peak shape/height	Acceptable	1
		Not acceptable	-1
5	Ion likelihood and/or adduct correlation	Adduct match or likely ion	1
		Unknown ion	0
		Unlikely ion	-1
		Adduct does not match	-10
6	RT likelihood	Possible	1
		Unknown	0
	OR	Unlikely	-1
	Comparison with known reference standard	RT within ± 0.5 min or RRT within ± 0.01	2
		RT > ± 0.5 min or RRT > ± 0.01	-10
7	Relevant Metlin matches (correct adduct only if available)	<=10	1
		11-30	0
		>30	-1
8	Geographical location or species	Possible	1
		Unknown	0
		Unlikely	-2

Criterion number	Criterion	Category	Score
9	Supporting information (in-source, MS-MS and/or MS^n fragments, positive and negative ion data present and both likely)	Supporting	1
		None	0
		Contradictory	-1

RT – Retention Time

RRT – Relative Retention Time (RT relative to a suitable internal standard)

Core Diet - Rearing substrate sample

Two features were scored as 9 or 10 (identity confirmed versus reference material). These were identified as:

- Solanidine. This is an alkaloid produced by plants of the family Solanaceae (including potato, tomato, egg plant). The concentration was estimated by reference to an external standard to be in the range 900 – 1620 µg/kg.
- α-chaconine. This is a glycoside of solanidine produced by plants of the family Solanaceae (including potato, tomato, egg plant). Identification was supported by the detection of three in-source fragments also found in the reference standard. The concentration was estimated by reference to an external standard to be in the range 650 – 1160 µg/kg.

One feature presented as two peaks was scored 5 and 6 for the individual peaks. This was tentatively identified as:

- Glycyrrhetinic acid. This is the aglycone of glycyrrhizin (the chief sweet-tasting constituent of liquorice root (Glycyrrhiza glabra). Although commonly used in flavouring, excessive consumption can result in hypertension and irregular heart rhythm and in extreme cases death.

Two additional features were scored as 5. These were tentatively identified as:

- Urushiol I. This is a catechol phytotoxin with allergenic properties, causing an allergic dermatitis reaction. It is usually present as part of a mixture of related compounds. It is found in a number of species, for example poison ivy, mango, and cashew.
- Emodin. This is classified as both a phytotoxin and a mycotoxin. It can be isolated from, for example, rhubarb and buckthorn and also from many species of fungi. However, emodin was not detected in the mycotoxin screen for this rearing substrate. This may be due to the use of different sub-samples in the different tests, but the toxin screen score of 5 also indicates a tentative identification, which would need further investigation using a known standard.

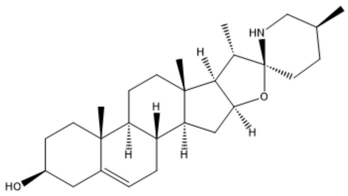
Core Diet larvae sample

One feature was scored as 9 (identity confirmed v reference material). This was identified as solanidine. The concentration was estimated by reference to an external standard to be in the range 1404 – 2229 µg/kg.

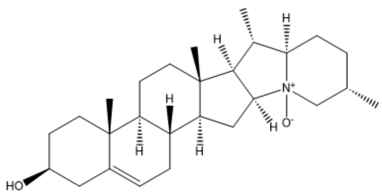
One feature presented as three peaks, each scored 7 (strong indication of identity in the absence of reference standard) respectively. Five possible related compounds were tentatively identified (see Figure 15), with no clear evidence as to which corresponds to which peak:

- Tomatidenol
- Solanidine N-oxide
- Rubijervine
- Leptinidine
- Solasodine

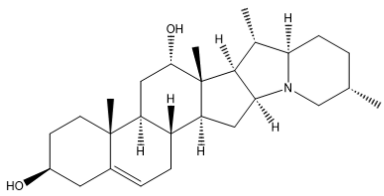
These five compounds are also produced by plants of the family Solanaceae and may be reasonably expected to occur alongside solanidine.



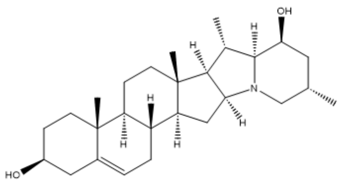
Tomatidenol



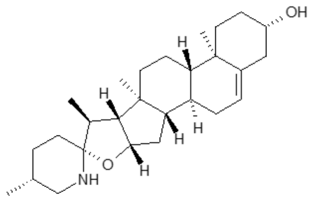
Solanidine N-oxide



Rubijervine



Leptinidine



Solasodine

Figure 15. Structures of related and tentatively identified compounds for core diet and core diet + 20% larvae and frass samples.

Core diet frass sample

Two features were scored as 9 or 10 (identity confirmed v reference material). These were identified as solanidine (concentration estimated by reference to an external standard to be in the range 1404 – 1513 µg/kg) and α-chaconine (identification was supported by the detection of three in-source fragments also found in the reference standard). The concentration of α-chaconine was estimated by reference to an external standard to be in the range 632 – 681 µg/kg.

Two features were scored as 8 (strong indication of identity in the absence of reference standard). These were identified as γ-chaconine (alternative ID γ-solanine) and ß$_1$-chaconine (alternative IDs ß$_1$-solanine, ß$_2$-chaconine, ß$_2$-solanine).

One feature presented as three peaks, each were scored 6 or 7 (strong indication of identity in the absence of reference standard) respectively. There were five possible related compounds tentatively identified (tomatidenol, solanidine N-oxide, rubijervine, leptinidine and Solasodine; see Figure 15), with no clear evidence as to which corresponds to which peak. All these compounds are also produced by plants of the family Solanaceae and may be reasonably expected to occur alongside solanidine and α-chaconine.

Two features were scored as 5. These were tentatively identified as emodin and glycyrrhetinic acid.

Core diet +20% rearing substrate sample
Two features were scored as 9 or 10 (identity confirmed v reference material). These were identified as solanidine (concentration to be in the range 473 – 996 µg/kg) and α-chaconine (concentration to be in the range 227 – 478 µg/kg).

One feature was scored as 6. This was tentatively identified as cicutoxin with an alternative ID of oenanthotoxin. These two compounds are isomeric and are classified as phytotoxins. They are neurotoxins causing death by respiratory paralysis resulting from disruption of the central nervous system. They are produced by members of the family Apiaceae (includes water hemlock, water dropwort), which are found in North America and parts of Europe.

Two features were scored as 5. These were tentatively identified as urushiol I and emodin.

Core diet + 20% larvae sample
One feature was scored as 9 (identity confirmed v reference material). This was identified as solanidine. The concentration was estimated by reference to an external standard to be in the range 813 – 1774 µg/kg.

One feature presented as two peaks, each was scored 6 or 7 (strong indication of identity in the absence of reference standard) respectively. There were five possible related compounds tentatively identified (tomatidenol, solanidine N-oxide, rubijervine, leptinidine and solasodine; see Figure 15), with no clear evidence as to which corresponds to which peak.

One feature was scored as 5. This was tentatively identified as fusaric acid, a mycotoxin produced by *Fusarium* species.

Core diet + 20% frass sample
Two features were scored as 9 or 10 (identity confirmed v reference material). These were identified as solanidine (concentration estimated to be in the range 1492 – 2325 µg/kg) and α-chaconine (concentration estimated to be in the range 1275 - 1995 µg/kg).

One feature was scored as 8 (strong indication of identity in the absence of reference standard). This was identified as γ-chaconine (alternative ID γ-solanine).

One feature presented as two peaks; each was scored as 7 (strong indication of identity in the absence of reference standard). There were five possible related compounds tentatively identified (tomatidenol, solanidine N-oxide, rubijervine, leptinidine and solasodine; see Figure 15), with no clear evidence as to which corresponds to which peak.

One feature was scored as 6. This was tentatively identified as cicutoxin (alternative ID oenanthotoxin).

Three features were scored as 5. These were tentatively identified as fusaric acid, emodin and flavipucine, classified as a mycotoxin.

Currently permitted rearing substrates: Results of note
Two glycoalkaloids solanidine and alpha-chaconine associated with plants of family S*olanaceae* (potato, tomato, eggplant etc) could be identified by comparison with a reference material. Residue levels in freeze-dried samples ranged from estimated 0.2 to 2.3 mg/kg. The solanine levels increased in the larvae and frass, compared to the substrate.

Alpha-chaconine levels were lower than the solanidine level in the same sample; larvae samples did not contain any alpha-chaconine.

The related compounds tomatidenol/solanidine N-oxide, rubijervine, leptinidine, solasodine, and gamma-chaconine associated with plants of the family *Solanaceae* were also tentatively identified.

The other five compounds (cicutoxin, urushiol I, emodin, fusaric acid, flavipucine) tentatively identified may warrant further investigation.

Currently there are no maximum levels of glycoalkaloids specified for animal feed. A risk assessment published by EFSA concluded that a risk characterisation of potato glycoalkoloids in feed for farm and companion animals was not possible due to insufficient data on potential adverse effects in these species (EFSA, 2020). Data on the presence of these compounds in animal feed is therefore required before an assessment of the levels found in insect larvae can be evaluated.

Currently non-permitted substrates

Catering rearing substrate sample

One feature was scored as 6 (strong indication of identity in the absence of reference standard). This was tentatively identified as sparteine. This is an alkaloid phytotoxin found in *Cytisus scoparius* (Common broom) and also in species of lupin (*Lupinus* genus). It acts as a sodium channel blocker.

Three additional features were scored as 5. These were tentatively identified as:

- 1,4-ipomeadiol - found in mould-damaged sweet potatoes (*Ipomoea batatas*). Known to cause pulmonary toxicity in cattle and rodents.
- 1-(3'-furyl)-6 7-dihydroxy-4 8-dimethylnonan-1-one - found in mould-damaged sweet potatoes.
- Aposcopolamine - a tropane alkaloid found in members of the Nightshade family (Solanaceae), in particular *Datura ferox* (Angel’s trumpets, fierce thornapple). An alternative identification for this feature was morphine (an opioid alkaloid).

Catering larvae sample

One feature was scored as 6 (strong indication of identity in the absence of reference standard). This was tentatively identified as muscimol. This is a psychoactive component of mushroom species such as fly agaric (*Amanita muscaria*).

Three additional features were scored as 5. These were tentatively identified as:

- Aposcopolamine.
- Fusaric acid - a mycotoxin produced by *Fusarium* species.
- Penigequinolone A - a mycotoxin produced by *Pencillium* spp.

Catering frass sample

One feature was scored as 9 (identity confirmed v reference material data). This was identified as alpha-chaconine.

One feature was scored as 7 (strong indication of identity in the absence of reference standard). This was tentatively identified as:

- Muscimol

Three features were scored as 6 (strong indication of identity in the absence of reference standard). These were tentatively identified as:

- Anagyrine - a phytotoxin produced by a number of plant species including members of the genus *Lupinus* (lupins) and *Anagyris foetida* (stinking bean trefoil). An alternative identification of verruculotoxin (a mycotoxin produced by *Penicillium verrucosum*) was scored as 5.
- Aposcopolamine.
- Flavipucine - a mycotoxin.

An additional seven features were scored as 5. These were tentatively identified as:

- Aphidicolin - a mycotoxin.
- Chanoclavine - an ergot alkaloid. Alternative identifications for this feature were the ergot alkaloids isochanoclavine, dihydroelymoclavine,

dihydroisolysergol, dihydrolysergol and fumigaclavine B and the roquefortine alkaloid roquefortine B, all scored as 5.

- Chlamydosporol - a mycotoxin.
- Deoxaphomin - a mycotoxin.
- Fusaric acid - a mycotoxin produced by *Fusarium* species.
- Indole-3-acetic acid - a plant hormone. This has been listed as mutagenic and potentially carcinogenic.
- 1-ipomeanol - found in mould-damaged sweet potatoes (*Ipomoea batatas*).

Manufacturing rearing substrate sample

Two features were scored as 7 (strong indication of identity in the absence of reference standard). These were tentatively identified as:

- 1,4-ipomeadiol - Found in mould-damaged sweet potatoes (*Ipomoea batatas*). Known to cause pulmonary toxicity in cattle and rodents.
- Galantamine - a phytotoxin found in *Narcissus* spp (e.g. daffodil). An alternative identification, also scored a 7, was pluviine, an isomeric phytotoxin also found in *Narcissus* spp.

One feature was scored as 6 (strong indication of identity in the absence of reference standard). This was tentatively identified as:

- Seneciphylline - a pyrrolizidine alkaloid found in e.g. *Jacobaea vulgaris* (ragwort). An alternative identification also scored as 6 was spartioidine, also a pyrrolizidine alkaloid found in e.g. *Jacobaea vulgaris* (ragwort).

An additional five features were scored as 5. These were tentatively identified as:

- Aposcopolamine.
- Agistatin A - a mycotoxin.
- Juglone - a phytotoxin produced by members of the Juglandaceae family (walnuts). Toxic to other plant species.
- Muscimol.
- Tryprostatin A - a mycotoxin.

Manufacturing larvae sample

One feature was scored as 7 (strong indication of identity in the absence of reference standard). This was tentatively identified as:

- Heliotrine - a pyrrolizidine alkaloid phytotoxin produced by plants of the genus *Heliotropium*.

One feature was scored as 6 (strong indication of identity in the absence of reference standard). This was tentatively identified as:

- Fusaric acid

An additional three features were scored as 5. These were tentatively identified as:

- 1,4-ipomeadiol.
- Aposcopolamine.
- Prop-2-ene carboxylic acid. This compound is a mycotoxin found in some mushrooms such as *Russula subnigricans*.

Manufacturing frass sample

One feature was scored as 6 (strong indication of identity in the absence of reference standard). This was tentatively identified as:

- Fusaric acid.

An additional five features were scored as 5. These were tentatively identified as:

- 1-myoporol - found in mould-damaged sweet potatoes (*Ipomoea batatas*). An alternative identification, 6-myoporol, was scored as 5.
- Aspergillic acid - a mycotoxin produced by *Aspergillus flavus*.
- Flavipucine.
- Indole-3-acetic acid.
- Muscimol.

Supermarket rearing substrate sample

One feature was scored as 9 (identity confirmed versus reference material data). This was identified as solanidine - an alkaloid produced by plants of the family Solanaceae (including potato, tomato, egg plant).

One feature was scored as 7 (strong indication of identity in the absence of reference standard). This was tentatively identified as galantamine - phytotoxin found in a number of *Narcissus* species, e.g daffodils. An alternative identification scored as 7 was pluviine, a phytotoxin often found alongside galantamine.

Five features were scored as 6 (indication of identity in the absence of reference standard). These were tentatively identified as:

- Emodin.
- Grayanotoxin III - a phytotoxin found in *Rhododendron* spp.
- Grayanotoxin IV - a phytotoxin found in *Rhododendron* spp.
- Menisdaurin - a phytotoxin found in *Ilex aquifolium* (common holly).
- Rubellin D - a mycotoxin. An alternative identification, rugulosin, a mycotoxin, was also scored as 6.

Five additional features were scored as 5. These were tentatively identified as:

- Chrysophanol - a hepatotoxic anthraquinone mycotoxin.
- Metatyrosine - a phytotoxin found e.g. in *Festuca arizonica* (pine grass, native to North America) and *Festuca rubra* (red fescue, widespread in the Northern hemisphere).
- Morphine - a morphinan alkaloid phytotoxin produced by *Papaver somniferum* (Opium poppy). Also used as a pharmaceutical and a drug of abuse.
- Thebaine - a morphinan alkaloid phytotoxin produced by *Papaver somniferum* (Opium poppy, minor component) and *Papaver bracteatum* (Persian poppy, major component). Also used as a drug of abuse and as a starting point in the synthesis of other opioids.
- Versiconol acetate - a mycotoxin.

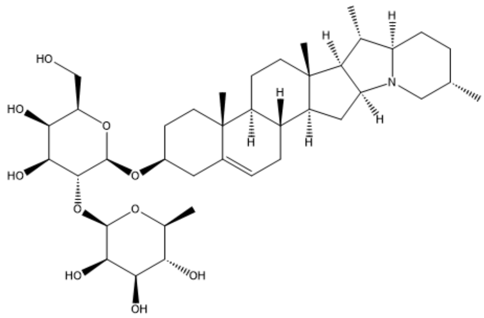
Supermarket larvae sample

Two features were scored as 9 (identity confirmed versus reference material data). These were identified as:

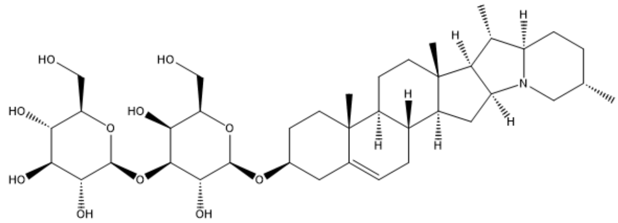
- Solanidine.
- Alpha-chaconine.

One feature was scored 8 (strong indication of identity in the absence of reference standard). There were four possible related compounds (Figure 16) tentatively identified all of which are alkaloids produced by plants of the family Solanaceae (including potato, tomato, egg plant). There was no clear evidence as to which compound corresponded to which peak:

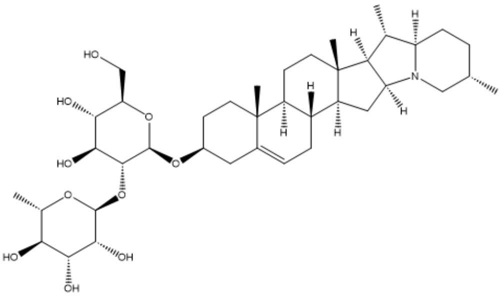
- ß^{1}-solanine.
- ß^{2}-solanine.
- ß^{1}-chaconine.
- ß^{2}-chaconine.



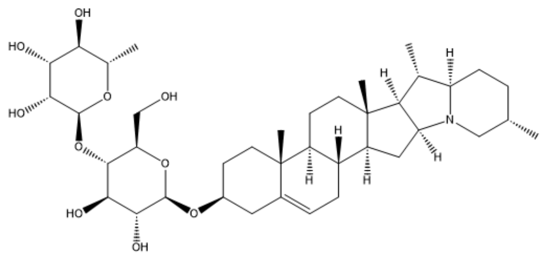
ß^{1}-solanine



ß^{2}-solanine



ß^{1}-chaconine



ß^{2}-chaconine

Figure 16. Structures of related and tentatively identified compounds for compounds found in supermarket larvae and frass samples.

Four additional features were scored as 5. These were tentatively identified as:

- Muscimol.
- Penigequinolone A - a mycotoxin produced by *Pencillium* spp.
- Scopine - a tropane alkaloid phytotoxin produced by a number of plant species including members of the genus *Madragora* and *Scopolia carniolica* (Henbane bell).

- Trypyoquivaline E - a mycotoxin. An alternative identification, tryptoquivaline H, was also scored as 5.

Supermarket frass sample

One feature was scored as 8 (strong indication of identity in the absence of reference standard). There are four possible related compounds tentatively identified (Figure 16), with no clear evidence as to which compound corresponds to which peak:

- ß^1-solanine.
- ß^2-solanine
- ß^1-chaconine.
- ß^2-chaconine.

One feature was scored as 7 (strong indication of identity in the absence of reference standard). This was tentatively identified as convalloside - a cardiac glycoside phytotoxin, found in *Convallaria majalis* (lily of the valley).

Five features were scored as 6 (indication of identity in the absence of reference standard). These were tentatively identified as:

- Emodin.
- Indole-3-acetic acid.
- Rubellin D - a mycotoxin. An alternative identification, rugulosin, a mycotoxin, was also scored as 6.
- Tropine - a tropane alkaloid found in *Atropa belladonna* (deadly nightshade) and *Datura stramonium* (devil's trumpet).
- Tryptophol - classified both as a mycotoxin and a phytotoxin. Produced by the fungus *Candida albicans* and also found in *Pinus sylvestris* (Scots pine).

Nine additional features were scored as 5. These were tentatively identified as:

- Anisomycin - a bacterial toxin with antibiotic action produced by *Streptomyces griseolus*.

- Aristolochic acid I - a member of a group of phytotoxins (carcinogenic, mutagenic and nephrotoxic) commonly found in plants of the family Aristolochiaceae (birthworts).
- Chanoclavine - an ergot alkaloid mycotoxin. Alternative identifications are the ergot alkaloids isochanoclavine, dihydroelymovlavine, dihydroisolysergol, dihydrolysergol and fumigaclavine B and the roquefortine alkaloid roquefortine B.
- Chlamydosporol - a mycotoxin.
- Cladosporin - a mycotoxin. An alternative identification is the mycotoxin curvularin.
- Coumarin - a phytotoxin found naturally in many plants such as strawberries, tonka bean, cinnamon etc.
- Domoic acid - a fycotoxin that accumulates in shellfish. A causative agent of amnesiac shellfish poisoning (ASP).
- Homolycorine - a phytotoxin found in plants of the genus *Narcissus* (e.g. daffodil).
- Tenuazonic acid - a mycotoxin.

Poultry manure rearing substrate sample
Three features were scored as 6 (indication of identity in the absence of reference standard) These were tentatively identified as:
- Emodin.
- Lupinine - a phytotoxin (hepato- and neurotoxin) found in plants of the genus *Lupinus* (Lupins).
- Onchidal - a neurotoxic shellfish toxin acting as an irreversible acetylcholinesterase inhibitor, produced by species of the genus *Onchidella* (sea slugs).

Eleven additional features were scored as 5. These were tentatively identified as:
- Dinophysistoxin-2 - a fycotoxin (algal toxin that accumulates in shellfish), a causative agent of diarrhetic shellfish poisoning. A related toxin, okadaic acid, is an alternative identification.
- Chrysophanol - a hepatotoxic anthraquinone mycotoxin.

- Conhydrine - an alkaloid phytotoxin found in *Conium maculatum* (poison hemlock, native to Europe and North Africa).
- Cucurbitacin D - a phytotoxin found in Cucurbitaceae spp. (e.g. squash, courgette, cucumber, watermelon).
- Cycloaspeptide F - a cyclic peptide mycotoxin.
- Digitoxin - a cardiac glycoside phytotoxin found in *Digitalis purpurea* (foxglove).
- Hebevinoside VI - a mycotoxin.
- Hebevinoside X - a mycotoxin related to the previous compound.
- Ipomeanine - a myco/phytotoxin, found in mould-damaged sweet potatoes (*Ipomoea batatas*).
- Monocerin - a mycotoxin.
- Versicolorin A - a mycotoxin.

Poultry manure larvae sample

One feature was scored as 5. This was tentatively identified as:

- Emodin.

Poultry manure frass sample

Four features were scored as 6 (indication of identity in the absence of reference standard). These were tentatively identified as:

- Emodin.
- Enniatin B - a cyclic peptide mycotoxin.
- Enniatin B1 - a cyclic peptide mycotoxin related to the previous compound. An alternative identification is enniatin B4.
- Scopine - a tropane alkaloid phytotoxin, produced by a number of plant species including members of the genus *Madragora*, and *Scopolia carniolica* (Henbane bell). Alternative identifications, scored as 5, are retronecine (a pyrrolizidine alkaloid phytotoxin) and scopoline (related to as an isomer of scopine).

Eleven additional features were scored as 5. These were tentatively identified as:

- Chrysophanol - a hepatotoxic anthraquinone mycotoxin.

- Citrinin - a mycotoxin produced by a number of fungal species e.g. *Penicillium*, *Aspergillus* spp.
- Cucurbitacin A - a phytotoxin found in *Cucurbitaceae* spp (e.g. squash, courgette, cucumber, watermelon).
- Cucurbitacin D - a phytotoxin found in *Cucurbitaceae* spp (e.g. squash, courgette, cucumber, watermelon). An alternative identification is cucurbitacin L.
- Cycloaspeptide F - a cyclic peptide mycotoxin.
- Desferrioxamine E - a bacterial toxin produced by *Streptomyces pilosus*.
- Enniatin A1 - a cyclic peptide mycotoxin. An alternative identification is Enniatin G.
- Hebevinoside III - a mycotoxin.
- Hebevinoside VI - a mycotoxin, related to the previous compound.
- Heliotrine - a pyrrolizidine alkaloid phytotoxin found in plants of the genus *Heliotropium* (wide-ranging and also used as garden plants).
- Lupinine.

Currently non-permitted rearing substrates: Results of note

It is noted that very few compounds were scored as 8 or 9 and therefore for which the identification can be reasonably assured. The compounds scored as 9 were identified as solanidine and alpha- chaconine.

As discussed under the results for the currently permitted substrates there are currently no maximum specified levels for these glycoalkaloids in animal feed, but it is considered that further data on the presence of these compounds in animal feed is required before an evaluation can be made.

Although most of the compounds were scored between 5 and 7 and therefore the tentative identifications may not provide correspond to the actual compound present, the results may provide a guide as to the type of compound that may be present. The majority of compounds found were tentatively identified as mycotoxins, particularly in the frass samples. Further analysis with standards would be needed to ascertain the identity of these compounds.

Generally, there were fewer compounds identified in the larvae than in the rearing substrate or frass.

Microbiological analysis

Commission Regulation (EU) No 142/2011 specifies that feed materials should comply with the following limits for microbiological contaminants during or upon withdrawal from storage:

- Salmonella spp: absence in 25 g n = 5; c = 0; m = 0; M = 0
- Enterobacteriaceae: n = 5; c = 2; m = 10; M = 300 in 1 g

Where:
n = number of samples to be tested,
c = number of samples where the number of bacteria expressed in colony forming units (CFU) is between m and M,
m = threshold value for the number of bacteria expressed in CFU that is considered satisfactory,
M = maximum value of the number of bacteria expressed in CFU.

Salmonella, *Campylobacter* and ESBL *E. coli* were not detected in any extracts from any samples. Staphylococci were reported as <10 cfu/g for supermarket surplus, larvae reared on supermarket surplus and the frass produced. Levels of Staphylococci in kitchen catering waste were high but did not vary considerably between the rearing substrate, the larvae and the frass (means of 7.715, 7.288 and 8.224 $\log_{10}$ cfu/g respectively).

The level of Enterobacteriaceae specified in Commission Regulation (EU) No 142/2011 was exceeded in larvae from all rearing substrates except the core diet and core diet +20%. Larvae reared on currently permitted substrates were culled by blanching in boiling water (approx. 100°C) and cooking until core temperature exceeded 75°C. The larvae reared on the currently non-permitted substrates were killed by blanching in water above 80°C for three minutes. This method was used to kill the larvae but not to serve as a method to reduce the microbiological load to the specified limits. There are different processing methods that are used to manufacture processed animal protein from insect larvae and these include different protocols to kill the larvae together with subsequent processing to produce insect protein. It is highly likely that these processing methods will reduce the microbiological load significantly. Therefore, in the context of this project these results are likely to represent a maximum microbiological load that would be reduced by further processing.

It should be noted that larvae produced from the core diet and core diet + 20% had been processed in accordance with the commercial providers standard procedures to ensure regulatory compliance.

Commission Regulation (EU) No 142/2011 specifies that processed manure taken during or immediately after processing should comply with the following limits for microbiological contaminants:

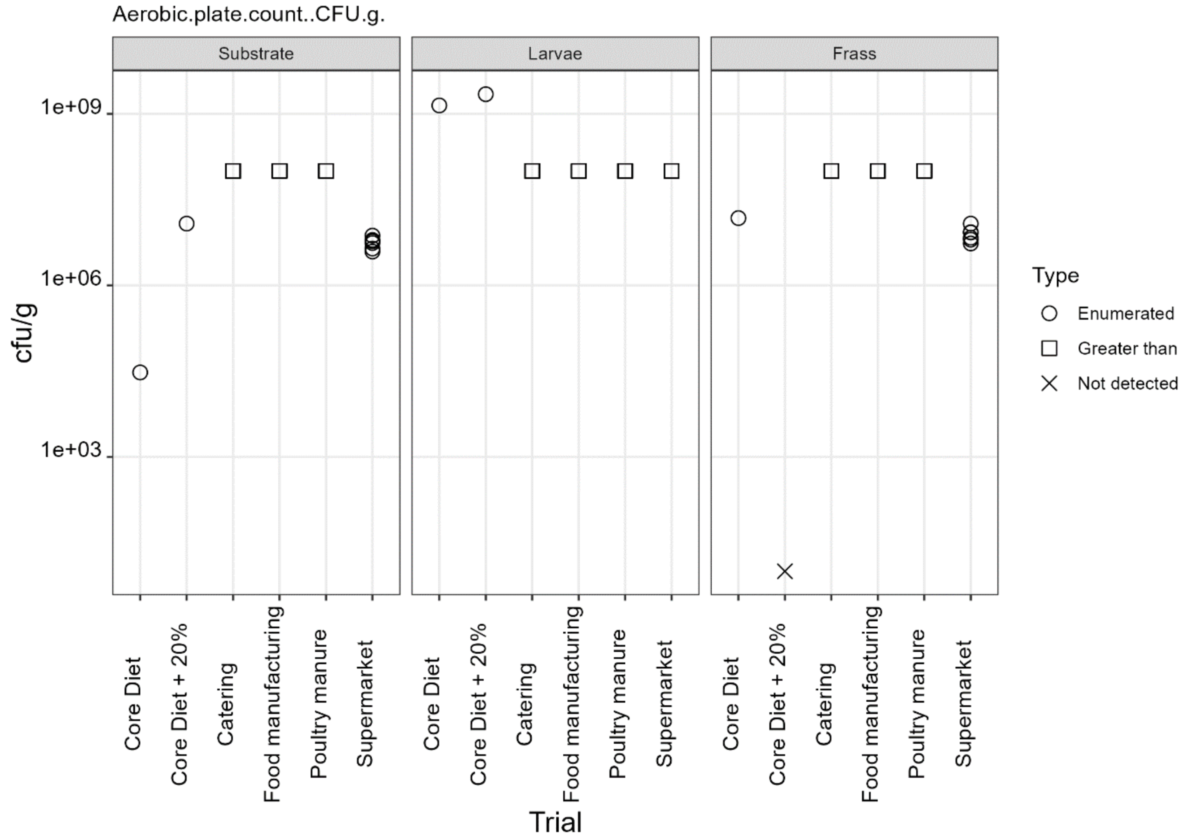
- *Salmonella* spp: absence in 25 g n = 5; c = 0; m = 0; M = 0
- *E. coli* or Enterococcaceae: n = 5; c = 5; m = 0; M = 1000 in 1 g

Where:
n = number of samples to be tested,
c = number of samples where the number of bacteria expressed in colony forming units (CFU) is between m and M,
m = threshold value for the number of bacteria expressed in CFU that is considered satisfactory,
M = maximum value of the number of bacteria expressed in CFU.

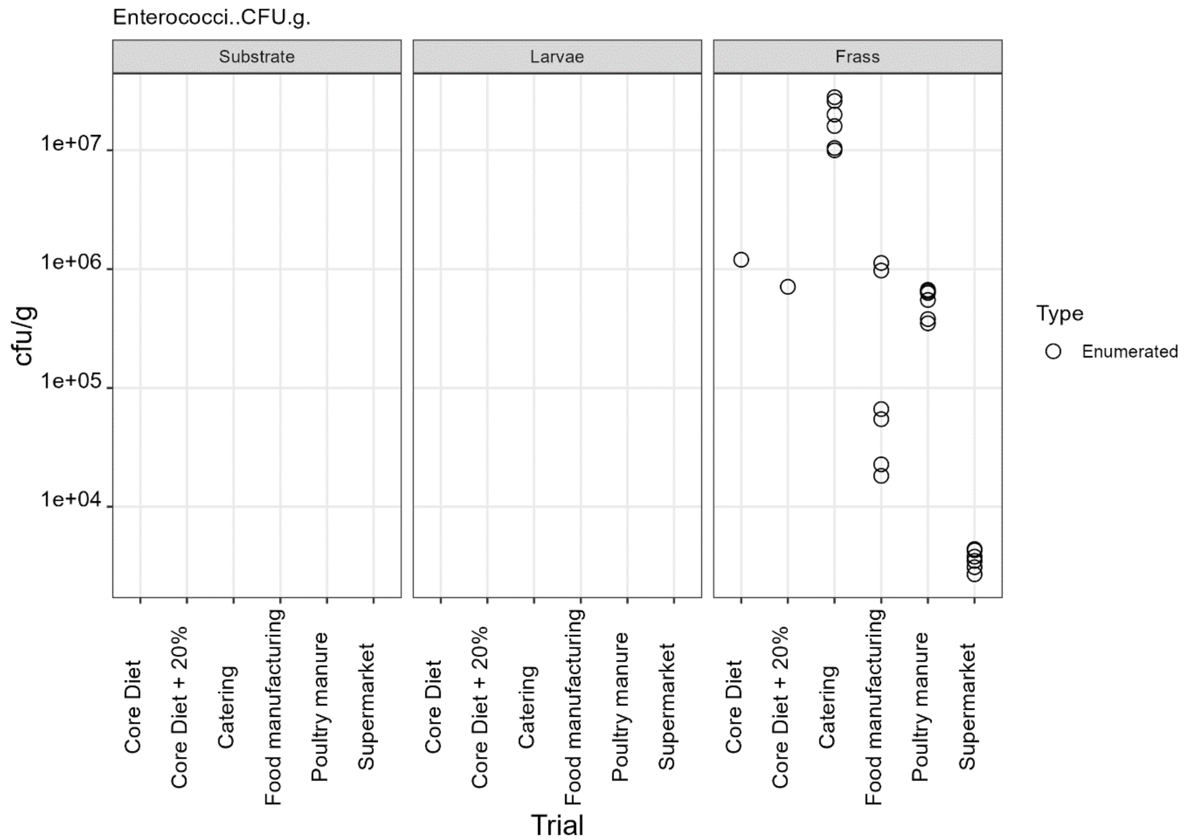
EU regulation 2019/1009 also specifies the same limits as those given above for pathogens in an organic fertiliser. The maximum value of 1000 CFU/g was exceeded for Enterococcaceae in frass samples from all rearing substrates but was only exceeded for *E. coli* in frass from catering waste. These results indicate that further processing of the frass would be required to conform to regulatory requirements.

Individual results are shown in Figure 17 and full results are shown in Appendix I.

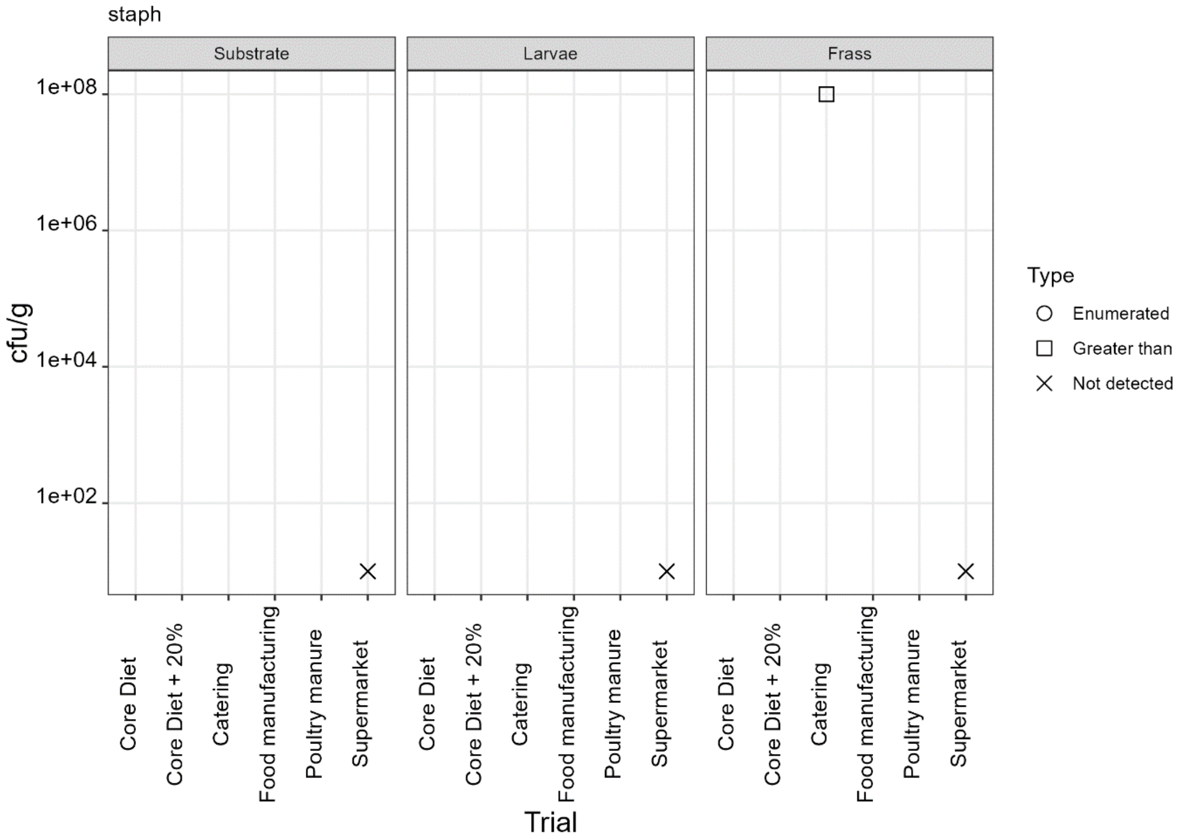
A.


Aerobic.plate.count..CFU.g.
Substrate
Larvae
Frass
1e+09
1e+06
1e+03
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

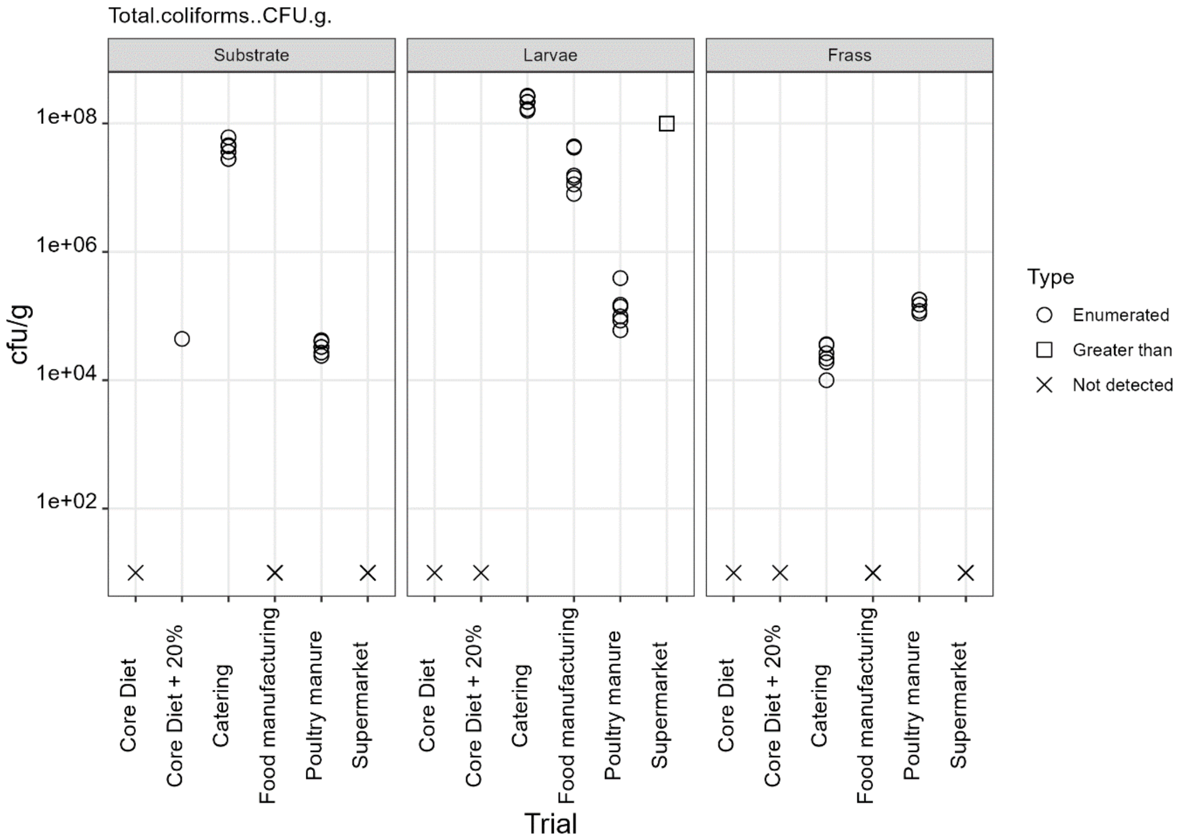
B.


Enterococci..CFU.g.
Substrate
Larvae
Frass
1e+07
1e+06
1e+05
1e+04
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated

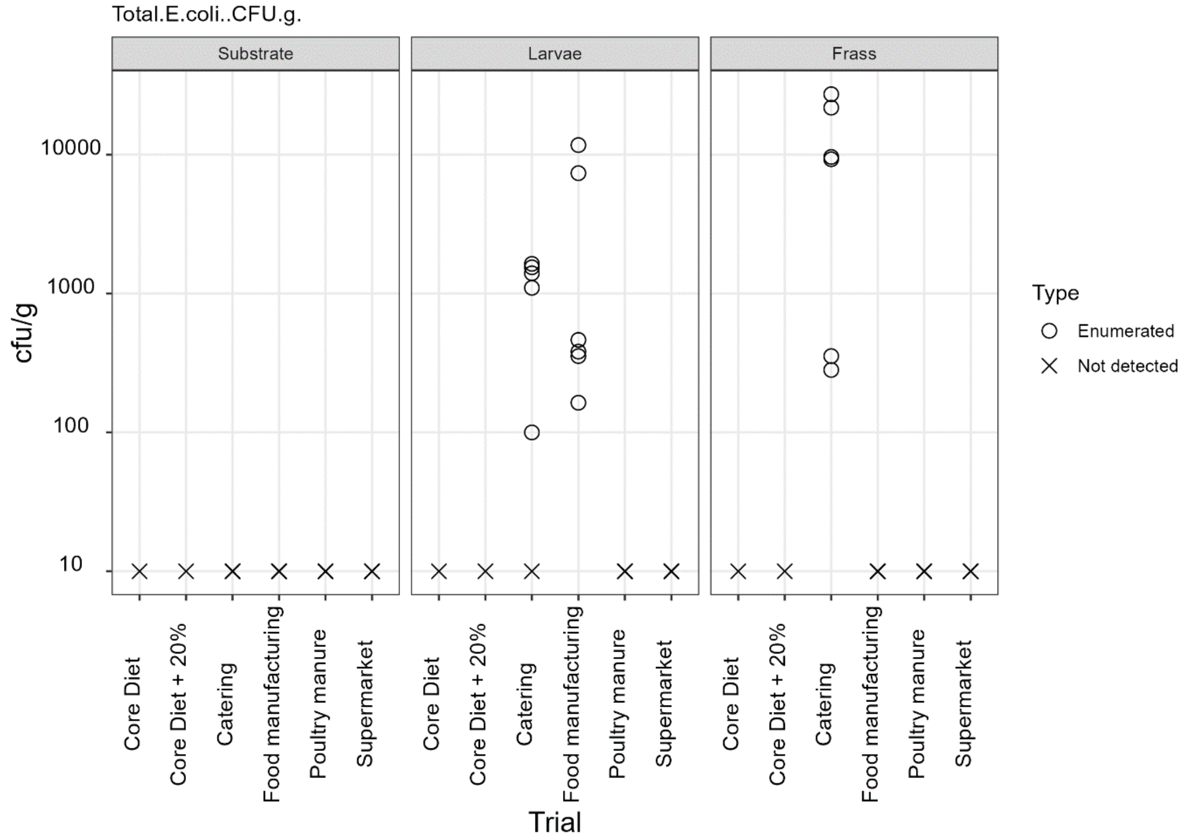
C.


staph
Substrate
Larvae
Frass
1e+08
1e+06
1e+04
1e+02
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

D.


Total.coliforms..CFU.g.
Substrate
Larvae
Frass
1e+08
1e+06
1e+04
1e+02
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

E.


Total.E.coli..CFU.g.
Substrate
Larvae
Frass
cfu/g
10000
1000
100
10
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Not detected

F.

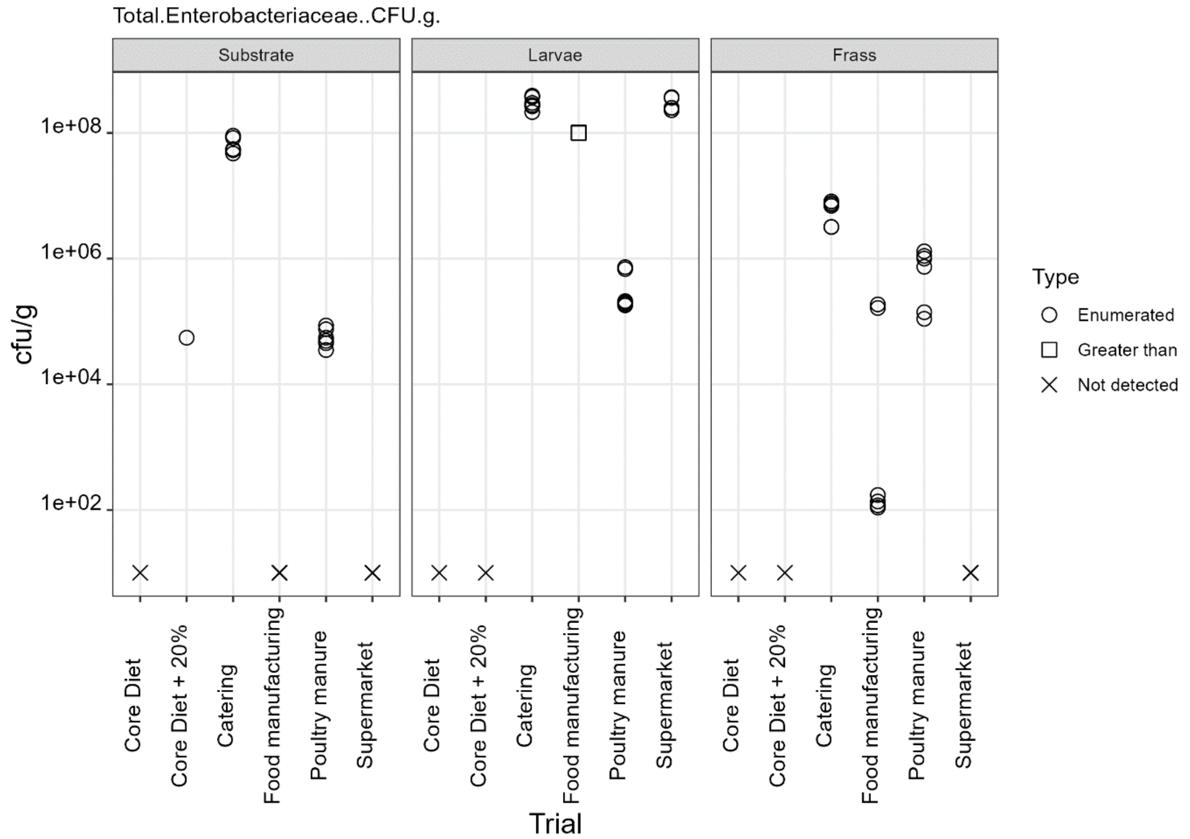

Total.Enterobacteriaceae..CFU.g.
Substrate
Larvae
Frass
cfu/g
1e+08
1e+06
1e+04
1e+02
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

Figure 17 (A–F). Enumeration of microorganisms found in rearing substrates, the larvae reared on the rearing substrates and the resultant frass. Points indicate results from samples (A, B1 and B2) with duplicate plates.

Non-targeted virus screen

Difficulty was experienced in extracting good quality RNA from the rearing substrate samples even after repeat extraction. A total of 848,589,172 read pairs passed the QC process with an average of 42 million read pairs per sample. The reads were assessed for duplicates prior to the normalised reads being assembled. Extracted RNA quantities, read numbers and normalised reads are presented in Table 8. As can be seen lower levels of RNA were extracted from the rearing substrate samples leading to lower levels of reads or lower diversity (low normalised reads percentages) in the resulting sequence data.

After QC trimming the resulting high-quality reads were assembled and compared to the GenBank NT database using BLASTN (Benson *et al*., 2015). Table 9 details all the viruses identified. They can be broadly classified into three groups based on their likely origin.

- Viruses associated with bacteria, fungi or other organisms (excluding plant and avian) associated with the rearing substrate or the insect rearing process.
- Plant viruses associated with the rearing substrate.
- Avian viruses associated with the rearing substrate.

Table 8. Sample RNA concentrations and sequencing read numbers and normalisation.

Sample description	Extracted RNA concentration (ng/µL)	Trimmed Reads	Normalised Reads	Normalised Reads %
Catering - rearing substrate	0.4	12,396,578	90,155	0.73%
Catering - larvae	1463.5	71,690,582	8,460,275	11.80%
Catering - frass	353.6	62,870,706	2,053,248	3.27%

Sample description	Extracted RNA concentration (ng/µL)	Trimmed Reads	Normalised Reads	Normalised Reads %
Manufacturing – rearing substrate	2.5	45,858,120	294,326	0.64%
Manufacturing - larvae	497.3	71,421,705	10,521,813	14.73%
Manufacturing - frass	20.1	42,555,102	997,698	2.34%
Core diet – rearing substrate	Below detection limit	28,380	19,041	67.09%
Core diet - larvae	661.2	80,697,482	11,724,404	14.53%
Core diet - frass	12.0	15,589,953	3,931,158	25.22%
Core diet + 20% - rearing substrate	2.3	3,434,617	51,050	1.49%
Core diet + 20% - larvae	863.2	72,826,591	8,688,848	11.93%
Core diet + 20% - frass	28.1	29,466,074	4,223,550	14.33%
Poultry manure – rearing substrate	1.2	93,511,206	3,996,685	4.27%
Poultry manure - larvae	1992.1	105,002,694	11,593,652	11.04%
Poultry manure - frass	301.7	78,255,487	9,024,768	11.53%
Supermarket – rearing substrate	0.3	6,508	5,413	83.17%
Supermarket - larvae	1126.8	58,543,188	6,390,309	10.92%
Supermarket - frass	5.5	21,493	15,582	72.50%
Extraction Blank	0.0	1,732,966	71,930	4.15%
Positive control (artificial RNA)	NT	2,679,740	36,100	1.35%

NT = not tested

Table 9. Details of viral RNA detected in samples.

Sample Description	Likely virus origin: Bacteria, fungi, other	Likely virus origin: Plant	Likely virus origin: Avian
Catering – rearing substrate	No virus detected	Pepino mosaic virus	No virus detected
Catering - larvae	Totiviridae, Levivirdae	Pepino mosaic virus, Tomato brown rugose fruit virus	No virus detected
Catering - frass	Agaricus bisporus virus 2 Totiviridae, Levivirdae	Pepino mosaic virus, Tomato brown rugose fruit virus, Turnip yellow virus, Lettuce big vein associated virus, Tomato spotted wilt virus, Carrot cryptic virus, Lettuce mottle virus, Watermelon mosaic virus, Turnip yellows associated virus	No virus detected
Manufacturing – rearing substrate	No virus detected	Pepino mosaic virus	No virus detected
Manufacturing - larvae	Leviviridae	No virus detected	No virus detected
Manufacturing - frass	bacteriophage Totiviridae, Naravirus, Levivirdae	possible novel Tombus virus	No virus detected
Core diet – rearing substrate	No virus detected	No virus detected	No virus detected
Core diet - larvae	bacteriophage Leviviridae	Pepino mosaic virus, Tomato brown rugose fruit virus	No virus detected

Sample Description	Likely virus origin: Bacteria, fungi, other	Likely virus origin: Plant	Likely virus origin: Avian
Core diet - frass	Hypovirus Leviviridae	Apple hammerhead viroid, Carrot mottle mimic virus satellite RNA, Grapevine leafroll-associated virus 3, Grapevine leafroll-associated virus 4, Potato mop-top virus, Tomato brown rugose fruit virus, PepMV, Potato virus S, Apple stem grooving virus, Carrot mottle virus, Carrot cryptic virus, Carrot toradovirus, Carrot red leaf virus, Potato leafroll virus, Zucchini yellow mosaic virus, Carrot mottle mimic virus satellite RNA	No virus detected
Core diet + 20% - rearing substrate	No virus detected	No virus detected	No virus detected
Core diet + 20% - larvae	bacteriophage	pepino mosaic virus, Tomato brown rugose fruit virus	No virus detected

Sample Description	Likely virus origin: Bacteria, fungi, other	Likely virus origin: Plant	Likely virus origin: Avian
Core diet +20% frass	bacteriophage Naravirus Totiviridae, Levivirdae	Potato mop top virus, Tomato brown rugose fruit virus, Pepino mosaic virus, Hop latent virus, Potato virus S, Carrot mottle virus, Citrus concave gum-associated virus, Carrot cryptic virus, Carrot toradovirus, Carrot red leaf virus, Potato leaf roll virus, Potato virus Y, Arracacha latent virus E associated RNA, Carrot mottle mimic virus satellite RNA, genome Apple hammerhead viroid	No virus detected

Sample Description	Likely virus origin: Bacteria, fungi, other	Likely virus origin: Plant	Likely virus origin: Avian
Poultry manure – rearing substrate	bacteriophage Naravirus, Mitovirus, Botouriaviridae, Partitivirus Levivirdae	Barley yellow dwarf virus	Gallus gallus enteric parvovirus, Rotavirus A, Rotavirus D, Rotavirus F, Avian orthoreovirus, unclassified Picobirnavirus (including Avian and Porcine associated), Infectious bronchitis virus, Chicken calicivirus chicken isolates of Anativirus, Gallivirus, chicken megrivirus , Sicinivirus, Avian nephritis virus
Poultry manure - larvae	bacteriophage Mitovirus	No virus detected	Megrivirus

Sample Description	Likely virus origin: Bacteria, fungi, other	Likely virus origin: Plant	Likely virus origin: Avian
Poultry manure - frass	bacteriophage	No virus detected	Infectious bronchitis virus, Megrivirus, Gallivirus, chicken isolates Anativirus, chicken isolates Sicinivirus, Avian nephritis virus
Supermarket – rearing substrate	No virus detected	No virus detected	No virus detected
Supermarket - larvae	No virus detected	pepino mosaic virus, Tomato brown rugose fruit virus, Tomato mosaic virus	No virus detected
Supermarket - frass	No virus detected	Citrus tristeza virus, Tomato mosaic virus	No virus detected
Extraction Blank	No virus detected	No virus detected	No virus detected
Positive control (artificial RNA)	No virus detected	No virus detected	No virus detected

A range of plant pathogenic viruses were detected in the plant based feed and associated larvae and frass. More were detected in the larvae and frass than in the original feed. A range of chicken pathogenic viruses were detected in the poultry manure with some also passing on into the larvae and frass.

The aim of this study was to assess the likelihood of pathogenic viruses passing through the BSF production system if introduced from the original feed stock. The areas of concern are human and livestock viruses passing into the insect protein samples which are destined for consumption and plant and human viruses passing into the frass which may be used as fertiliser and spread on fields. The presence of viruses was assessed based on the presence of viral RNA detected by sequencing. This is the only method available which can assess the wide range of potential viruses which might be present, but it should be noted that presence of viral RNA in a sample although indicative of the original presence of a virus does not necessarily equate to the presence of an infectious virus in a particular sample.

The viruses detected were split into groups based on their likely origin. No human or non-avian livestock viruses were detected, which is not an unexpected result as the rearing substrates were from food or food grade manufacturing surplus or poultry manure and although the potential for such viruses exists it would be expected to be at very low level. In the absence of such viruses, it is difficult to assess the potential for their transmission though the system but by looking at the presence of viruses from similar classes some relevant information can be inferred.

Regarding plant virus presence, it is noticeable that more viruses were detected in the frass than in the original rearing substrate. A reason for this may be due to the diverse nature of the rearing substrates resulting in difficulty extracting RNA from these samples. This led to fewer reads and lower diversity of reads, which is likely to have reduced the detection efficiency for viruses (Pecman *et al*., 2018). Due to the diverse nature of the rearing substrates, 2.5 g samples of this matrix may be insufficient to be a true representation of viruses present in the whole sample. The frass is also likely to have been made more homogenous by the activity of the BSF larvae during development so the frass samples could be more representative of the diversity of the original sample. The frass and larvae particularly the catering surplus and core diet samples contain RNA from a range of common viruses known to infect vegetables such as tomatoes, potatoes, carrot, and lettuce. Significantly RNA from these viruses was present in the frass and larvae samples.

Based on studies of viral survival from disinfection (Noble *et al*., 2009) and composting experiments (Kerins *et al*., 2018) many of the viruses detected are unlikely to be viable after passing through the BSF digestive system or after pasteurisation of the frass. Others are transmitted via insects while feeding. This is unlikely / impossible from leaf debris in frass / larvae. This leaves only two organisms of concern. *Apple hammerhead viroid* which is not currently present in the UK and particularly *Tomato brown rugose fruit virus*. This virus is very stable (Skelton *et al*., 2023) and of ongoing concern for Defra and the UK tomato industry. Tomato brown rugose fruit virus was detected in the catering, supermarket and core diet samples. The detection of *Tomato brown rugose fruit virus* was not unexpected and is in agreement with recent work done at Fera on imported tomatoes (publication in preparation), which frequently found the virus on supermarket bought tomatoes and results from a similar study done in the USA (Yilmaz & Batuman, 2023).

Considering the avian viruses, the poultry manure is likely to have been much better homogenised by the chickens than the food waste. A diverse range of viruses including *Infectious bronchitis virus*, Rota viruses, enteric *parvovirus*, *Megrivirus*, *Gallivirus* and *Avian nephritis virus* were found in the rearing substrate but less seems to have carried through to the larvae with only *Megrivirus* still detected. More viruses were detectable in the frass with RNA from *Infectious bronchitis virus, Megrivirus, Gallivirus, Anativirus, Sicinivirus* and *Avian nephritis virus* present. As with the plant viruses the presence of RNA does not confirm the presence of infectious viral particles. The authors are not experts in avian viruses but from a literature search the viruses detected appear to be common in UK poultry and do not appear to be a risk to humans or other livestock.

The viral sequences detected in the frass and larvae come from a wide range of single and double stranded RNA based viruses and include at least one enveloped virus and one viroid. This suggests that RNA from many viruses could survive the BSF rearing process. What this screening has not shown is whether any of these viruses remain viable and are likely to be a risk to humans, animals or plants. Infection studies would need to be carried out to determine if this is a risk. With the high levels detected, known stability and threat to the tomato industry, *Tomato brown rugose fruit virus* would seem an ideal candidate for such infection studies.

In summary, RNA from a range of avian and plant viruses was detected in the frass and larvae of BSF. It is not known if any of these viruses were still infectious but if they were they may pose a risk to animal and plant health and by inference open the possibility that if human viruses had been present, they also might have been a risk.

Assessment of the extent to which the results are representative of the substrate, larvae and frass.

A priority for assessment of chemical and microbiological safety is ensuring that experimental findings for each rearing substrate are, as far as possible, representative of the long run average of the real-world systems for which they are models. Achieving this rests on the analysis of representative samples. Representativeness means that if the long-run average of the concentration of a hazard in the real-world system is above the limit of detection, or other critical concentration then we should detect its presence in this study. It also means that for a particular hazard in a substrate, the probability that a detectable quantity is presented for analysis should depend *only* on the size of the sample presented for analysis and the long-run mean quantity of the hazard (definition adapted from “Object of sampling” ISTA 2022).

In addition to gaining a measure of the mean hazard, a more completely representative assessment would include an estimation of the size of the variation in the concentration of hazards between manufacturers, batches of material and at smaller scales. This is particularly relevant for potential acute hazards. However, this would entail the analysis of a large number of analytical samples at great cost. Hence, the aim of the current assessment was to provide information about the mean presence of hazards that is robust to the variation, rather than to describe how variable individual instances of the material may be. The goal of the sampling designs used in this project was to deploy limited resources (sample handling capacity; number of analytical tests) as efficiently as possible to provide a reliable estimate of the mean presence of hazards and, in particular, to ensure that any localised risks (e.g. those associated with only a subset of the material in a particular substrate group) have a sufficient chance of being detected (Lundy and

Parrella, 2015, Varelas, 2019) with sufficient replicate samples and testing to provide assurance that the mean estimates are robust.

Analyses were undertaken with the aim of determining the average concentration of substances and microbiological content in substrate, larvae and frass for each of catering, manufacturing and supermarket surplus and poultry manure. An A sample and B sample were formed from independent sets of primary samples. Two separate sub-samples were taken from the B sample. Samples were analysed in duplicate. (For PFAS, toxin screen and non-targeted viral screen all samples were combined into a C sample which was analysed once).

The average concentration is estimated as:

$$\text{Estimate} = (A + ((B1 + B2)) \div 2$$

Equation 1

Hence, if the standard deviation of between-sample variation (A, B) is σ_B and the standard deviation of duplicates taken from an independent sample is σ_W then the standard error of the estimated average for the type and source is:

$$se = \sqrt{(\sigma_B^2)/2 + (3.\sigma_W^2)/8}$$

Equation 2

We can say that an upper limit for a value of se for a useful measurement result which provides evidence that an average concentration is above a limit of assurance that an average concentration is below a limit is:

$$se \le |\text{limit} - \text{estimate}| \div 2$$

Equation 3

The critical difference (p=0.05) for the difference between the mean of B1, B2 and A (i.e. the observed difference between independent samples) is given by:

$$diff_{95} \le 2\sqrt{2.\sigma_B^2 + (3.\sigma_W^2)/2}$$

Equation 4

We were unable to undertake enough replicate analyses to estimate values of σ_B and σ_W within the budget of the project. Hence, we are limited to identifying cases where the variation between results appears to be large compared with the variation we need to be confident about whether a mean is above or below a limit. This is because the results produced by only two independent sample may be close to each other by chance even when the underlying variation is large.

The variation we observe between samples has two sources:

- Between-sample variation: this is variation in the concentration of analyte between samples despite our efforts to create samples that contained the analyte at the mean concentration in the bulk.
- Analytical variation: the variation we might see between measurement results even where the concentration in samples is the same.

We expect that analytical variation for chemicals will be no higher than approximately 20% relative standard deviation, based on the Modified Horwitz Relative standard deviation of 22% for analytes at very low concentrations (Thompson, 2000). We also expect that the analytical variation for microbiological analytes will be no higher than a standard deviation of 0.5 Log_{10} units for microbiological analytes (Public Health England). Hence, where between-sample variation is no larger than analytical variation we expect that for the particular patterns of replication that we employed that for chemical analytes, the difference between replicate samples (divided by the mean result) to be no larger than 0.58 (95% confidence, estimated by simulation). For microbiological counts we expect the difference between duplicate samples to be less than 1.44 Log_{10} units. Table 10 shows instances where the difference is exceeded.

The majority of between replicate variation was found for the rearing substrate. As previously hypothesised, this is likely to be due to the rearing substrate being less homogenous than the larvae and the frass. It is therefore recommended that when assessing contaminants in rearing substrate a greater number of samples are assessed.

Table 10 (A–O). Chemicals and rearing substrate, larvae or frass where the between replicate variation exceeds that expected based on the between sample relative standard deviation being no higher than 20% for chemical contaminants and an underlying standard deviation of 0.5 Log_{10} units for microbiological contaminants.

A. Metals: Rearing substrate

Trial	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Catering	Sodium	6	6	14380	20400	-0.04868	-0.6638
Catering	Aluminium	6	6	4.762	6.9	-0.1155	0.8767
Catering	Titanium	6	4	0.25	0.5	0	1.2
Catering	Vanadium	6	5	0.01625	0.03	-0.3077	1.077
Catering	Total chromium	6	6	0.15	0.2	0	0.6667
Catering	Cobalt	6	6	0.01387	0.019	-0.03605	0.6669
Catering	Arsenic	6	6	0.07475	0.139	-0.02676	1.679
Catering	Yttrium	6	6	0.002125	0.003	-0.2353	0.8235
Catering	Zirconium	6	2	0.006	0.016	0	2
Catering	Tin	6	6	2.968	4	0.01011	0.6924
Catering	Antimony	6	5	0.00425	0.006	-0.4706	0.5882
Catering	Lanthanum	6	6	0.003	0.004	0	0.6667
Catering	Cerium	6	6	0.005	0.007	0	0.8
Catering	Neodymium	6	6	0.002	0.003	0	1
Catering	Tungsten	6	2	0.002	0.004	0	2
Catering	Thallium	6	3	0.002	0.006	1.5	-2
Catering	Lead	6	6	0.008375	0.011	0.0597	0.6269
Catering	Bismuth	6	6	0.00625	0.01	0.48	1.2
Catering	Thorium	6	1	0.00025	0.001	0	2

Trial	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Manufacturing	Calcium	6	6	826.2	1210	-0.03026	-0.8745
Manufacturing	Titanium	6	2	0.05	0.2	-4	-2
Manufacturing	Vanadium	6	4	0.01	0.02	0	-2
Manufacturing	Cobalt	6	1	0.000625	0.005	4	-2
Manufacturing	Nickel	6	4	0.02625	0.06	0.1905	-2
Manufacturing	Strontium	6	6	0.7012	1	-0.0927	-0.7594
Manufacturing	Yttrium	6	6	0.0015	0.002	0	-0.6667
Manufacturing	Zirconium	6	1	0.001125	0.009	4	-2
Manufacturing	Molybdenum	6	6	0.07488	0.107	-0.0601	-0.7579
Manufacturing	Cadmium	6	6	0.01025	0.014	0	-0.7317
Manufacturing	Barium	6	6	0.4063	0.56	-0.1108	-0.6461
Manufacturing	Lanthanum	6	6	0.00175	0.003	0.5714	-0.8571
Manufacturing	Cerium	6	6	0.00325	0.006	0.9231	-0.7692
Manufacturing	Neodymium	6	5	0.00125	0.003	1.6	-1.2
Manufacturing	Hafnium	6	1	5.00E-04	0.002	0	2
Manufacturing	Platinum	6	1	0.00025	0.001	0	2
Manufacturing	Thorium	6	2	0.00025	0.001	4	-2
Manufacturing	Uranium	6	6	0.002125	0.004	-0.2353	-1.059
Poultry manure	Iron	6	6	2076	3830	-0.02119	1.618
Supermarket	Praseodymium	6	2	0.00025	0.001	4	-2
Supermarket	Gadolinium	6	2	0.001125	0.007	-4	-2
Supermarket	Platinum	6	4	0.009375	0.019	0.16	1.84
Supermarket	Thorium	6	2	0.00025	0.001	4	-2

B. Metals: Larvae

Trial	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Catering	Yttrium	6	2	5.00E-04	0.002	-4	-2
Catering	Niobium	6	3	0.00075	0.002	-1.333	-2
Catering	Lanthanum	6	2	5.00E-04	0.002	-4	-2
Catering	Cerium	6	6	0.001875	0.004	-0.8	-0.9333
Catering	Neodymium	6	2	0.00025	0.001	-4	-2
Manufacturing	Lithium	6	5	0.004	0.006	0	-0.75
Manufacturing	Titanium	6	1	0.025	0.2	4	-2
Manufacturing	Total chromium	6	2	0.05	0.1	0	2
Manufacturing	Arsenic	6	3	0.002125	0.006	-1.647	-2
Manufacturing	Niobium	6	2	5.00E-04	0.002	-4	-2
Manufacturing	Lanthanum	6	6	0.00175	0.003	0.5714	-0.8571
Manufacturing	Cerium	6	6	0.003	0.005	0.6667	-0.6667
Manufacturing	Neodymium	6	6	0.0015	0.002	0	-0.6667
Manufacturing	Platinum	6	2	0.00175	0.007	4	-2
Supermarket	Zirconium	6	5	0.01375	0.02	-0.3636	0.9091
Supermarket	Niobium	6	2	5.00E-04	0.002	4	-2

C. Metals: Frass

Trial	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Catering	Total chromium	6	6	0.15	0.2	0	-0.6667
Manufacturing	Titanium	6	3	0.175	0.3	-0.5714	1.429
Manufacturing	Total chromium	6	2	0.125	0.3	0	2
Manufacturing	Arsenic	6	1	0.00125	0.005	0	2
Manufacturing	Platinum	6	2	0.00125	0.005	-4	-2
Poultry manure	Calcium	6	6	9442	12800	-0.04872	0.7112
Poultry manure	Palladium	6	2	0.005	0.01	0	2
Supermarket	Europium	6	1	0.000125	0.001	4	-2
Supermarket	Gadolinium	6	6	0.004	0.008	0.25	0.75
Supermarket	Erbium	6	2	0.00025	0.001	0	-2

D. Mycotoxins: Rearing substrate

Trial	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Roquefortine C	6	3	9.75	18.2	-.6399	1.36
Catering	Cyclopiazonic Acid	6	1	21.38	171	-3.999	-2
Manufacturing	Moniliformin	6	6	225.1	394.6	0.292	-1.063

E. Mycotoxins: Larvae

Trial	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Manufacturing	Wortmannin	6	4	86.62	180.4	- 0.114	-2

F. PAHs: Rearing substrate

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Substrate	Acenaphthene	6	5	0.28	0.39	0.03571	-0.6429
Catering	Substrate	Fluoranthene	6	6	1.661	2.33	0.1776	0.6668
Catering	Substrate	Pyrene	6	5	2.234	3.18	0.8393	0.8024
Catering	Substrate	X5.methylchrysene	6	1	0.0075	0.03	0	2

G. PAHs: Larvae

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Larvae	Acenaphthene	6	1	0.35	1.4	0	2
Catering	Larvae	Fluorene	6	2	0.7512	3.07	-4	-2
Catering	Larvae	Fluoranthene	6	4	0.64	0.99	-0.9375	1.062
Catering	Larvae	Pyrene	6	4	1.651	2.65	-0.7965	1.204
Catering	Larvae	Benzo.e.pyrene	6	2	0.02	0.04	0	2
Catering	Larvae	Benzo.ghi.perylene	6	4	0.05625	0.1	-0.4444	1.556
Catering	Larvae	PAH.4.SUM.Upper	6	6	0.4725	1.02	1.651	-0.6455

H. Nitrates/nitrites: Rearing substrate

Trial	Analyte	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Catering	Nitrite	6	2	29.55	60.98	0	2
Manufacturing	Nitrate	6	6	22.1	37.17	-0.02296	-1.308

I. Nitrates/nitrites: Frass

Trial	Analyte	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Poultry manure	Nitrite	6	3	5.061	15.21	-0.4214	-0.5841

J. Pesticides: Rearing substrate

Trial	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Catering	2.phenylphenol	6	4	0.006112	0.0127	-0.05726	-2
Catering	azoxystrobin	6	2	0.01112	0.02257	0	2
Catering	DDAC	6	2	0.06132	0.12635	0	2
Catering	fludioxonil	6	2	0.009833	0.0199	0	2
Catering	imazalil	6	2	0.02436	0.05106	0	2
Catering	pyrimethanil	6	2	0.04922	0.09875	0	2
Catering	thiabendazole	6	2	0.01659	0.03323	0	1.999

Trial	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Manufacturing	BAC12	6	4	0.02899	0.0615	-0.1845	-2
Manufacturing	DDAC	6	4	0.09175	0.19395	-0.2027	-2
supermarket	DDAC	6	6	0.2693	0.37	0.02488	0.653
supermarket	pyraclostrobin	6	5	0.007761	0.01057	0.02641	-0.6897

K. Pesticides: Larvae

Trial	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Poultry manure	2.phenylphenol	6	4	0.03687	0.0789	-0.1469	-2
Poultry manure	diphenylamine	6	2	0.005558	0.01162	0	2
supermarket	diphenylamine	6	2	0.006452	0.01313	0	2

L. Pesticides: Frass

Trial	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Within replicate difference	Between replicate difference
Poultry manure	BAC16	6	4	0.02658	0.0556	0.02107	-2

M. Veterinary medicines: Rearing substrate

Trial	Veterinary medicine	Number of injections	Detecte d	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Dinitrocarbanilide nicarbazin.	6	4	12.25	27	0.08163	-2

N. Veterinary medicines: Frass

Trial	Vet. Med.	Number of injections	Detecte d	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Narasin	6	6	5.09	20	-2.211	-0.8599

O. Microbiological counts: Frass (expected difference between duplicate samples to be less than 1.44 Log_{10} units, based on an underlying standard deviation of 0.5 Log_{10} units)

Trial	Organism	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Within replicate difference	Between replicate difference
Catering	Total *E.coli*	6	6	3.71	4.436	0.532	1.887
Manufacturing	Total Enterobacteriaceae	6	6	2.885	5.27	-1.659	3.056

Conclusions and recommendations for further studies.

This study has evaluated the chemical and microbiological contaminants in currently non-permitted rearing substrates for BSF larvae for production of protein for animal feed, the larvae produced and the remaining residue from the process (frass). The materials tested represented four different categories of substrate that are suitable for rearing of BSF. However, only a single source from each category was evaluated in this study and the samples obtained were taken over a short time period. This should be kept in mind when assessing the results.

Analytical methods screened for 745 chemical analytes, the presence and enumeration of key microbial organisms was assessed, and non-targeted screens were used to assess the presence of natural toxins and viral RNA that were present in the samples.

A summary of the number of chemical compounds found in the different sample types is provided in Tables 11-13. It should be noted that the same compound is not necessarily present in the rearing substrate, the larvae and the frass.

Table 11. Number of compounds detected in samples of currently permitted and non-permitted rearing substrates.

	Permitted		Non-permitted			
	Core diet	Core diet + 20%	Catering	Manufacturing	Poultry manure	Supermarket
Heavy metals (cadmium, arsenic, mercury or lead)	3	3	3	2	3	3
Veterinary medicines	0	0	2	0	3	2
Pesticides	5	7	8	2	7	12
Mycotoxins	0	2	2	1	3	4
PAHs	NF	NF	16	NF	NF	NF
Nitrite	1	1	1	1	1	1
PFAS	2	4	3	2	9	4

NF = not found

Table 12. Number of compounds detected in larvae reared on currently permitted and non-permitted substrates.

	Permitted		Non-permitted			
	Core diet	Core diet + 20%	Catering	Manufacturing	Poultry manure	Supermarket
Heavy metals (cadmium, arsenic, mercury or lead)	3	3	3	3	3	3
Veterinary medicines	0	0	0	2	3	0
Pesticides	1	0	0	0	6	10
Mycotoxins	1	0	0	1	0	0
PAHs	NF	NF	7	NF	NF	NF
Nitrite	0	1	0	0	1	1
PFAS	3	3	3	2	9	3

NF = not found

Table 13. Number of compounds detected in samples of frass produced from currently permitted and non-permitted rearing substrates.

	Permitted		Non-permitted			
	Core diet	Core diet + 20%	Catering	Manufacturing	Poultry manure	Supermarket
Heavy metals (cadmium, arsenic, mercury or lead)	3	3	3	3	3	3
Veterinary medicines	0	0	2	0	3	4
Pesticides	2	0	1	0	7	10
Mycotoxins	1	1	1	1	2	3
PAHs	NF	NF	15	NF	NF	NF
Nitrite	0	0	0	0	1	1
PFAS	1	2	3	3	5	3

NF = not found

Of the chemical analytes screened for a total of 101 were found in the larvae. The majority of these were metals (58). Of the analytes found, there were no exceedances for any of the chemicals where maximum limits are specified in feed materials of animal origin. However, some pesticide residues exceeded the MRLs for terrestrial invertebrates. This may have implications if the larvae were to be used as food.

The only regulatory limit exceeded for feed ingredients of animal origin, was for the presence of Enterobacteriaceae in larvae reared on all four of the currently non-permitted substrates. As previously discussed, this was likely to be due to the minimal processing used to kill the larvae. The regulatory levels specified refer to samples taken after the application of a processing method and therefore the results from this study illustrate that processing is required to reduce the microbial load. It is likely that processing methods typically used by industry for the production of insect protein would significantly reduce the level of these organisms, as demonstrated by the results from the baseline samples. However, this would need to be confirmed and supported by HACCP and GMP procedures.

Although the levels of contaminants were below regulatory limits for feeding stuffs of animal origin, there was evidence of bioaccumulation in the larvae for some compounds. Cadmium was shown to bioaccumulate in BSF larvae as previously reported. For metals, there was also evidence of bioaccumulation of lead, calcium, phosphorus and some other metals. Although this may not have a direct safety concern, this may have implications (positive or negative) for some metals that serve as macro- or micronutrients. There was also evidence of bioaccumulation of DDAC and haloxyfop in larvae reared on poultry manure. Other contaminants such as mycotoxins and PAHs also gave indication for potential bioaccumulation but variation between samples requires that further testing would be needed to ascertain whether this is the case.

As far as the authors are aware this is the first study that has examined the presence of PFAS through the insect bioconversion process using naturally occurring levels of these compounds. Some compounds were found in the rearing substrate, the larvae and the frass, but a more extensive study is recommended to confirm these findings. Further optimisation of the method is required to analyse the diverse range of substrates that may be used as a rearing substrate for insect bioconversion.

Commission Regulation (EU) No 142/2011 specifies that processed manure taken during or immediately after processing should comply with limits Salmonella spp. and *Escherichia coli* or Enterococcaceae. The maximum value of 1000 cfu/g was exceeded for Enterococcaceae in frass samples from all rearing substrates but was only exceeded for *E. coli* in frass from catering waste. These results indicate that further processing of the frass from currently permitted and non-permitted substrates is required to conform to regulatory requirements.

The non-targeted viral screen confirmed the presence of RNA from plant and animal pathogens in larvae and frass, but the infectivity of these viruses could not be elucidated from this study. Infection studies should be carried out to determine if this is a risk, particularly for plant pathogens when frass is used as a fertiliser.

The non-targeted toxin screen demonstrated the presence of natural toxins such as solanidine in larvae. Currently there are no regulatory limits for the presence in animal feed and an EFSA risk assessment concluded that a risk characterisation of potato glycoalkaloids in feed for farm and companion animals was not possible due to insufficient data on potential adverse effects in these species (EFSA, 2020). Data on the presence of these compounds in a range of animal feeds is therefore required before an assessment of the levels found in insect larvae can be evaluated. The non-targeted toxin screen also tentatively identified other natural toxins in larvae reared on currently permitted and non-permitted substrates, but scores for these were generally low (5-7) and further work would be needed to confirm the identity of these compounds.

It was noted that several compounds were not detected in the rearing substrate but were detected in the larvae and/or the frass. It is considered that this may be due to the greater homogeneity of the larvae and the frass samples compared with the rearing substrate such that there may have been a more even distribution of any contaminant present.

Examination of the variation in results between replicates showed that the majority of between replicate variation was found for the rearing substrate. This is likely to be due to the rearing substrate being less homogenous than the larvae and the frass. It is recommended that when assessing contaminants in rearing substrate a greater number of samples are assessed.

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Appendix A - Summary of comments from consultee questionnaire

The question posed for each of the potential substrate categories was:

- Do you have any concerns for the use of this substrate from the point of view of:
  - Market acceptance
  - Substrate availability
  - Safety

The following sections provide the comments for each category where the response was "yes" and further details were provided. In addition, the comments on specific examples of the category type for inclusion in testing are provided.

Mixed food surplus from retail, which includes ABPs

Concerns:

- Inclusion of plastic and other waste packaging.
- Microbial contamination may be an issue particularly if surplus is stored for long periods of time. Salmonella may be present.
- I think the key objective in the first instance is to work to continue to reduce food surplus, additionally would this detract from food banks? I think market acceptance could be an issue but if its good enough for humans, why would it not be good enough for insects? In terms of safety, we would need to understand the potential pathogen risk. Additionally, would consistency of substrate be an issue?
- Need to rule out whether insects can transmit TSE if to be used in ruminant and poultry feed. Risk is low for this substrate given that any ABP present will be TSE free anyway due to existing feed regulations and risk is not applicable if end use is fish feed. Good potential here if found to be safe due to large volumes available.
- Acceptability/market pull will be the defining factor.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Must include representative ABPs - e.g. meat-containing sandwiches, ready meals / processed foods, raw meats etc.; at levels representative of average retailer waste compositions (e.g. if 8-10% of waste occurring in retailer X, that should be level we test in a diet).
- Preferably waste which has no secondary use as of yet (i.e. challenging to be unprocessed to pet food or animal food).
- Mixed streams that may include meat products.
- Examples to be tested should be segregated into Mixed food surplus which contains poultry, pig and fish and that which contains ruminant ABPs. Time and method of storage before testing should be considered.
- Foods where the ABP component is in low quantity, for example in-store prepared and packaged sandwich waste, or long shelf-life foods like pizza with meat topping.

Mixed food manufacturing surplus, which includes ABPs

Concerns:

- Would need for this to result in EU approved end-products.
- Microbial contamination may be an issue particularly if surplus is stored at ambient temp for long periods of time. Salmonella may be present.
- I think the key objective in the first instance is to work to continue to reduce food surplus, additionally would this detract from food banks? I think market acceptance could be an issue but if its good enough for humans, why would it not be good enough for insects? In terms of safety, we would need to understand the potential pathogen risk. Additionally, would consistency of substrate be an issue?
- As before re. surplus retail, but slightly reduced safety risk due to sterile conditions in manufacturing sites (i.e. retail waste can include food past sell by date / left out of fridges or freezers).
- Acceptability is critical.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- In many ways, risk profile of this and supermarket wastes containing ABPs is the same. Key difference is operational / logistics - supermarkets will need depackaging, this one likely won't.
- As for answer 1 -examples should be segregated 1. those that contain chicken, pig and fish and 2. this that contain ruminant (e.g. steak, lamb). In addition, attention should be paid to how long the surplus has been left before testing as this may affect safety: testing "fresh" versus "stored" should be considered.
- Rendered products.
- As with surplus from retail.

Mixed food surplus hospitality and food service that includes ABPs

Concerns:

- Substrate availability > a single restaurant may produce XXkg waste per day, are existing logistics in place to converge these wastes already in place? otherwise logistics could become challenging.
- Debris of glass or plastic waste in the left-overs, i.e. any non-organic matter which may be hazardous for on processing into animal feed / application as fertiliser of frass from rearing on such substrates.
- We need to ensure our end-products will be suitable for use across the EU as the UK is not 100% of the end-market.
- Microbial contamination may be an issue particularly if surplus is stored at ambient temp for long periods of time. Salmonella may be present.
- As with retail including ABP.
- Acceptability is key.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- As for Qs 1 & 2 examples should be segregated - attention should be paid to how long the waste has been left before testing as this may affect safety: testing "fresh" versus "stored" should be considered.
- As with retail including ABP.

Food waste separated from domestic waste that includes ABPs

Concerns:

- Safety > Probably highest risk organic substrate in consideration by insect sector - largely due to the unknown unknowns of what could end up in the mix (batteries > heavy metals; biohazardous wastes, heavily spoiled meats/food > pathogenic bacteria, Salmonella).
- Market acceptance > derived larvae should be useful in non-food/non-feed applications; but hard to see how this would be possible for food / feed unless meticulously separated.
- Unknown source of material and contaminants potentially harmful to insect larvae.
- Harder to prove safety as handling of substrate is not as observable as with outputs of factories for example. May be decomposing faster bearing higher risk of mycotoxins for example. Harder to know what is included in substrate and therefore hard to communicate to end-customer, i.e. transparency may be harder as far as feedstock is concerned. Maybe more as a route into functional products as opposed to animal feed into human.
- We need to ensure our end-products will be suitable for use across the EU as the UK is not 100% of the end-market.
- Would need to have handling to ensure safety for material handling. Likely run through current waste management facilities first.
- Microbial contamination may be an issue particularly if surplus is stored at ambient temp for long periods of time. Salmonella could be present in food waste.
- Post consumer food waste offers good opportunity as so readily available, however traceability much more challenging and safety/contamination risks high even if food waste is separated from other domestic waste. Robust traceability really important for any feed/food products.
- Contamination will be an issue.

- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety. It would be important to support more councils to separate and collect domestic food waste to use as substrate.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- As for Q1-3 segregation should be considered.
- Fruit and vegetable waste without ABP as a first step, later expanding to include ABP if traceability can be assured.
- Household food waste bins

Poultry manure – layers

Concerns:

- Acceptance of food/feed applications of manure grown insects > cultural barriers potentially.
- Poultry manure - BSF - poultry feed cycle = market sensitivities. Potential pathogen issues.
- Would only consider if output from processing is for functional use. Safety and marketability will be hard to achieve.
- We need to ensure our end-products will be suitable for use across the EU as the UK is not 100% of the end-market. Potential end-market perception issue.
- Salmonella may be present.
- I think market acceptance would be the biggest hurdle in this instance. In terms of safety, we would need to understand the potential pathogen risk. I think in terms of availability and consistency of the substrate might actually be the main benefit of its potential use.
- Higher safety risk due than the achievable category due to manures being used, so stress on need for appropriate safety checks (and hence why this commission is very important and can help to address this question).
- Acceptance.

- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety. This is a priority substrate for us as it provides the volumes needed to replace soy used in feed. At the least, this this substrate can be used for pet food if acceptance remains low.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Composted and non-composted manure should be tested.
- Broilers and breeder.
- This substrate is a priority as it solves multiple environmental challenges, including water pollution.

Poultry manure – broilers

Concerns:

- Same to layers; albeit cycles vary (how old manure will be), it is also largely impacted by farmer system of manure removal.
- Same as for layers.
- Same as before, functional use only.
- We need to ensure our end-products will be suitable for use across the EU as the UK is not 100% of the end-market. Potential end-market perception issue.
- Salmonella may be present.
- I think market acceptance would be the biggest hurdle in this instance. In terms of safety, we would need to understand the potential pathogen risk. I think in terms of availability and consistency of the substrate might actually be the main benefit of its potential use.
- As with layers response.
- Acceptance.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptance can be built over time once the science shows safety. This is a priority substrate for us as it provides the volumes needed to

replace soy used in feed. At the least, this substrate can be used for pet food if acceptance remains low.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Composted and non-composted manure should be tested.
- This substrate is a priority as it solves multiple environmental challenges, including reducing water pollution.

Pig manure

Concerns:

- Same at other manures.
- Same as for poultry.
- Same as before - functional use only.
- Unlikely to be a good feedstock. Too many issues from a market perception, consistency, etc.
- Pigs eat a wide range of things -manure could contain microbiological hazards, including Salmonella.
- Safety component linked to TSE needs to be addressed/ruled out.
- Acceptance – consumers.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety. At the least, this this substrate can be used for pet food if acceptance remains low.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Composted versus non composted manure.

Cattle manure

Concerns:

- Poor performance on cow manures, much less nutrient dense compared to monogastric (broiler and pig) manures.
- Same concerns as for poultry/pig manures.
- Functional use only.
- Too many issues...
- As for previous manure questions presence of microbes may be an issue.
- Cattle link to TSE has high reputational risk for supply chain. Given the availability of other manures with reduced TSE link (i.e. poultry) could be better to focus on other manures. However this study would offer great value if the TSE risk can be addressed. One option is for insects fed on cattle manures to categorically not be used to feed cattle/dairy cows (i.e. used for fish feed instead).
- Consumers.
- The key measure for us is confirmation that this substrate is safe to use. Market and social acceptability can be built over time once the science shows safety. At the least, this this substrate can be used for pet food if acceptance remains low. The challenge of this substrate would be collection from extensive farming systems.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Composted and non-composted manure should be tested.

Anaerobic digestate – food based

Concerns:

- Very poor performance on conversion from trials in the past, likely uneconomical.
- Needs more explaining on this being safe for use and helpful from environmental perspective. No concerns other than additional education.
- Would need to understand nutritional (and antinutritional) factors.
- There may be safety risks associated with the use of non-pasteurised digestate.
- Main challenge on safety is traceability, but if solution found could be good source of substrate, especially given that current uses of digestate less

preferable/lower value than insect farming (and problems with excess nitrogen on land and eutrophication due to overuse of food based digestate).

- Consumers acceptance.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Pasteurised and non-pasteurised.

Anaerobic digestate – manure based

Concerns:

- Same concerns as for previous manures.
- Functional use only.
- There may be safety risks associated with the use of non-pasteurised digestate.
- Higher risk of contamination due to manures.
- Consumers.

Are there any specific examples of this substrate type that you would like to see tested within this category?

- Pasteurised and non-pasteurised.

Are there any substrate types which haven't been mentioned that you would ideally like to be examined?

- In order to create alternative feed sources that can reduce the pressure on soy from Brazil, all substrates should be considered. The key measure would be to understand the safety of the substrates from a scientific perspective. Once the first step of safety is addressed, the issues of social and market acceptance can be addressed through education, awareness and campaigns.

Do you have any further thoughts or considerations on this project you would like to share with us?

- If legislation did approve manure related substrates it is likely there would be pre-use requirements, such as heat treatment, odour treatments etc that would incur significant costs.
- Assessment should take place on larvae POST processing. The processing line will heat treat the product and therefore eliminate certain risk factors

which may present themselves in the unprocessed larvae. End-consumer, i.e. animal feed producer, will be evaluating the final product which they receive, not the larvae PRE processing. As long as the product (meal / oil / fertiliser) all adhere to safety standards and provide the relevant nutritional profile, there should be no consideration given to PRE-product stage (i.e. at harvesting).

- EU access and end-market perception are key challenges. Access to lower cost feedstocks are key opportunities.
- Every mixed waste stream will of course differ in nutritional content and this may affect the ability of the insects to develop- ideally the substrates to be tested would be comparable in terms of protein, fat etc but this is of course not possible if testing e.g. manure alone versus a mixed food waste stream (unless rearing samples are supplemented in order to reach comparability and this is not within the remit of the project) -care should be taken to analyse insect and frass samples obtained from comparable rearing efficiencies such that risks are assessed in an industry relevant manner.
- Need to consider the context in which the farmed insects will be used when considering what substrates are/aren't safe to use. If used feed, lower risks if used in poultry feed (i.e., vs cattle), negligible risk if used in fish feed. Consumer awareness of feed supply chains is low, and therefore insect fed would likely be viewed positively given that grazing on insects is part of freshwater fish + poultry + pig natural behaviour. Consumers unlikely to be concerned with what the insects themselves are fed given public awareness of feed supply chains is low, however it still needs to be safe and traceable.
- Insect farms should be viewed through the lens of alternative waste processing to AD, offering some advantages that AD does not, including upcycling material into a higher value end products relative to AD sludge. For example, value of kg of insect protein likely greater than value of kg of digestate. In terms of circular economy, circular food systems, and efficient resource use, insect farms also preferable to AD as closes loops in chains. Waste from insect farms (i.e., frass and to an extent chitin although this is also a co-product in its own right) is minimal and also marketable, particularly the frass, which is likely to support soil regeneration to a far better degree than AD sludge too.

- Given the importance of brand identity in foods I think there is a clear work programme to establish how consumers will react to some substrates - Popoff et al is a great example. Maybe get insects established as feeds using acceptable wastes before pushing the envelope/a study is required to understand market before extension.
- We would appreciate a prioritisation of the highest volume and more readily gathered substrate in this review. We would prioritise chicken manure from both layers and broilers as this is from intensive farming systems and have been linked to other environmental problems - such a river pollution. They can also offer the volume needed to replace the 3 million tonnes of soy from Brazil.

Appendix B - Metals

The aim of the analysis was to

- estimate the quantity of each element in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of (bio)accumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

Individual results are shown in the figures and the table shows the calculated quantities.

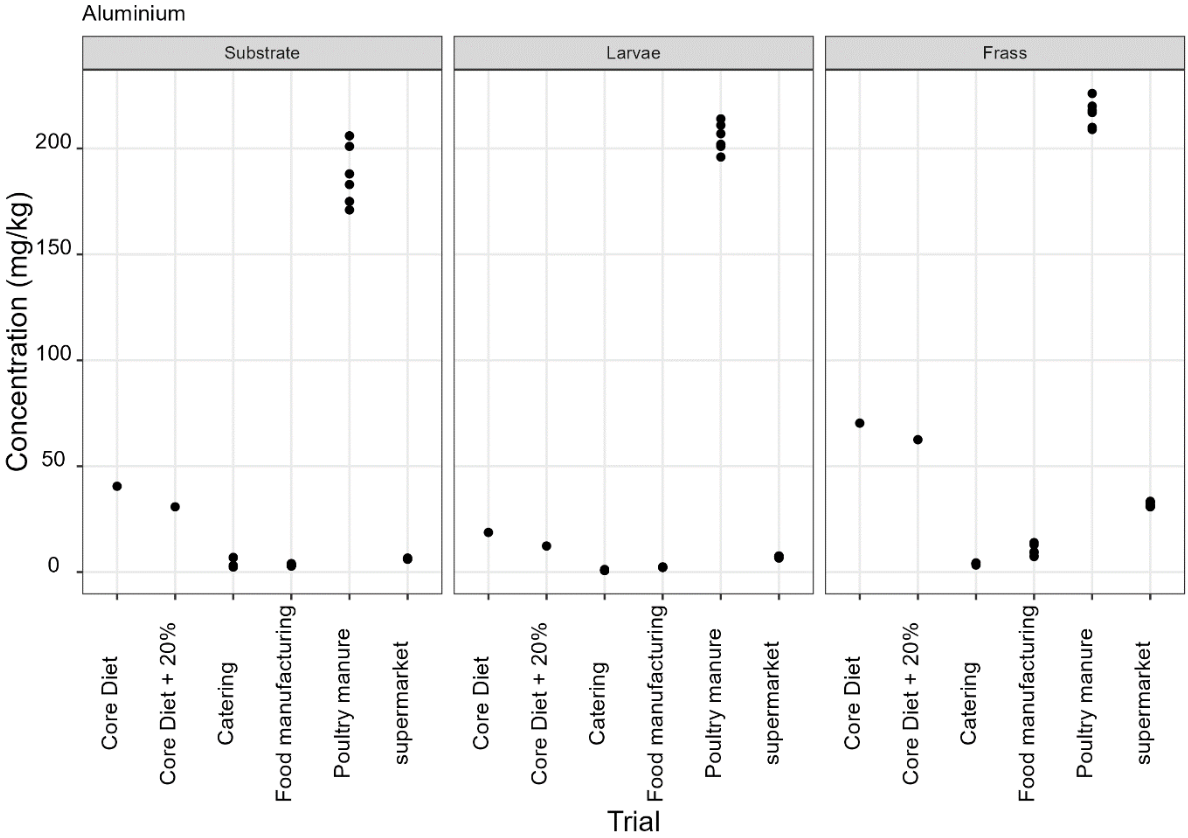
The reporting limits are shown below:

Table I. Reporting limits (limit of detection) for metals analysis.

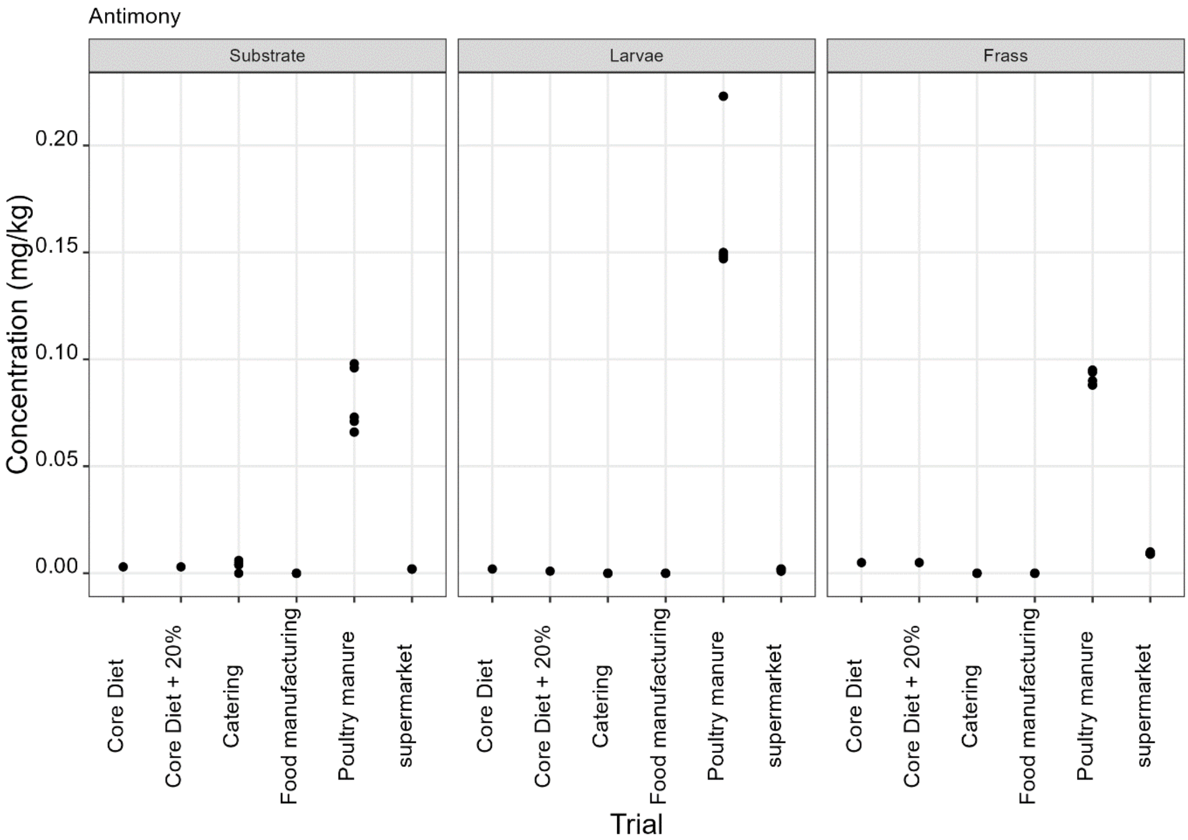
Metal	LoD mg/kg	Metal	LoD mg/kg	Metal	LoD mg/kg	Metal	LoD mg/kg
Lithium	0.005	Manganese	0.05	Molybdenum	0.005	Samarium	0.001
Beryllium	0.001	Iron	0.5	Ruthenium	0.001	Europium	0.001
Boron	1	Cobalt	0.005	Rhodium	0.005	Gadolinium	0.001
Sodium	5	Nickel	0.05	Palladium	0.02	Terbium	0.001
Magnesium	1	Copper	0.05	Silver	0.01	Dysprosium	0.001
Aluminium	0.5	Zinc	0.2	Cadmium	0.005	Holmium	0.001
Silicon	100	Gallium	0.01	Tin	0.02	Erbium	0.001
Phosphorus	10	Germanium	0.02	Antimony	0.002	Thulium	0.001
Sulfur	50	Arsenic	0.005	Tellurium	0.05	Ytterbium	0.001
Potassium	5	Selenium	0.01	Caesium	0.001	Lutetium	0.001
Calcium	5	Rubidium	0.01	Barium	0.01	Hafnium	0.001
Scandium	0.005	Strontium	0.05	Lanthanum	0.001	Tantalum	0.001
Titanium	0.2	Yttrium	0.001	Cerium	0.001	Tungsten	0.002
Vanadium	0.01	Zirconium	0.005	Praseodymium	0.001	Rhenium	0.001
Chromium	0.1	Niobium	0.002	Neodymium	0.001	Osmium	0.004
Iridium	0.001	Platinum	0.002	Gold	0.005	Mercury	0.01

Metal	LoD mg/kg	Metal	LoD mg/kg	Metal	LoD mg/kg	Metal	LoD mg/kg
Thallium	0.005	Lead	0.005	Bismuth	0.001	Thorium	0.001
Uranium	0.001						

1.


Aluminium
Substrate
Larvae
Frass
Concentration (mg/kg)
0
50
100
150
200
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

2.


Antimony
Substrate
Larvae
Frass
Concentration (mg/kg)
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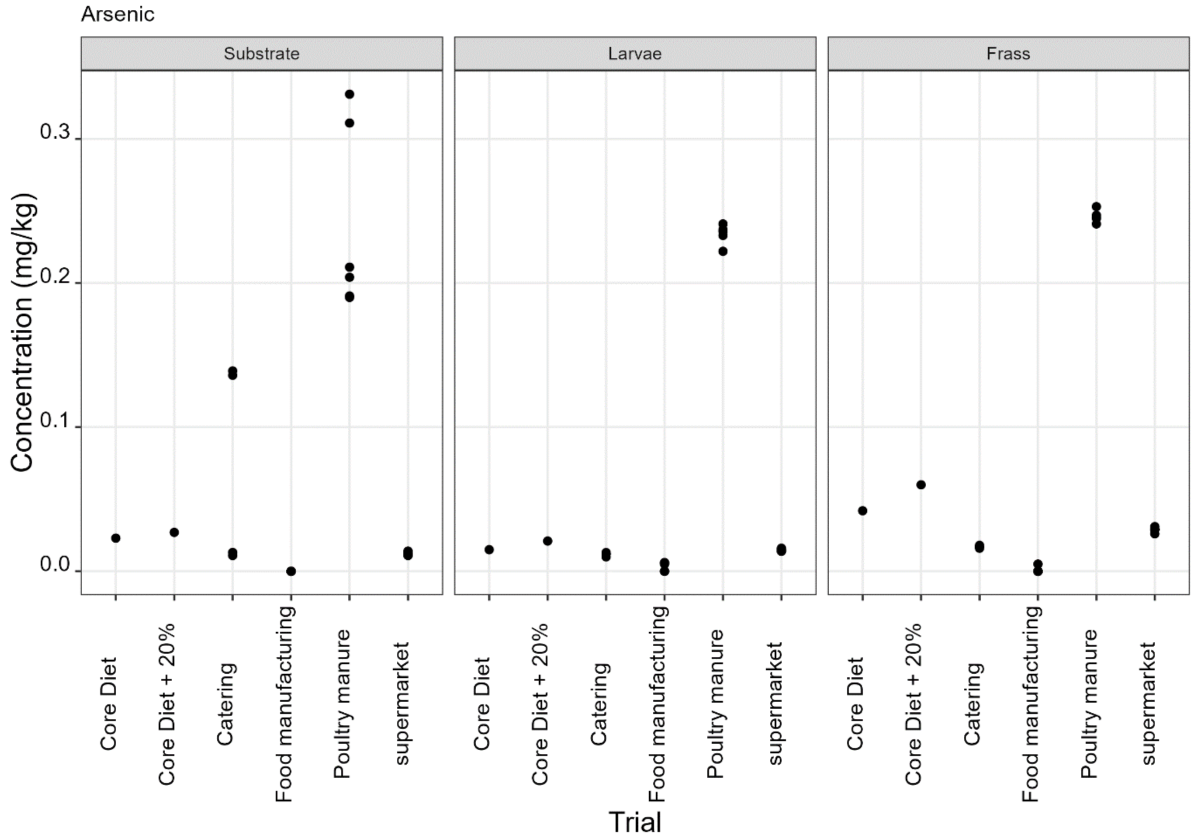
0.05
0.10
0.15
0.20
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

3.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Substrate
Larvae
Frass
Concentration (mg/kg)
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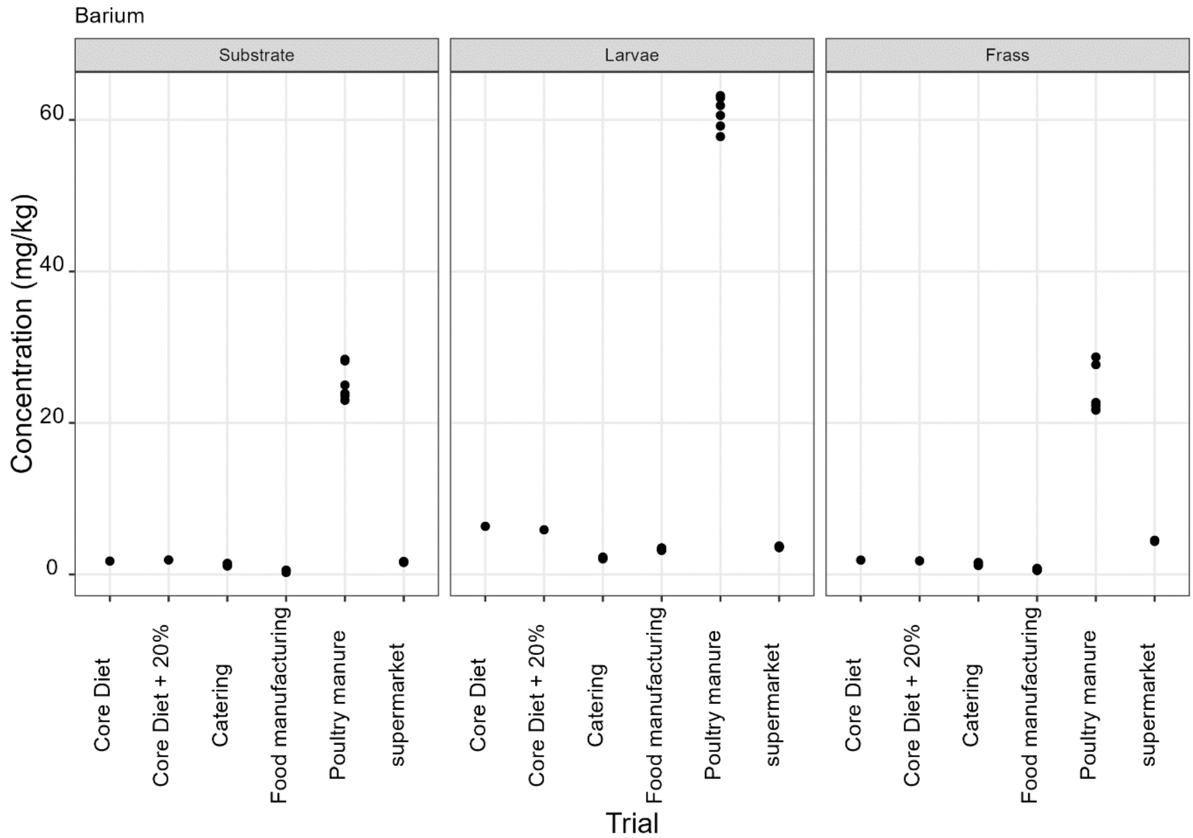
0.2
0.3
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

4.


Barium
Substrate
Larvae
Frass
Concentration (mg/kg)
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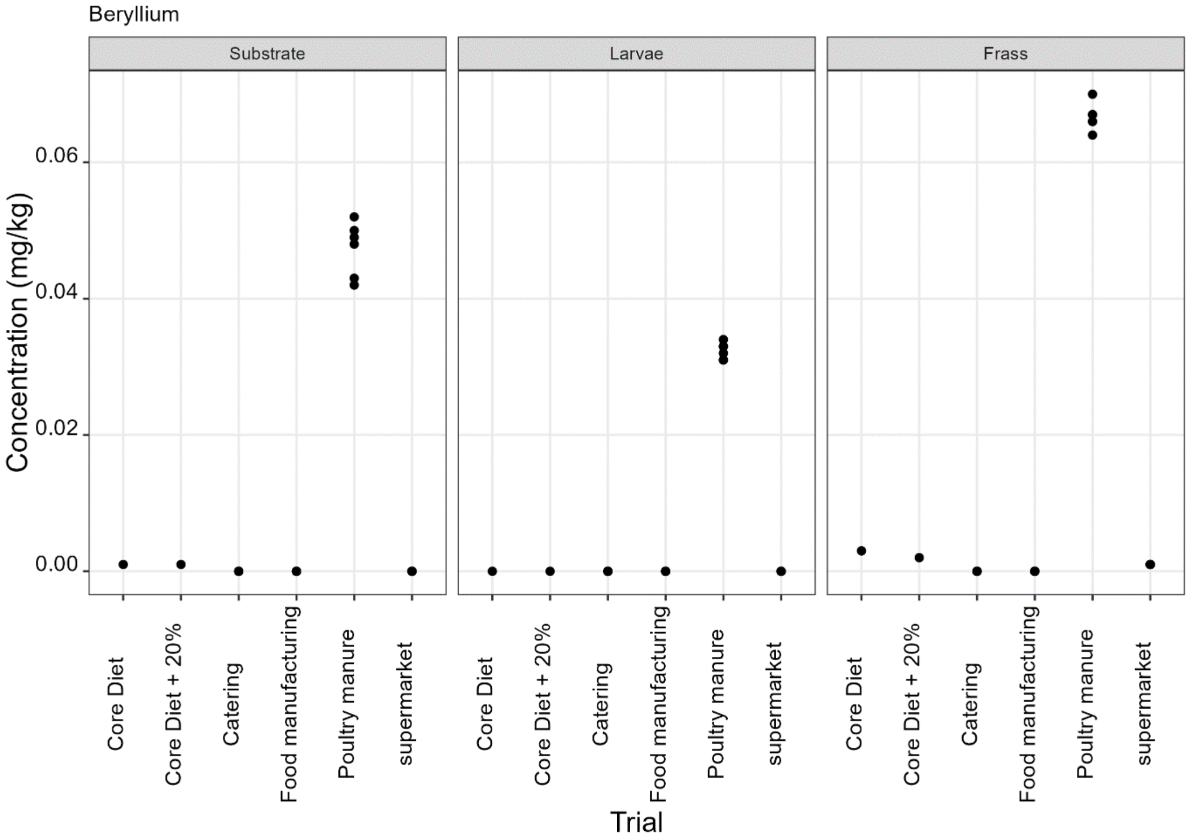
20
40
60
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

5.


Beryllium
Substrate
Larvae
Frass
Concentration (mg/kg)
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0.04
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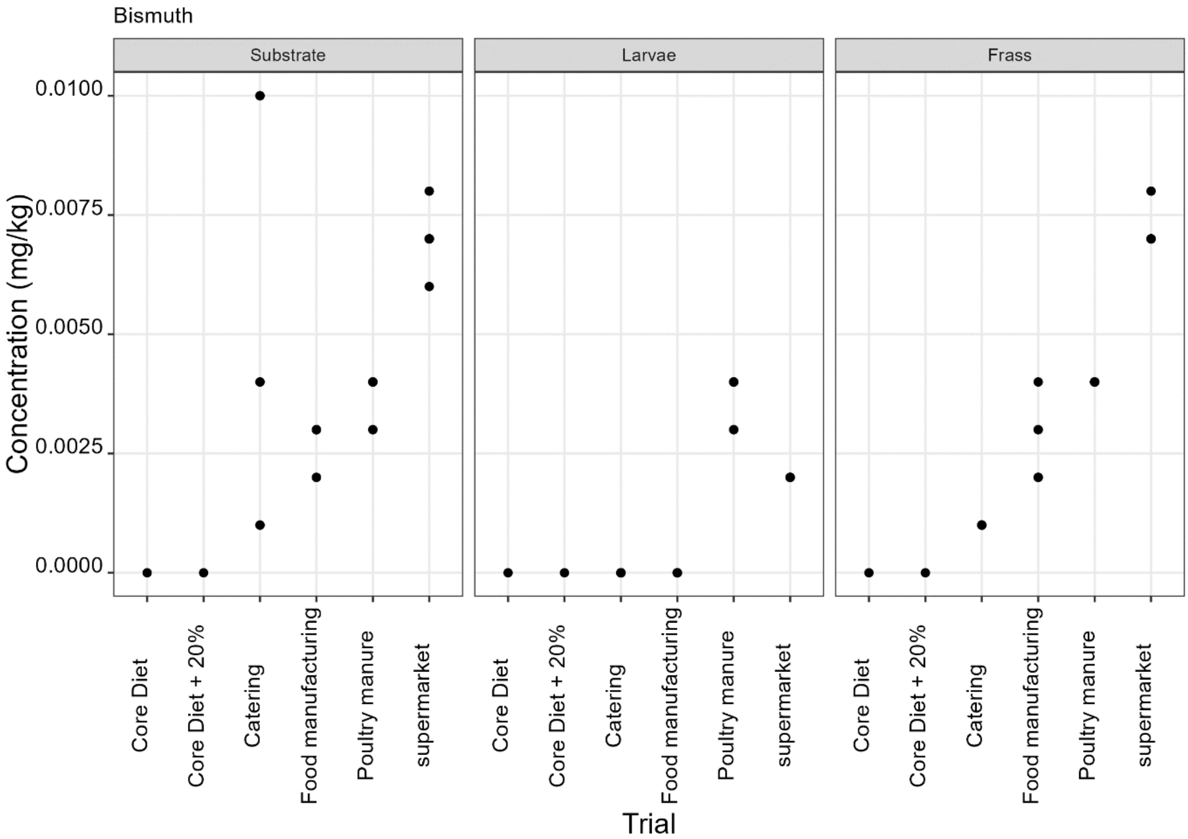
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

6.


Bismuth
Substrate
Larvae
Frass
Concentration (mg/kg)
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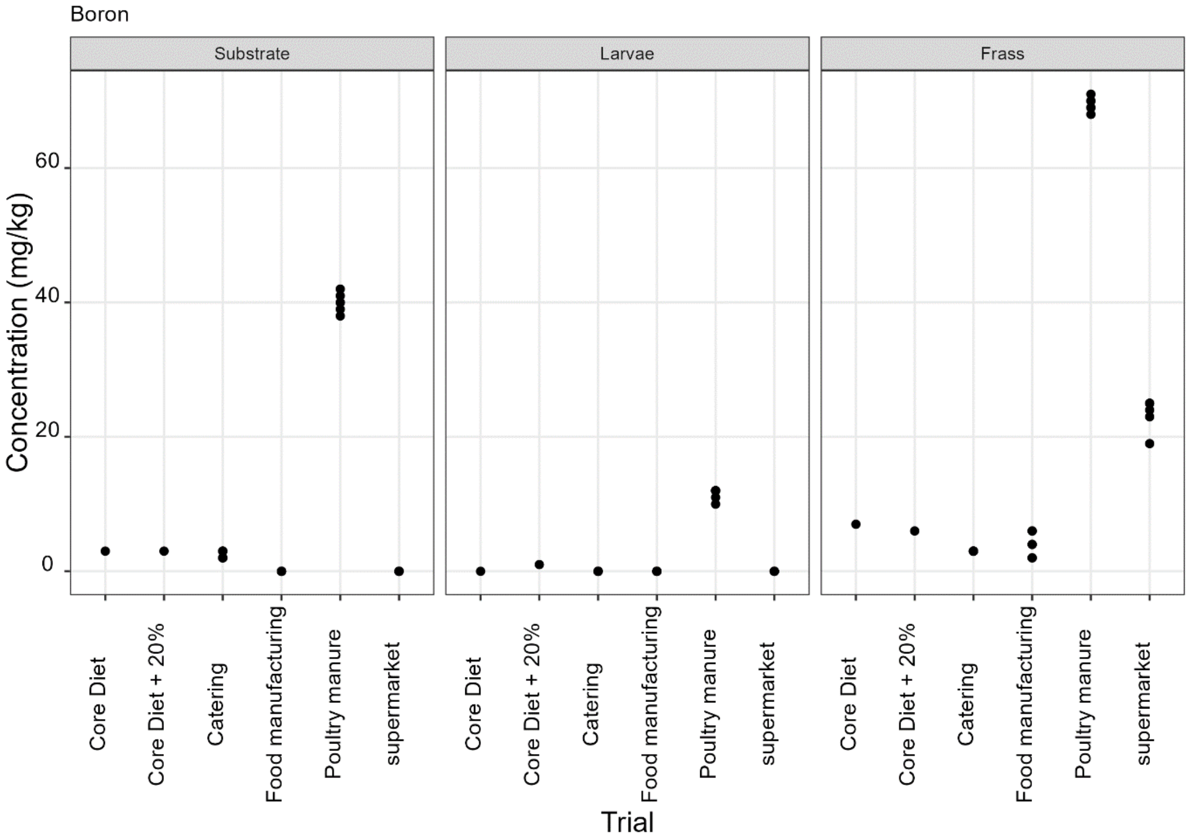
0.0075
0.0050
0.0025
0.0000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

7.


Boron
Substrate
Larvae
Frass
Concentration (mg/kg)
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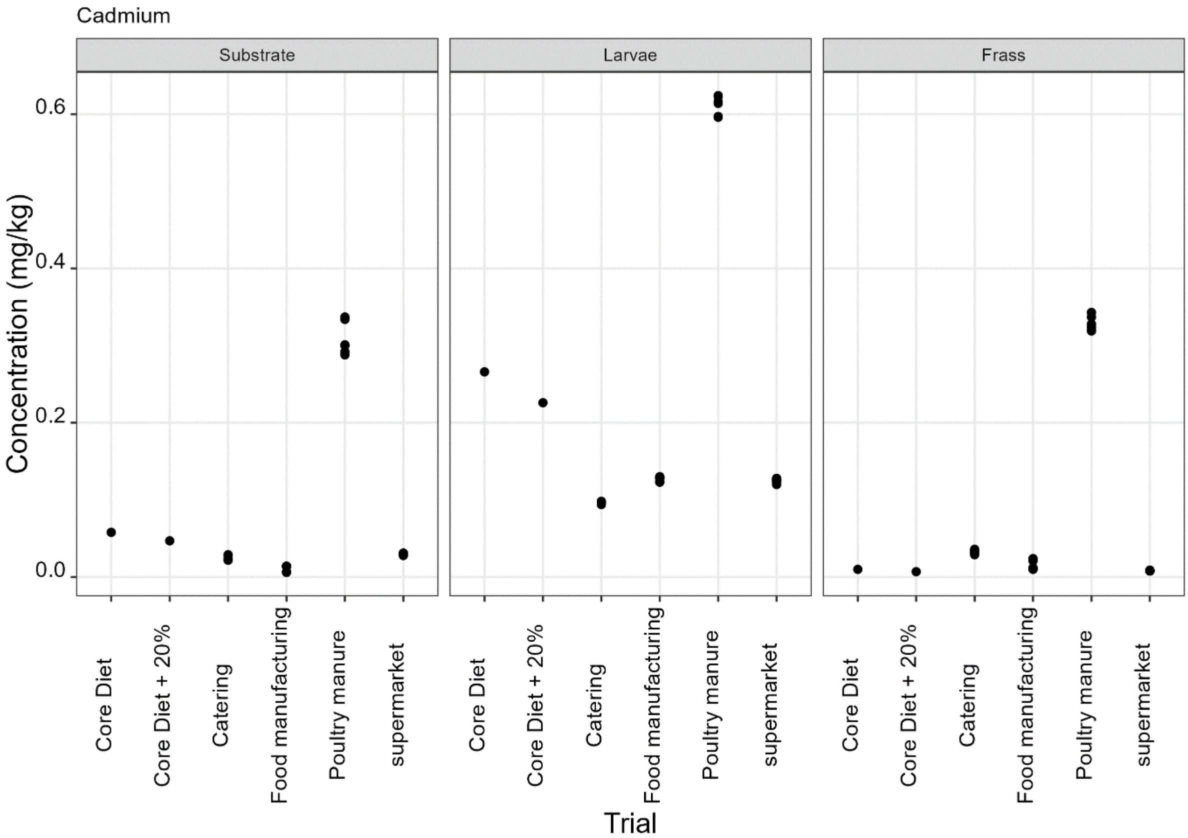
20
40
60
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

8.


Cadmium
Substrate
Larvae
Frass
Concentration (mg/kg)
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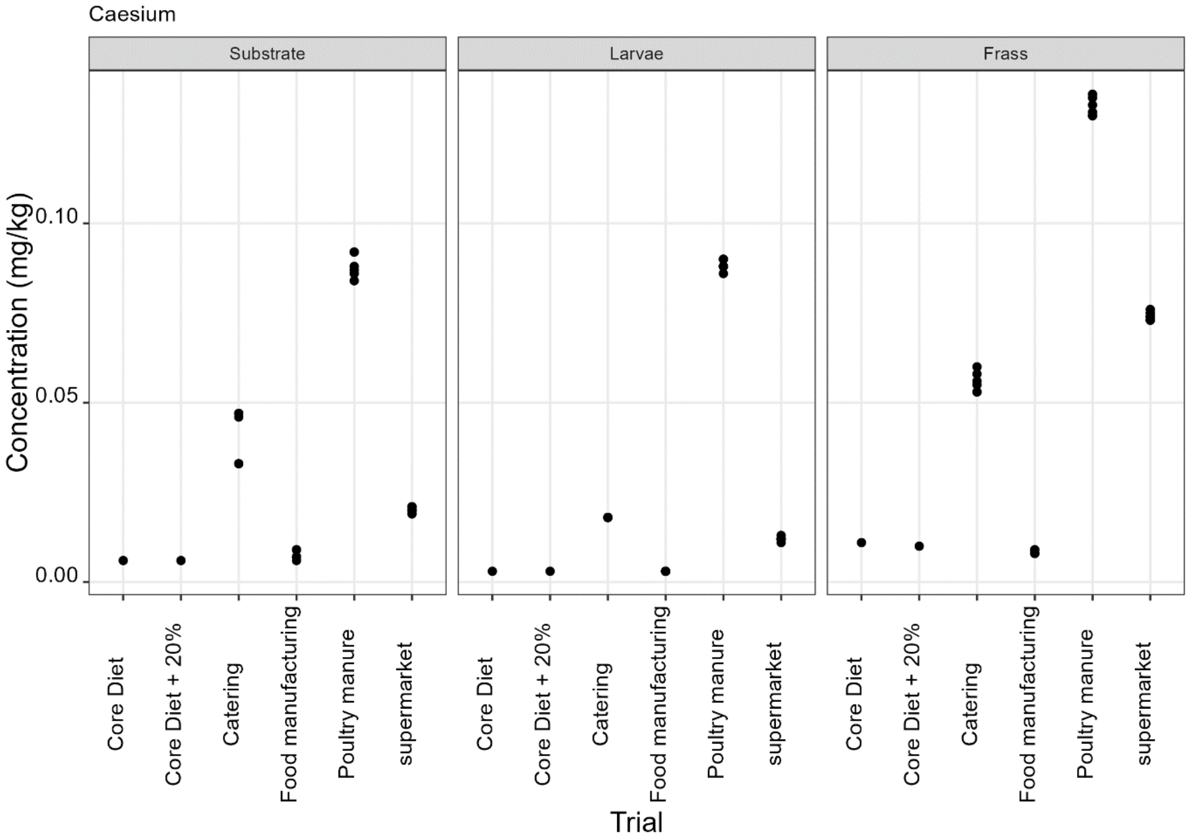
0.2
0.4
0.6
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

9.


Caesium
Substrate
Larvae
Frass
Concentration (mg/kg)
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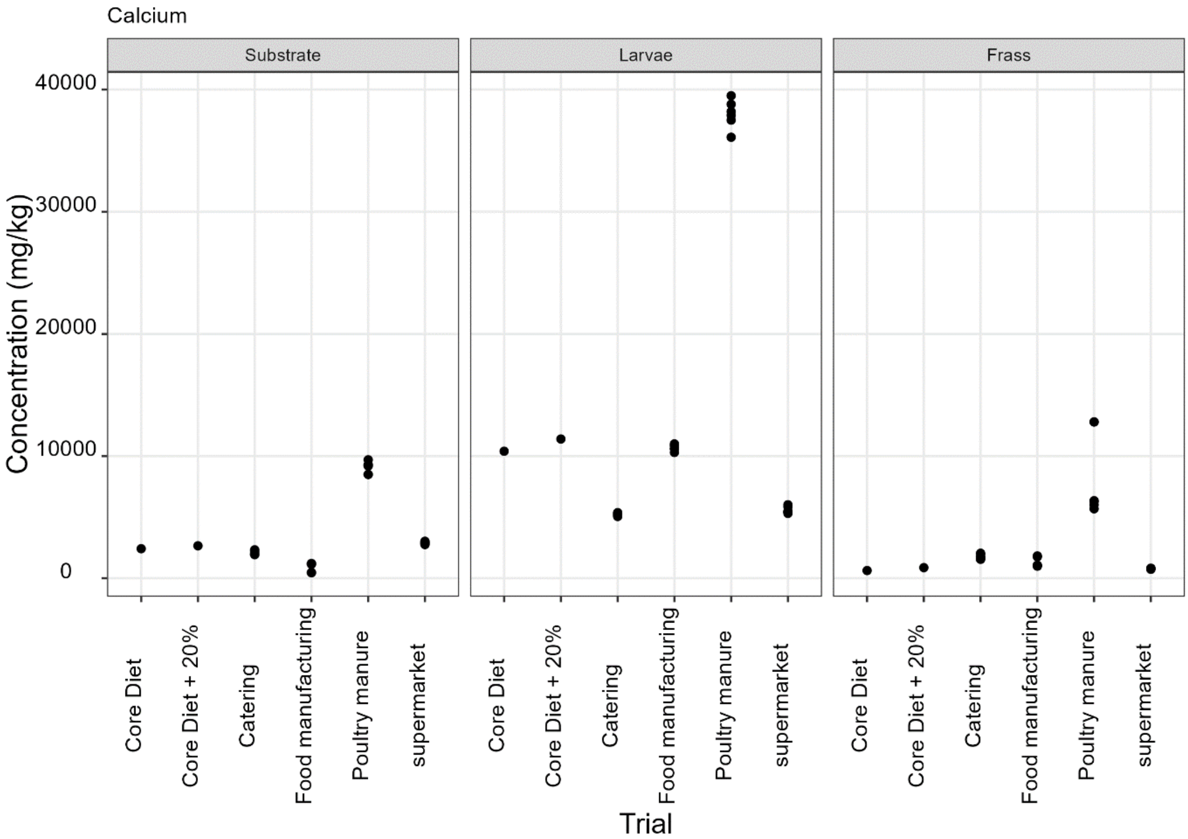
0.05
0.10
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

10.


Calcium
Substrate
Larvae
Frass
Concentration (mg/kg)
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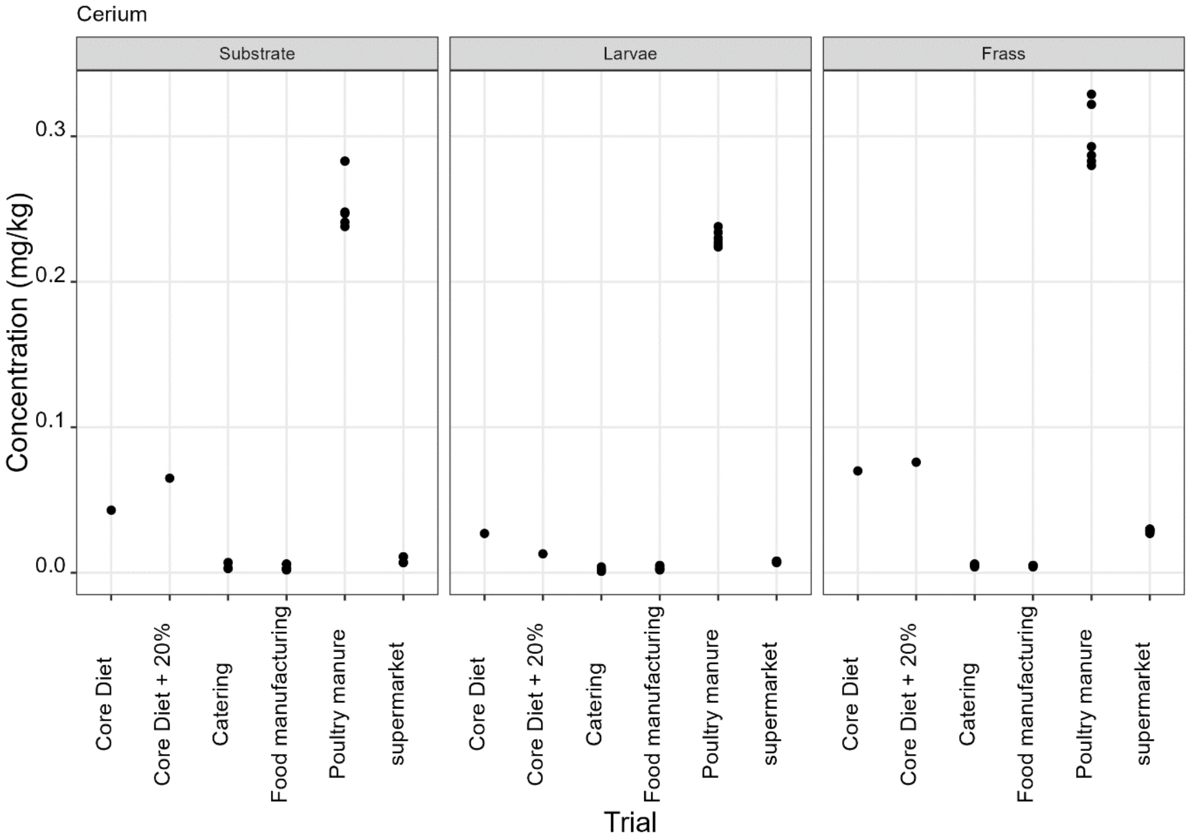
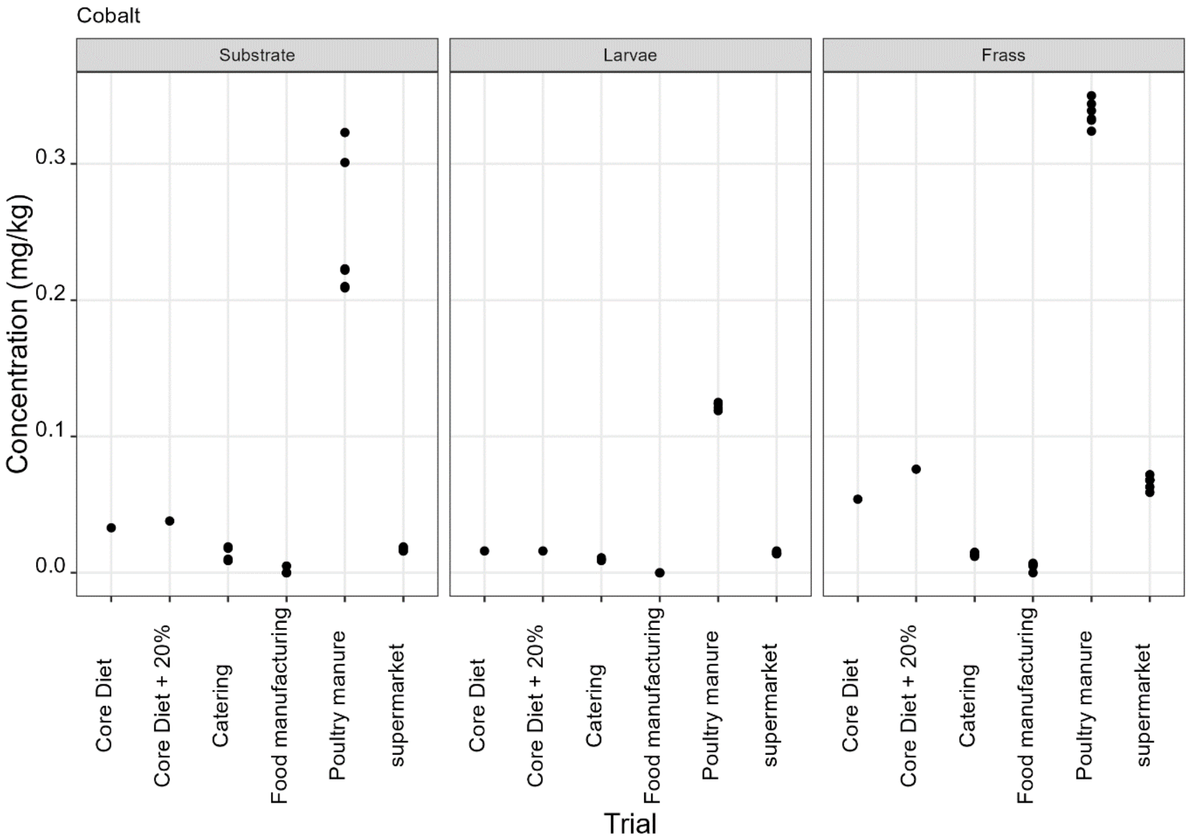
10000
20000
30000
40000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

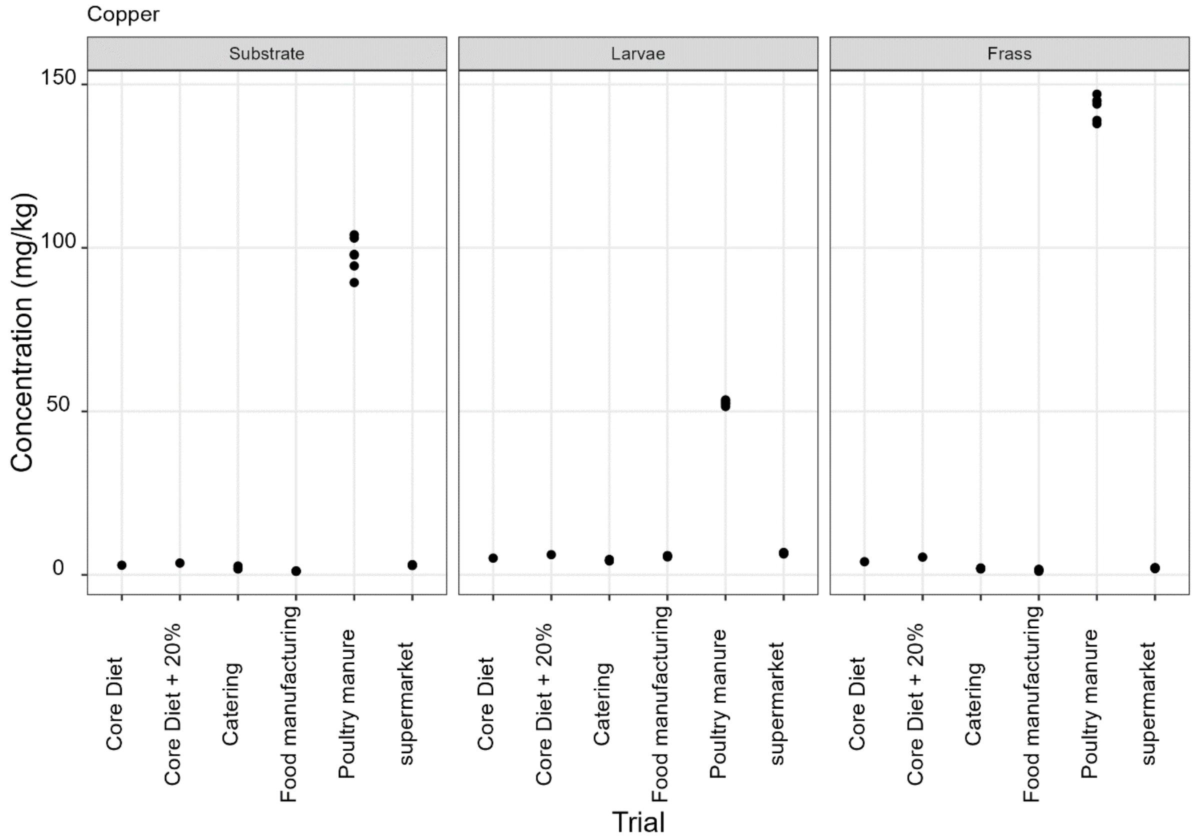
11.


Cerium
Substrate
Larvae
Frass
0.3
0.2
0.1
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

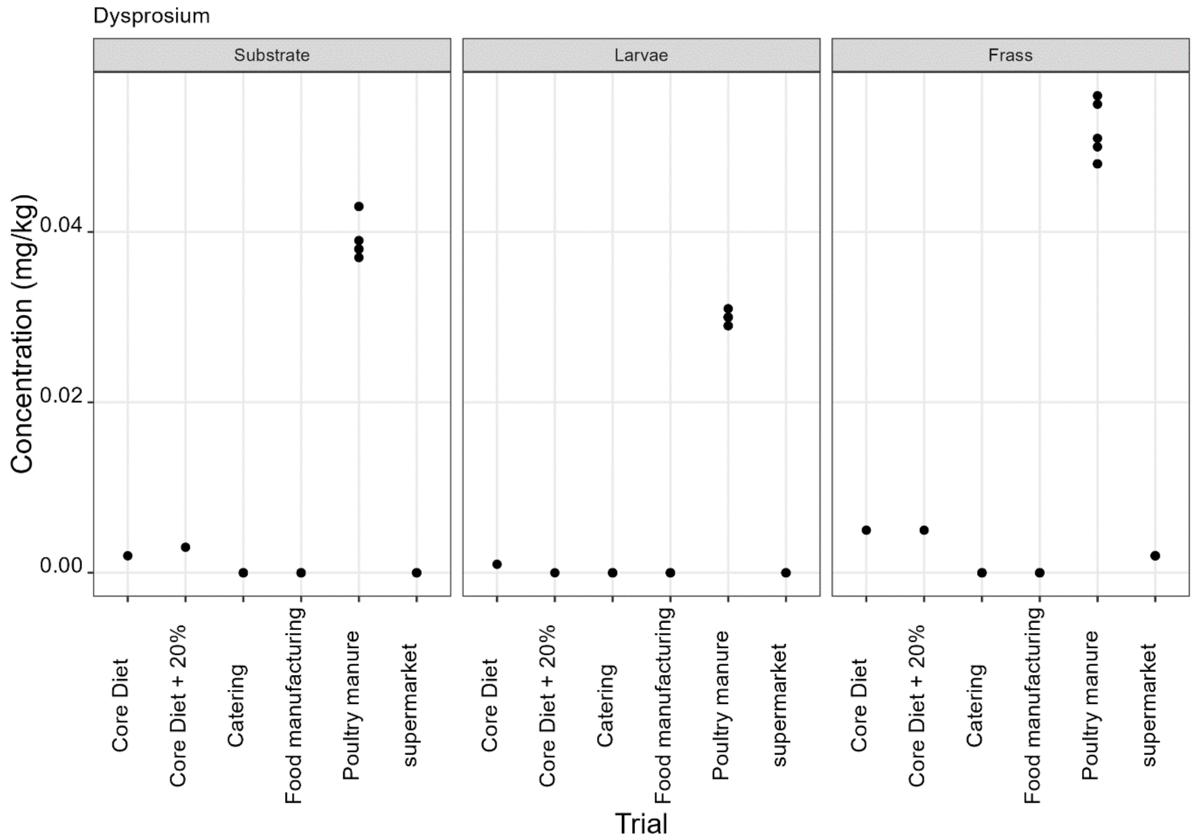
12.


Cobalt
Substrate
Larvae
Frass
0.3
0.2
0.1
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

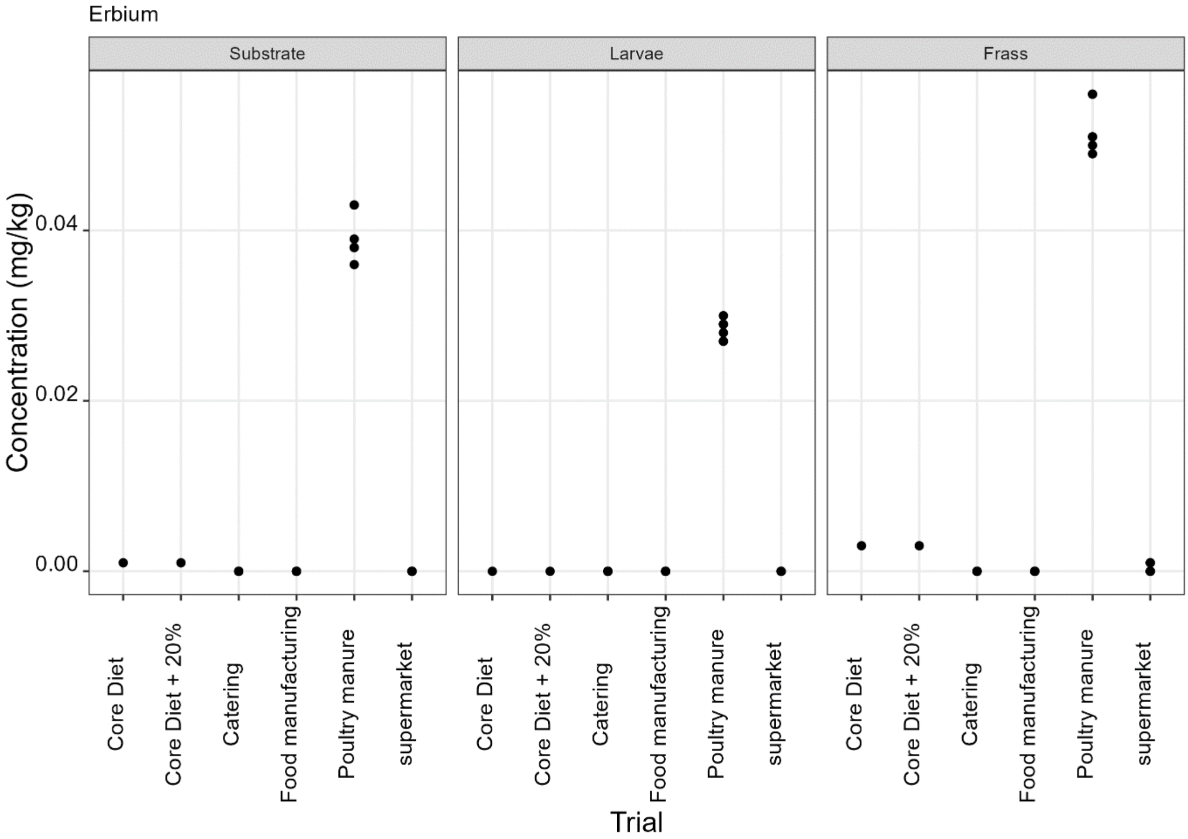
13.


Copper
Substrate
Larvae
Frass
Concentration (mg/kg)
150
100
50
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

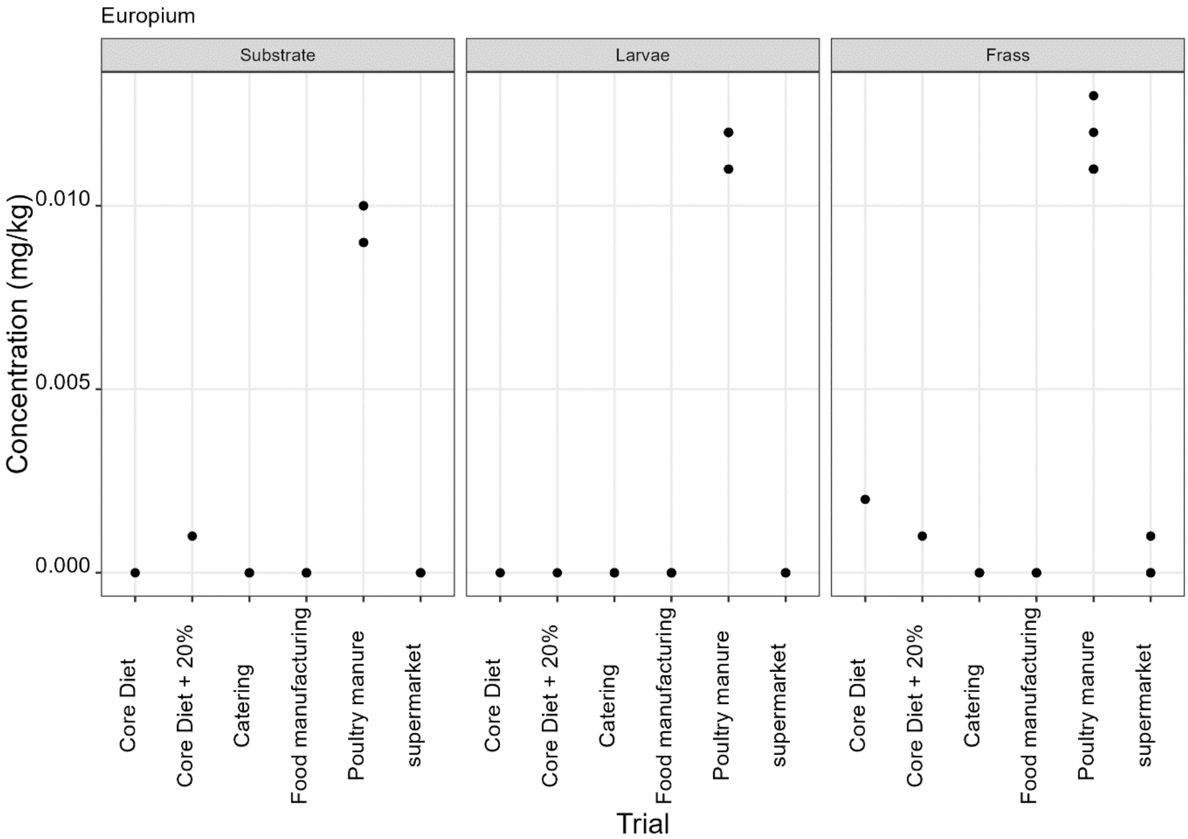
14.


Dysprosium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

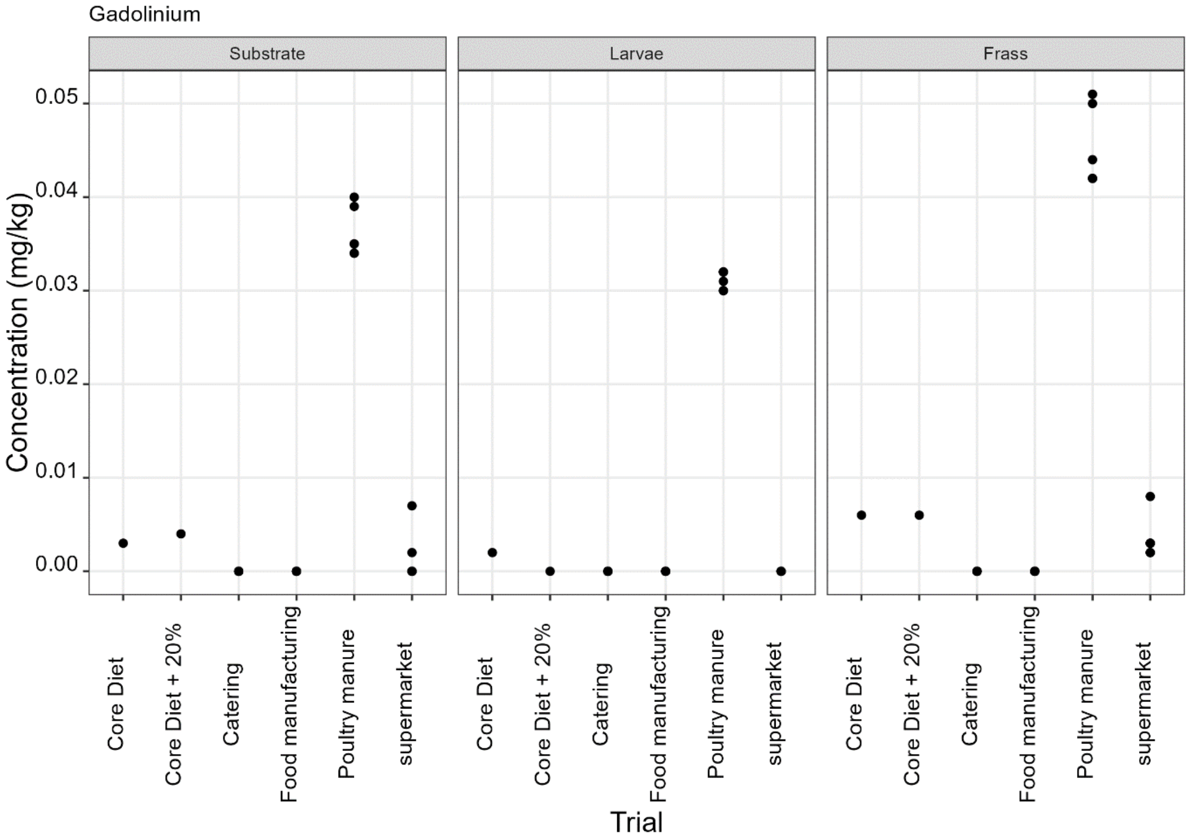
15.


Erbium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

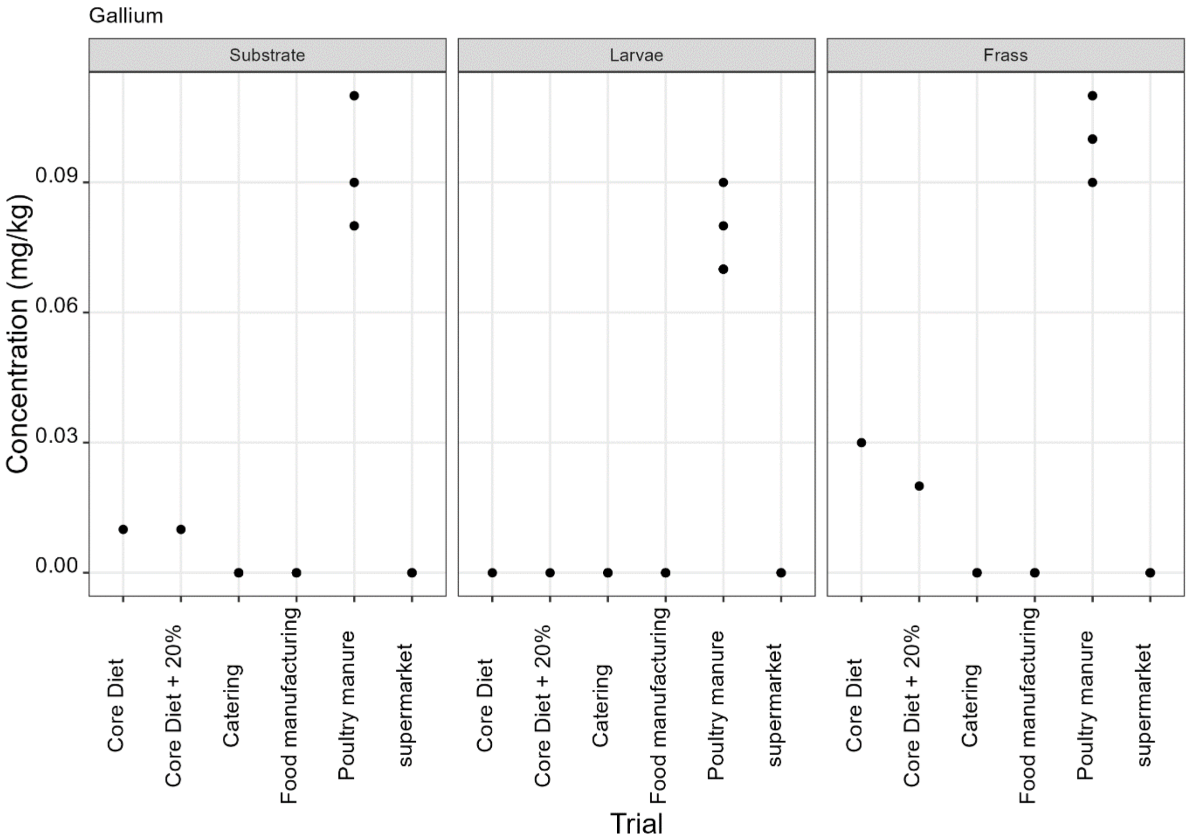
16.


Europium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

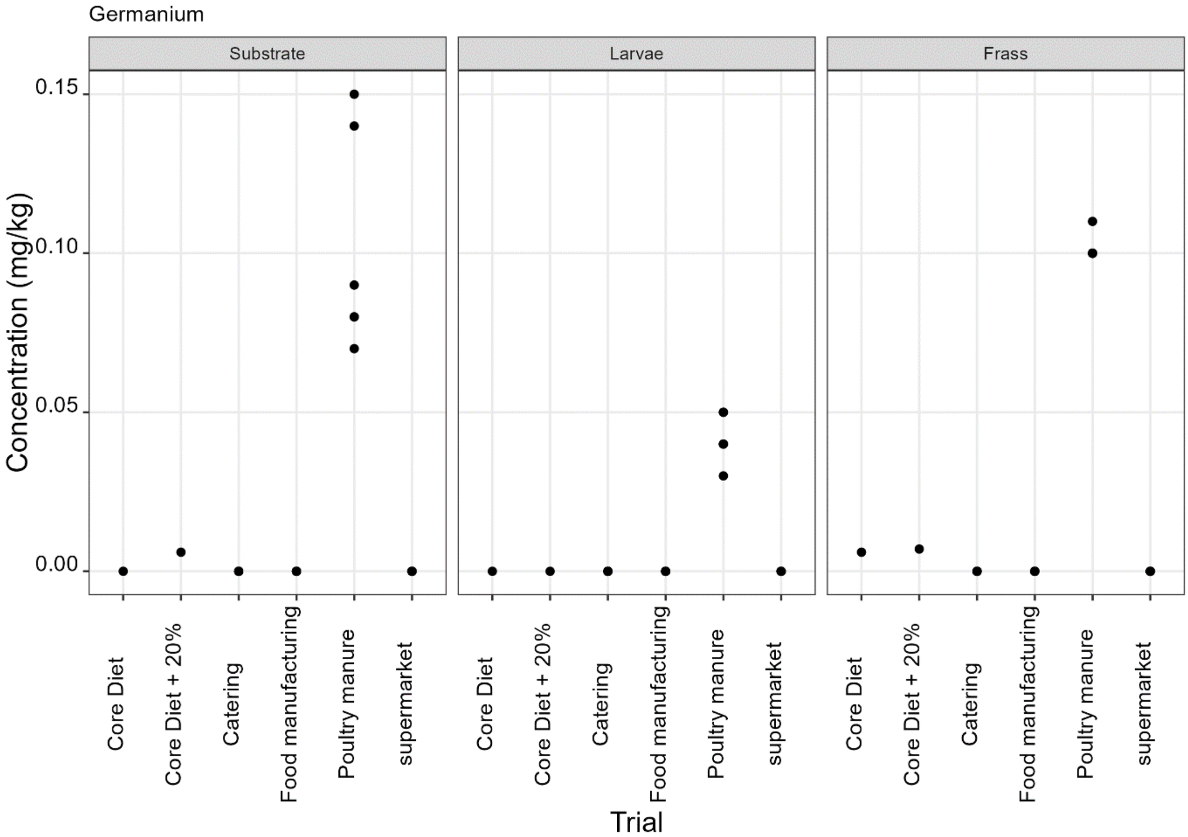
17.


Gadolinium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.00
0.01
0.02
0.03
0.04
0.05
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

18.

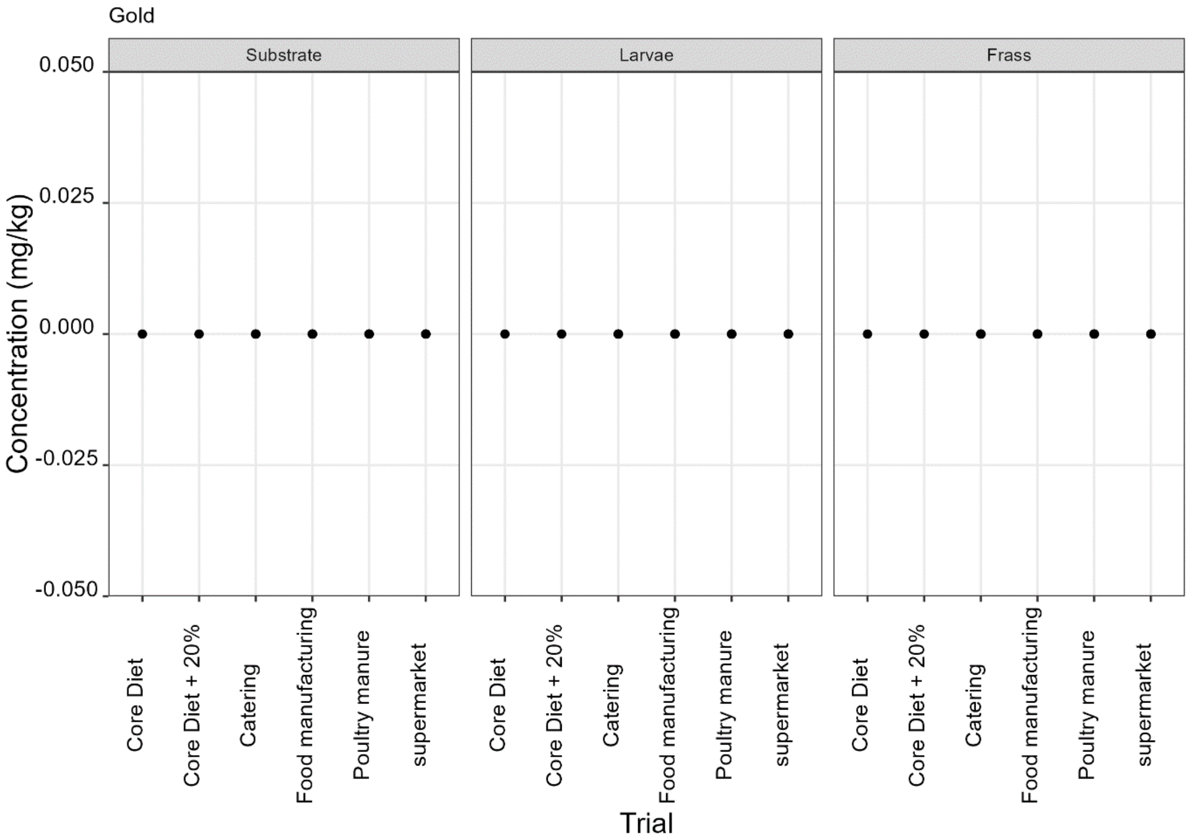

Gallium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.00
0.03
0.06
0.09
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

19.



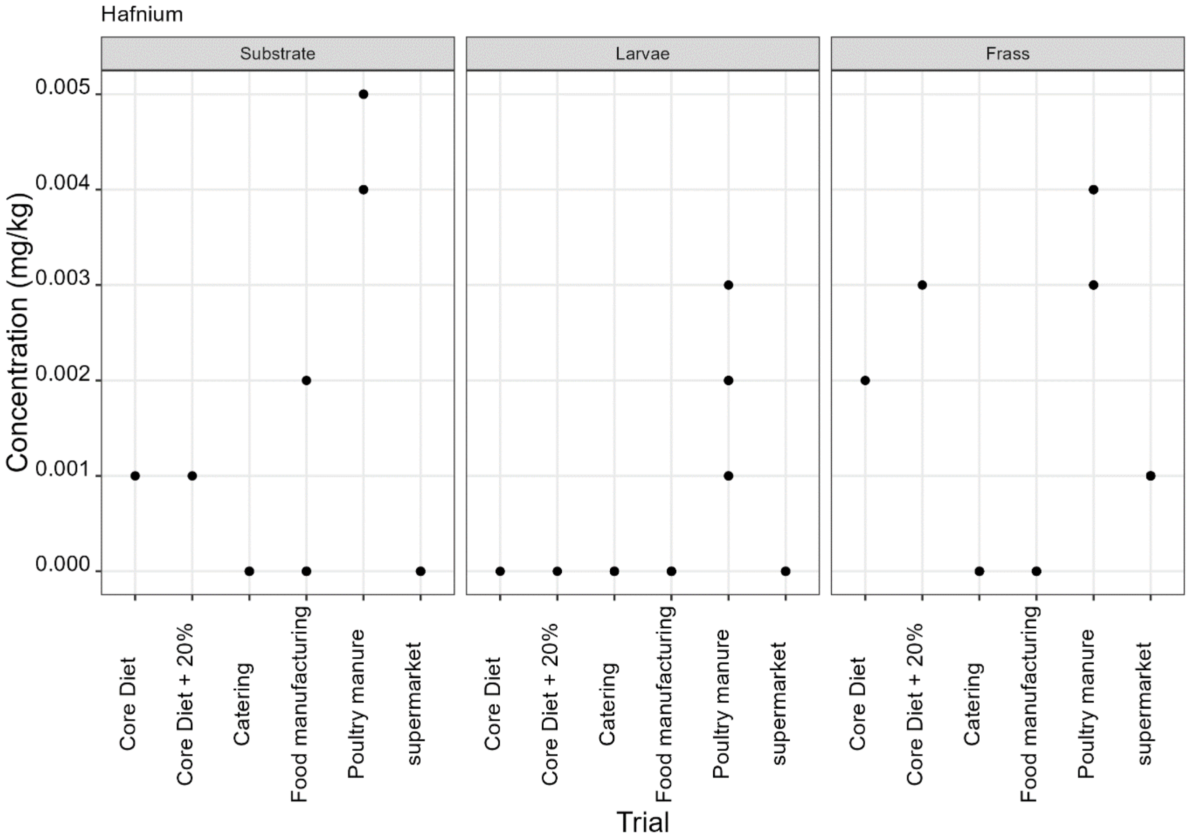
Germanium
Substrate
Larvae
Frass
0.15
0.10
0.05
0.00
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

20.

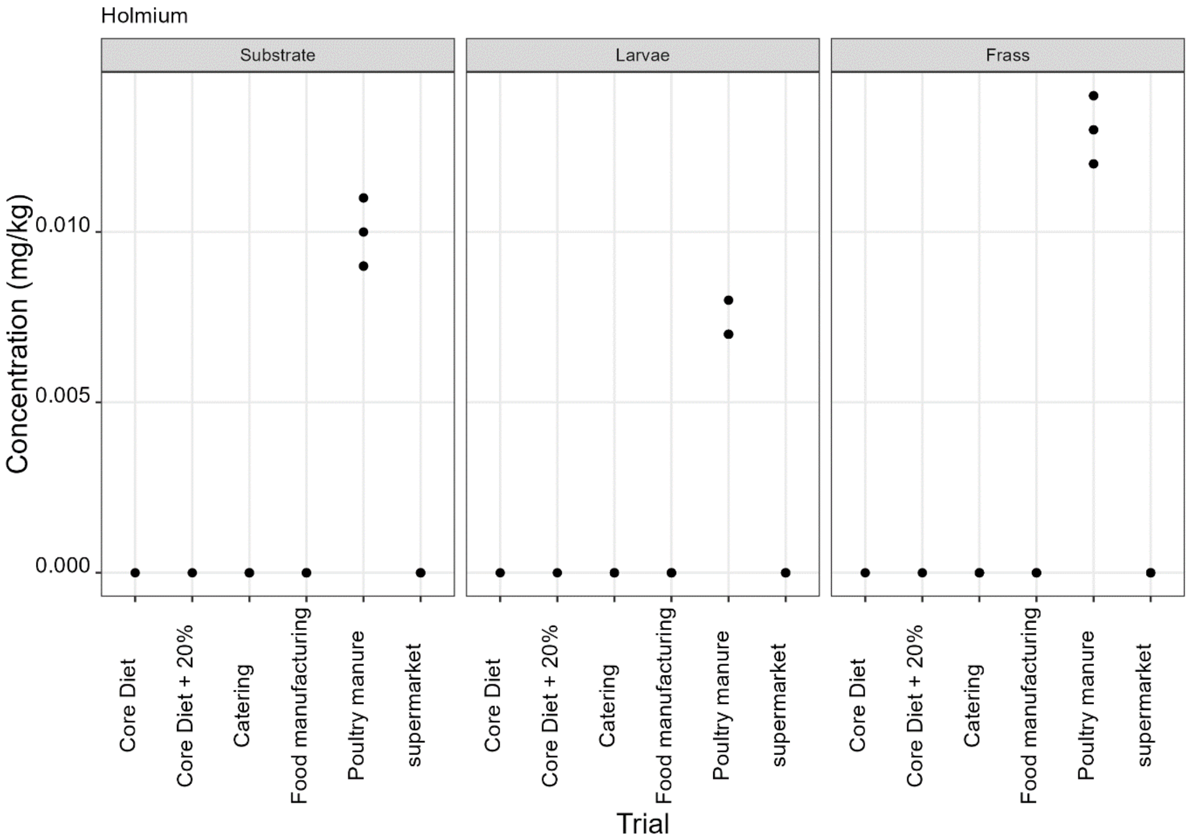


Gold
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

21.


Hafnium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.005
0.004
0.003
0.002
0.001
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

22.


Holmium
Substrate
Larvae
Frass
Concentration (mg/kg)
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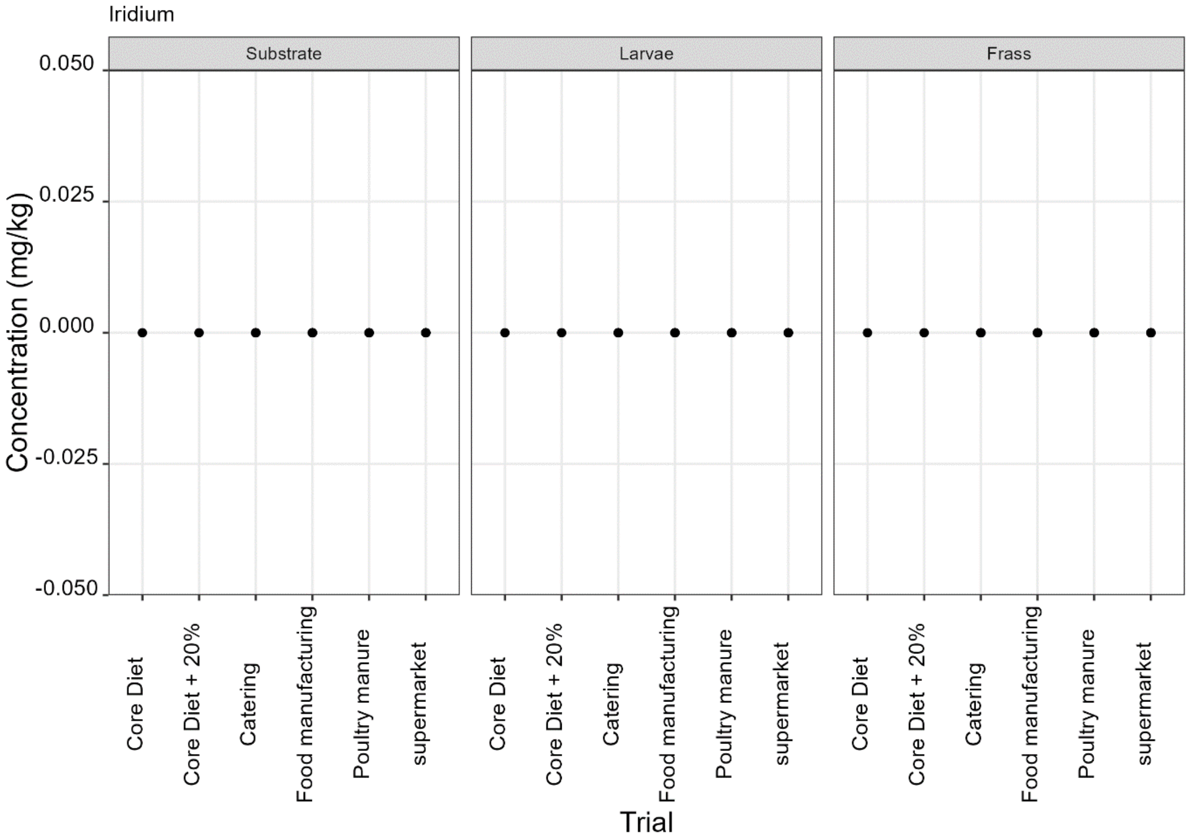
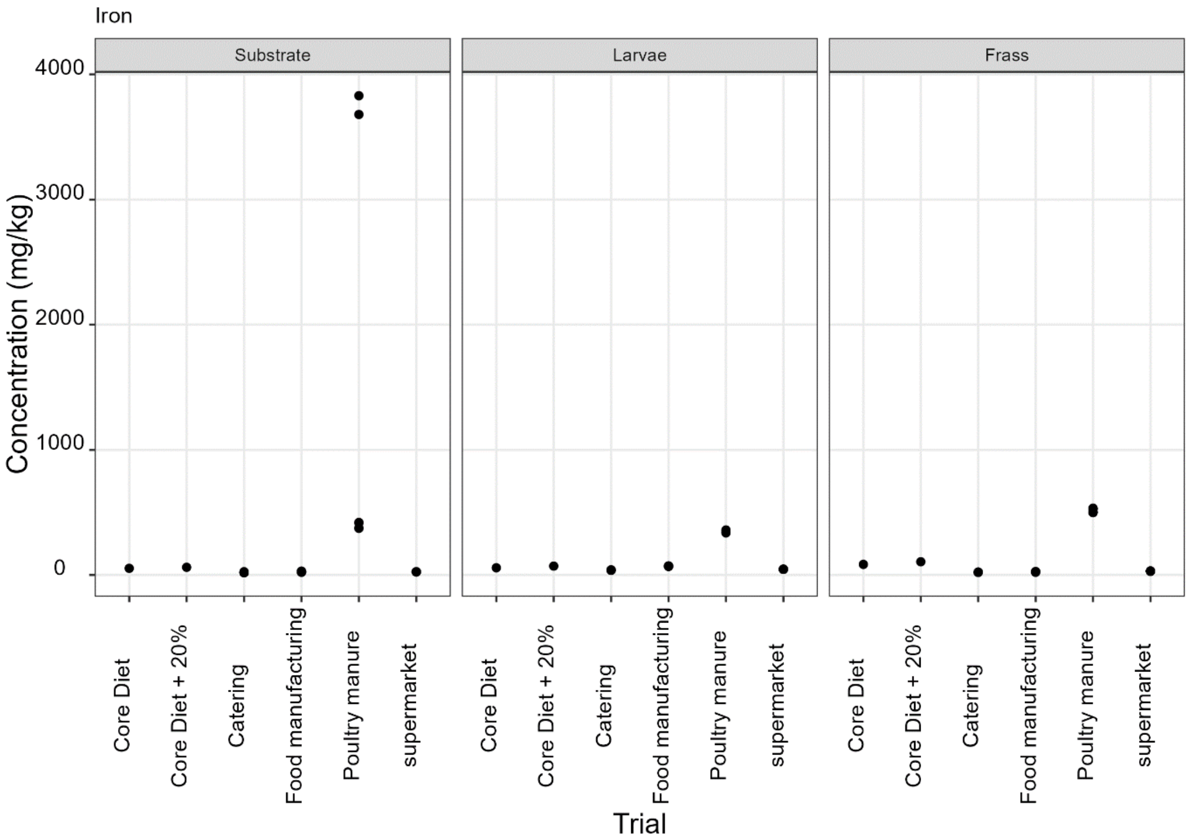
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

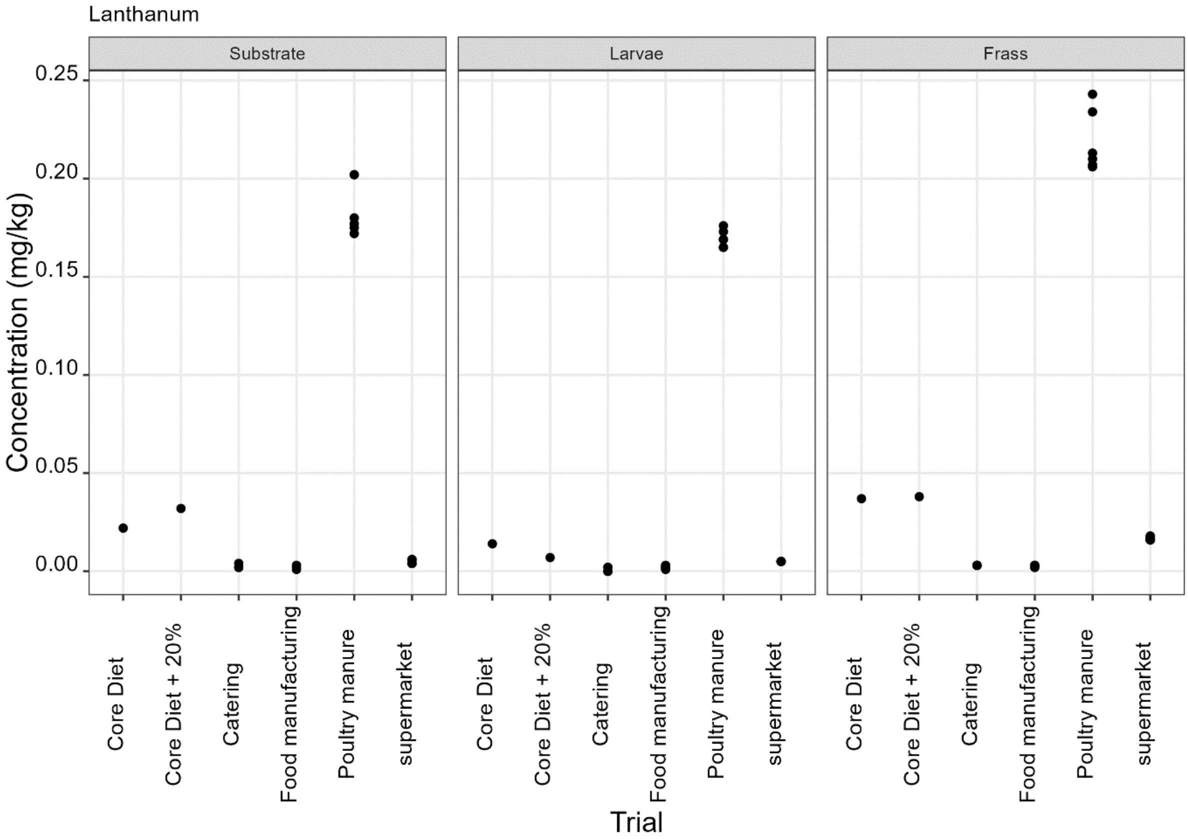
23.


Iridium
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

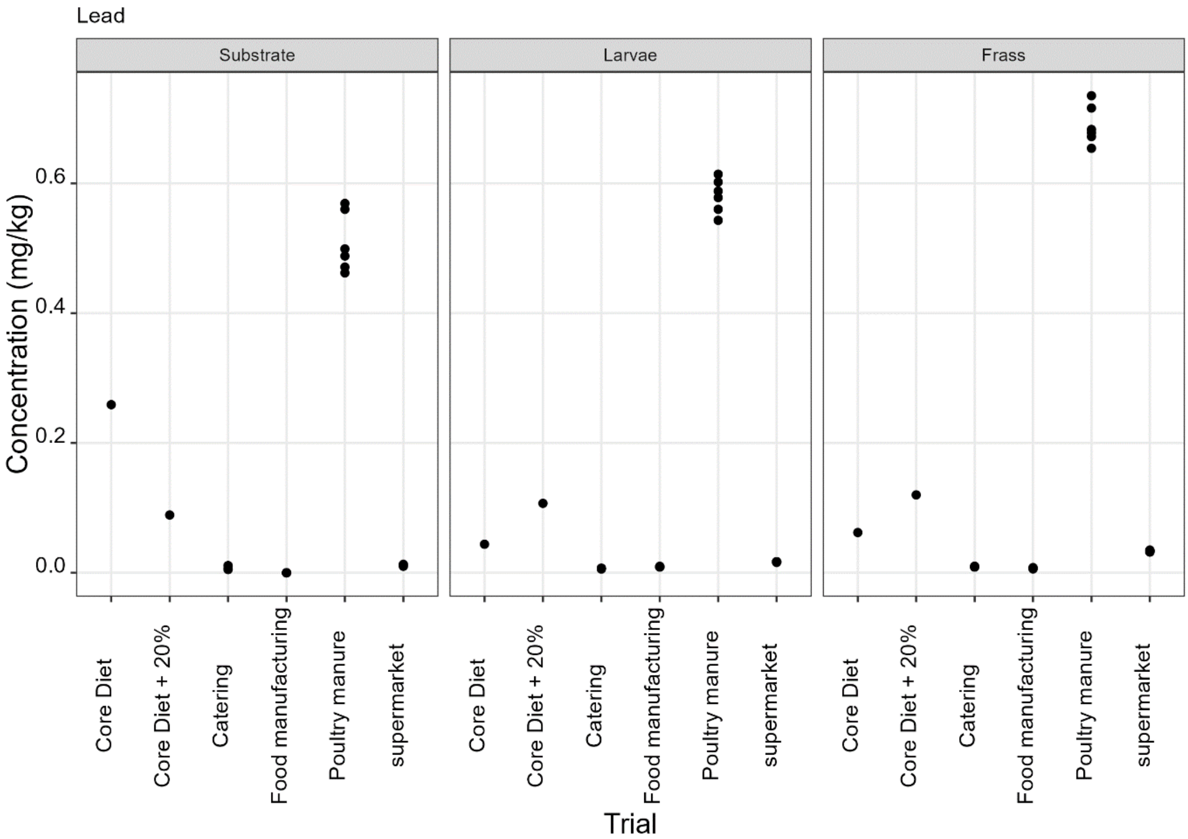
24.


Iron
Substrate
Larvae
Frass
4000
3000
2000
1000
0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

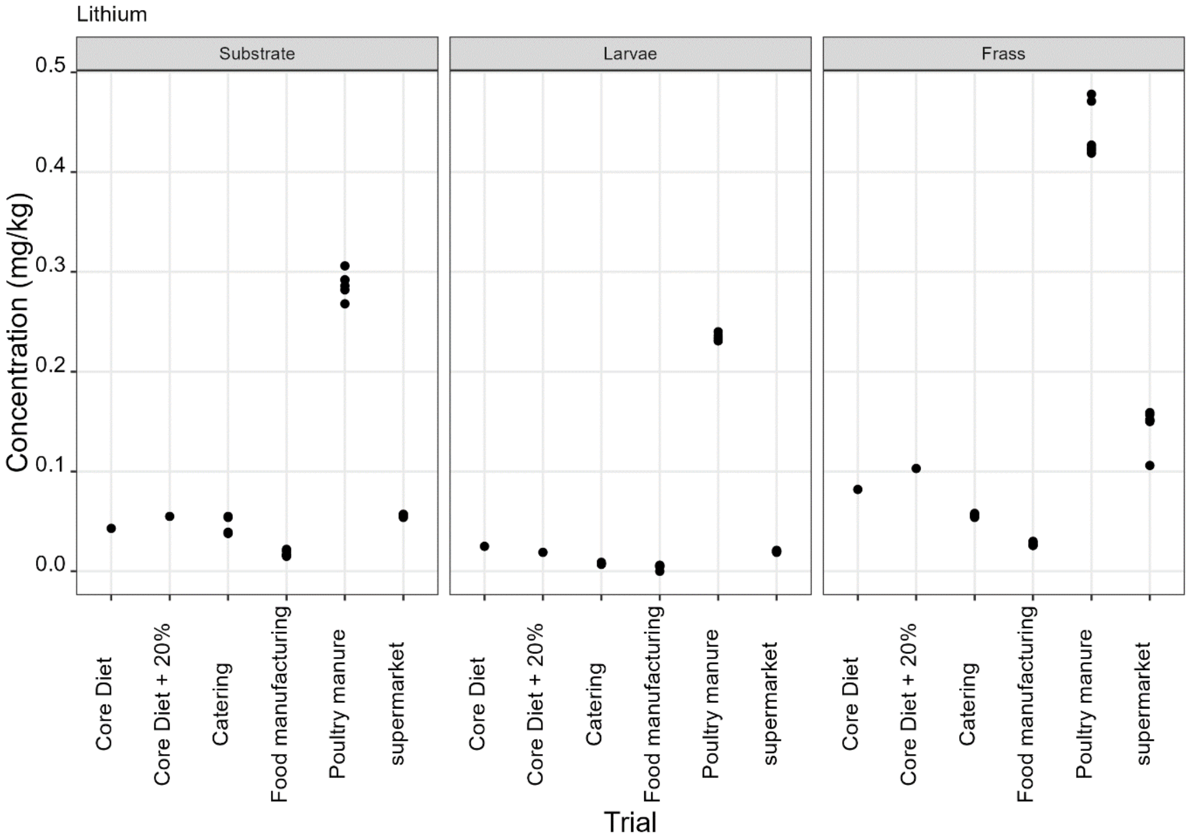
25.


Lanthanum
Substrate
Larvae
Frass
Concentration (mg/kg)
0.25
0.20
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

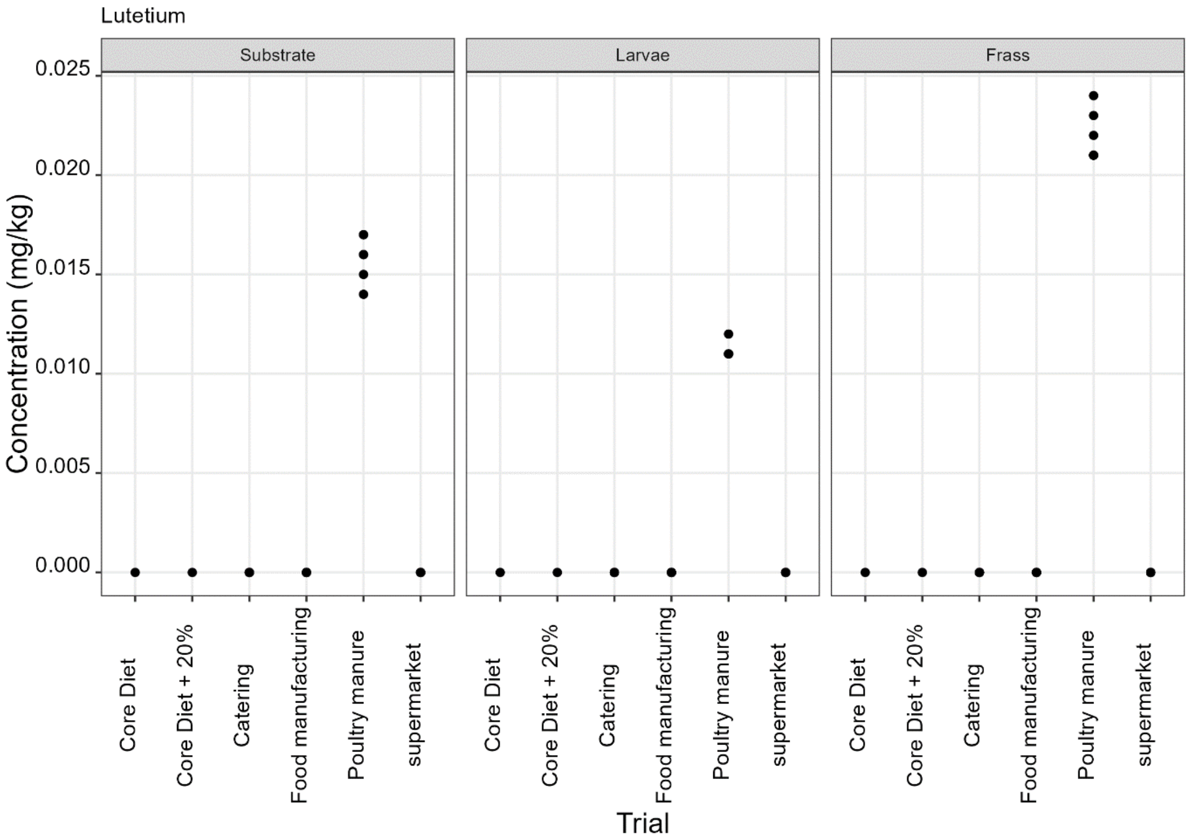
26.


Lead
Substrate
Larvae
Frass
Concentration (mg/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

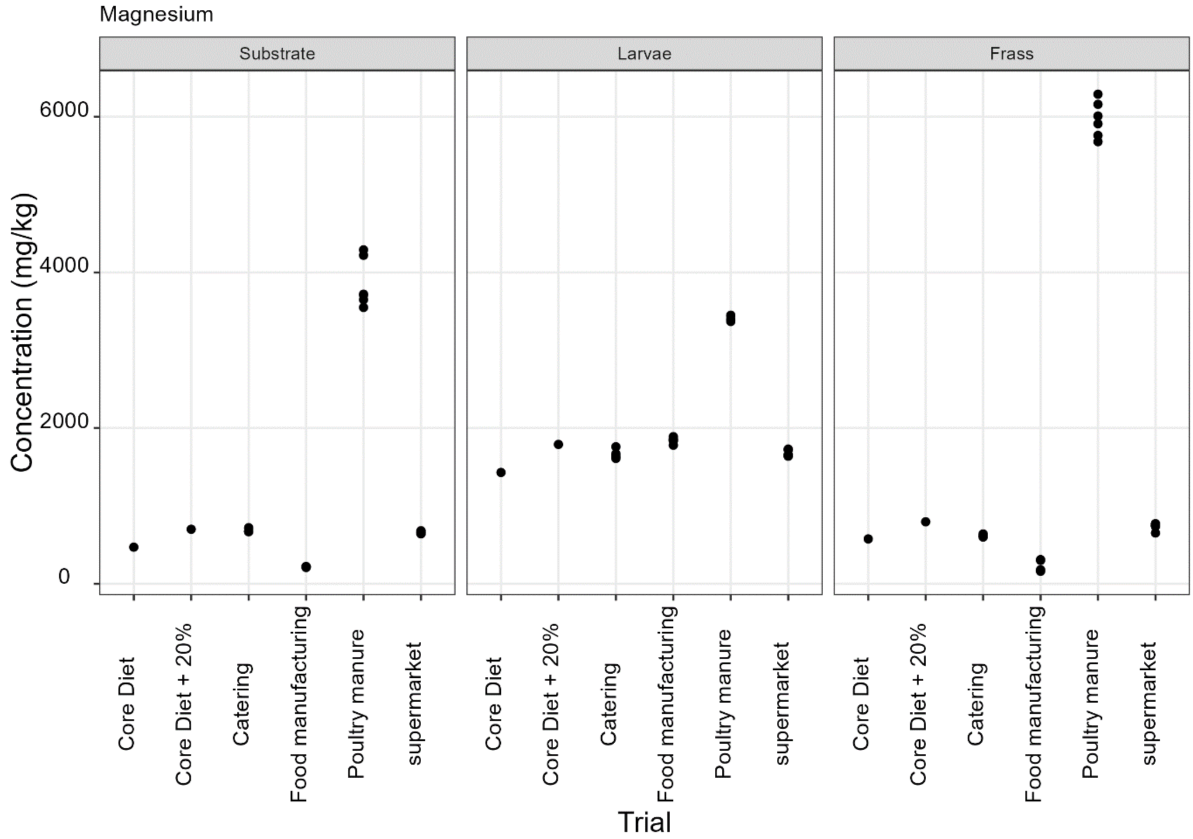
27.


Lithium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.5
0.4
0.3
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

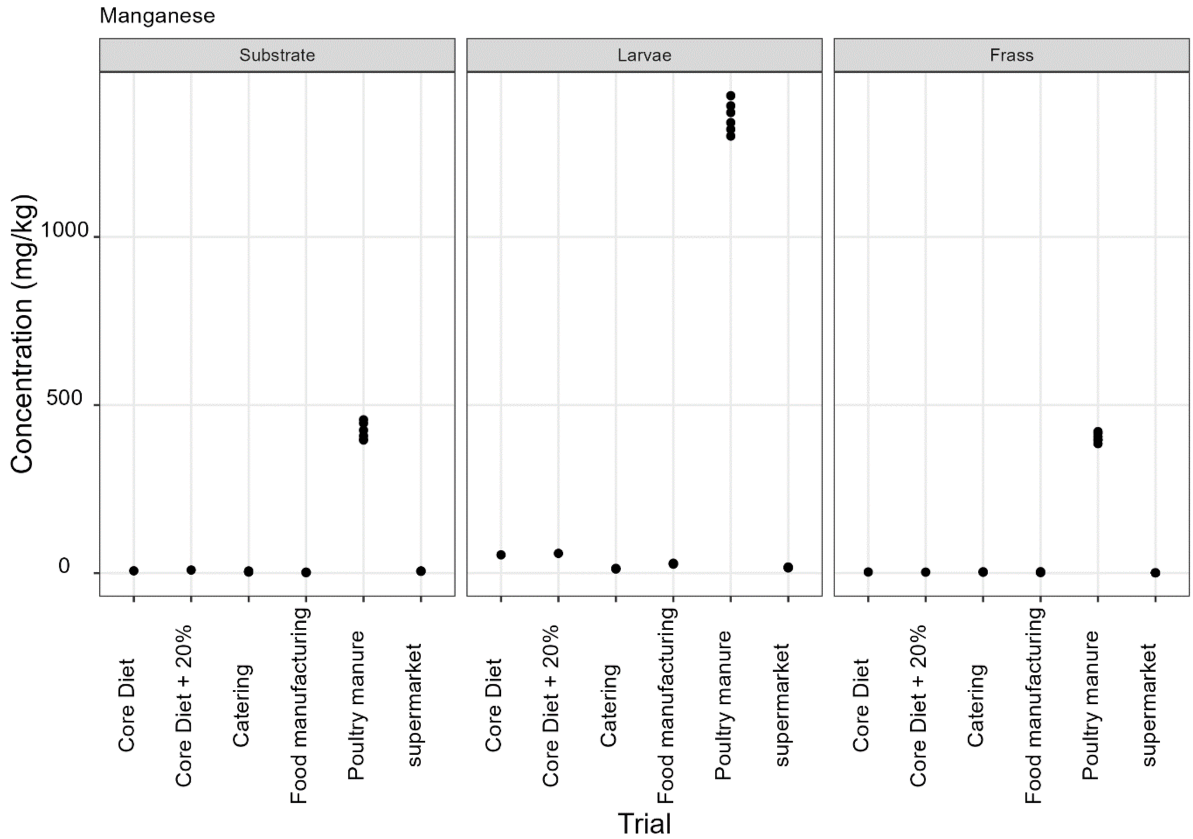
28.


Lutetium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.025
0.020
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

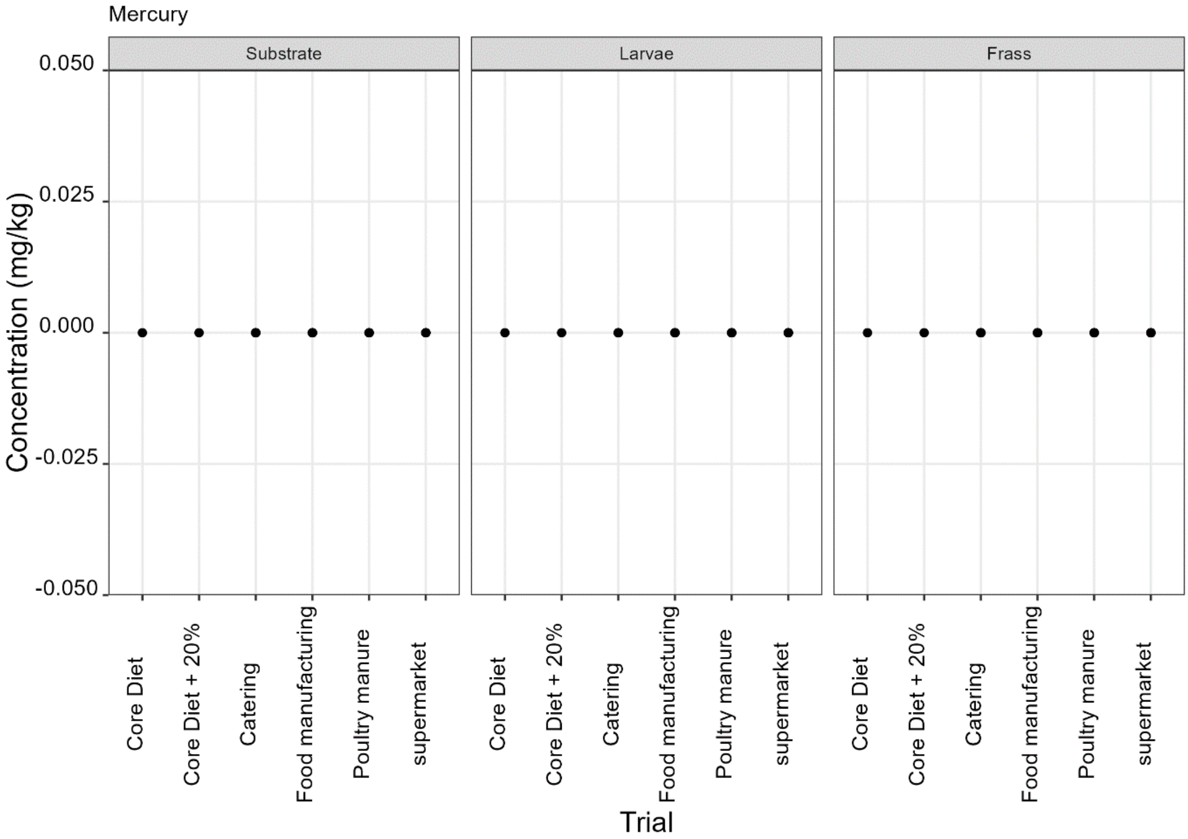
29.


Magnesium
Substrate
Larvae
Frass
Concentration (mg/kg)
6000
4000
2000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

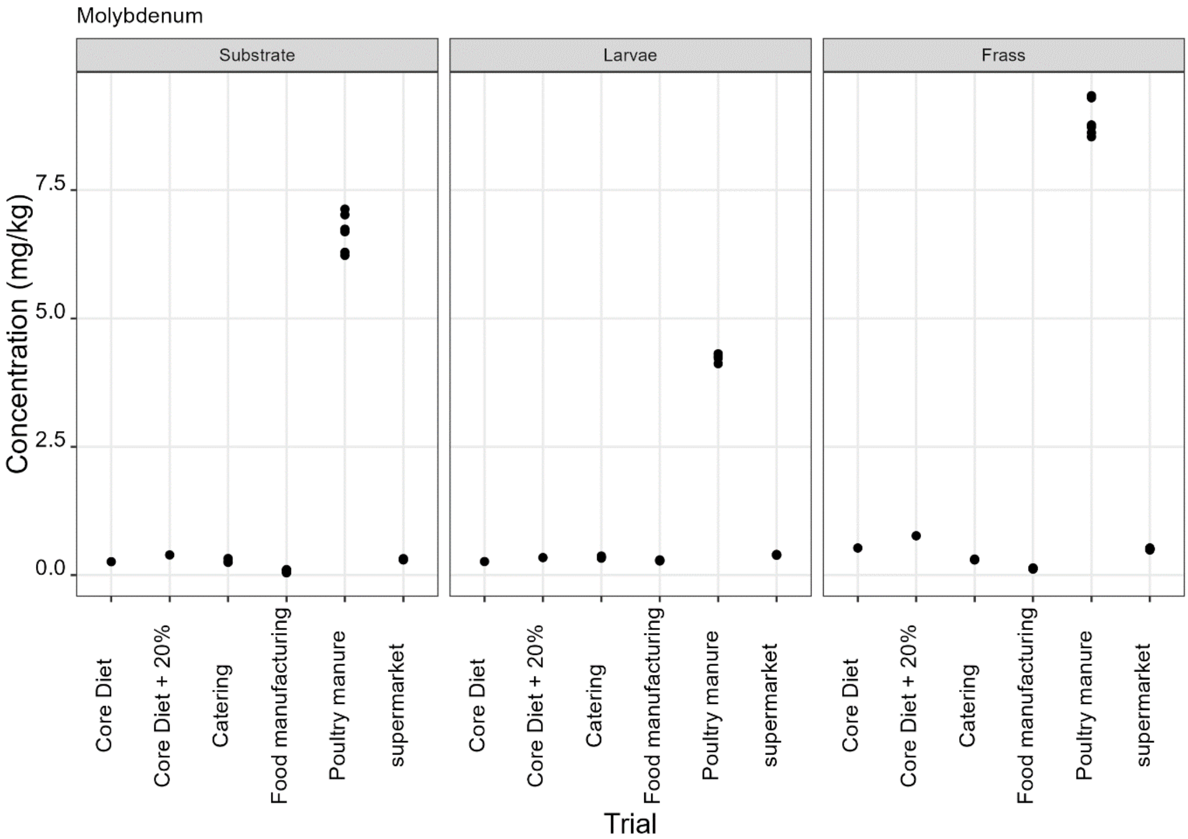
30.


Manganese
Substrate
Larvae
Frass
Concentration (mg/kg)
1000
500
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

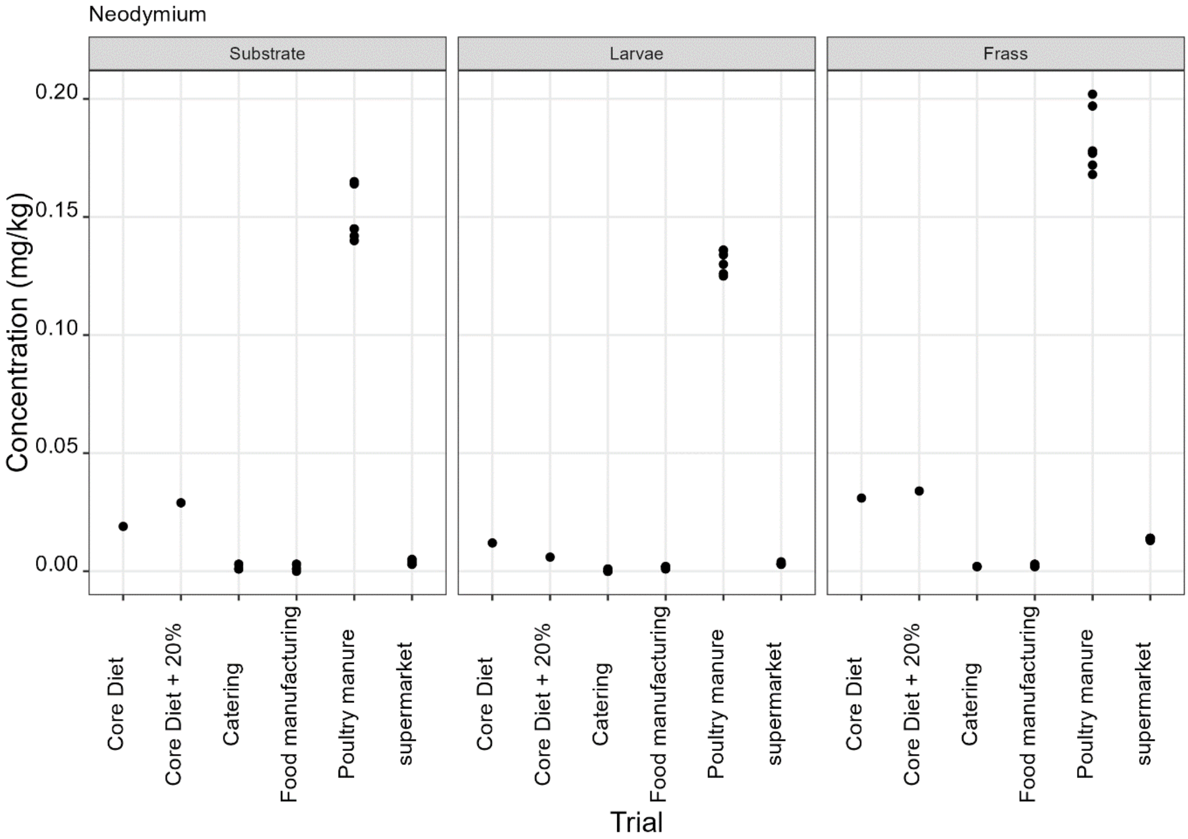
31.


Mercury
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

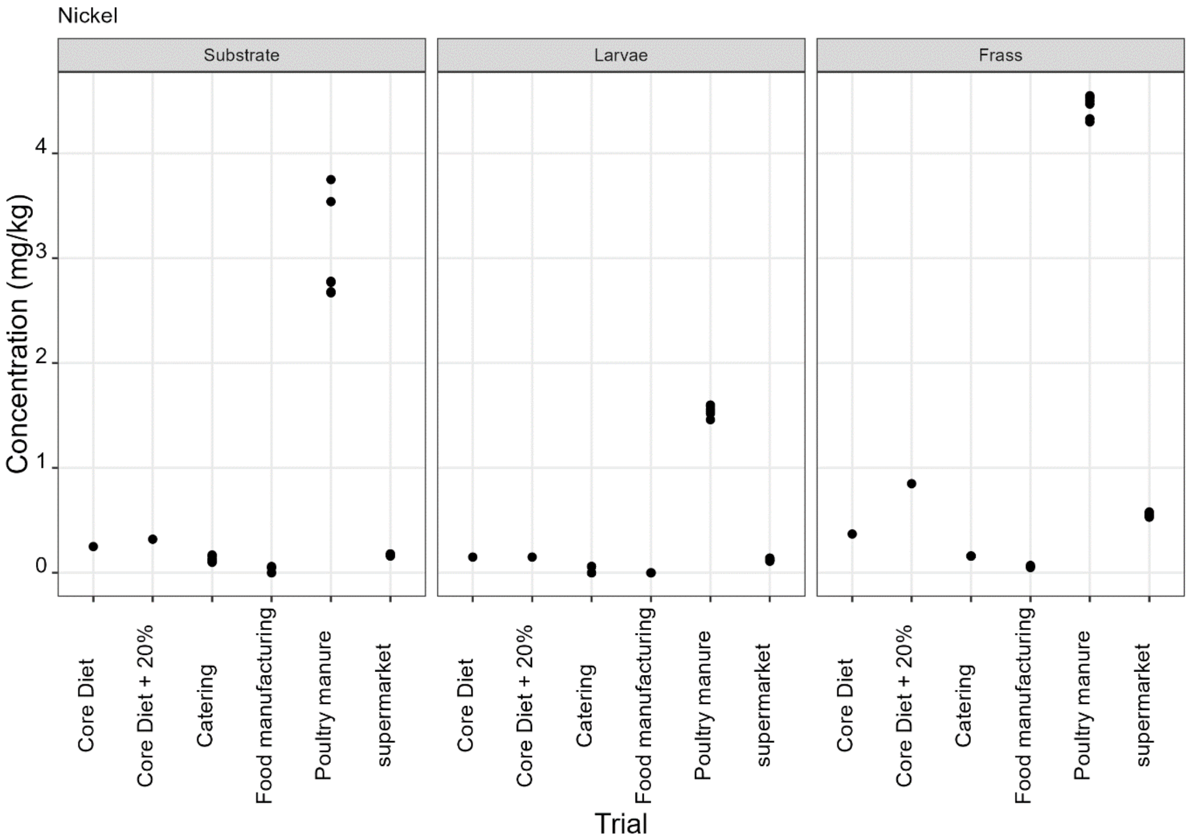
32.


Molybdenum
Substrate
Larvae
Frass
7.5
5.0
2.5
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

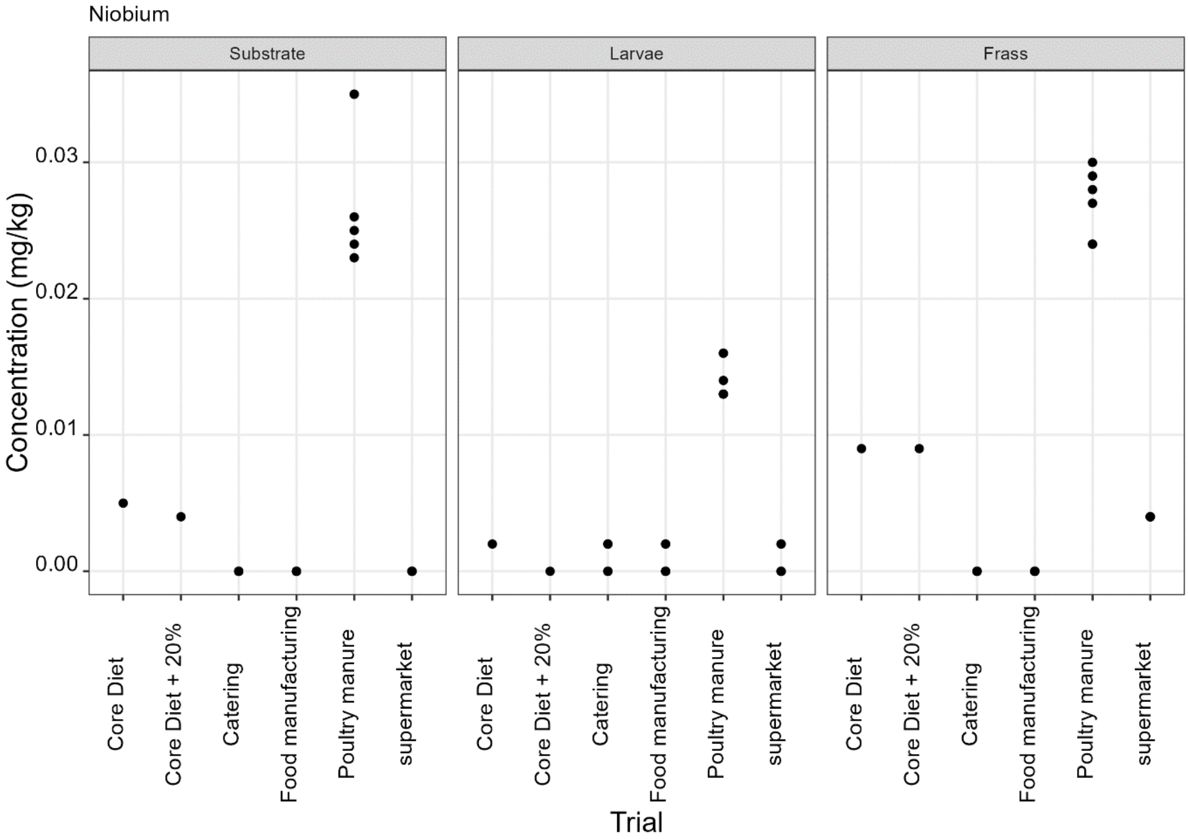
33.


Neodymium
Substrate
Larvae
Frass
0.20
0.15
0.10
0.05
0.00
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

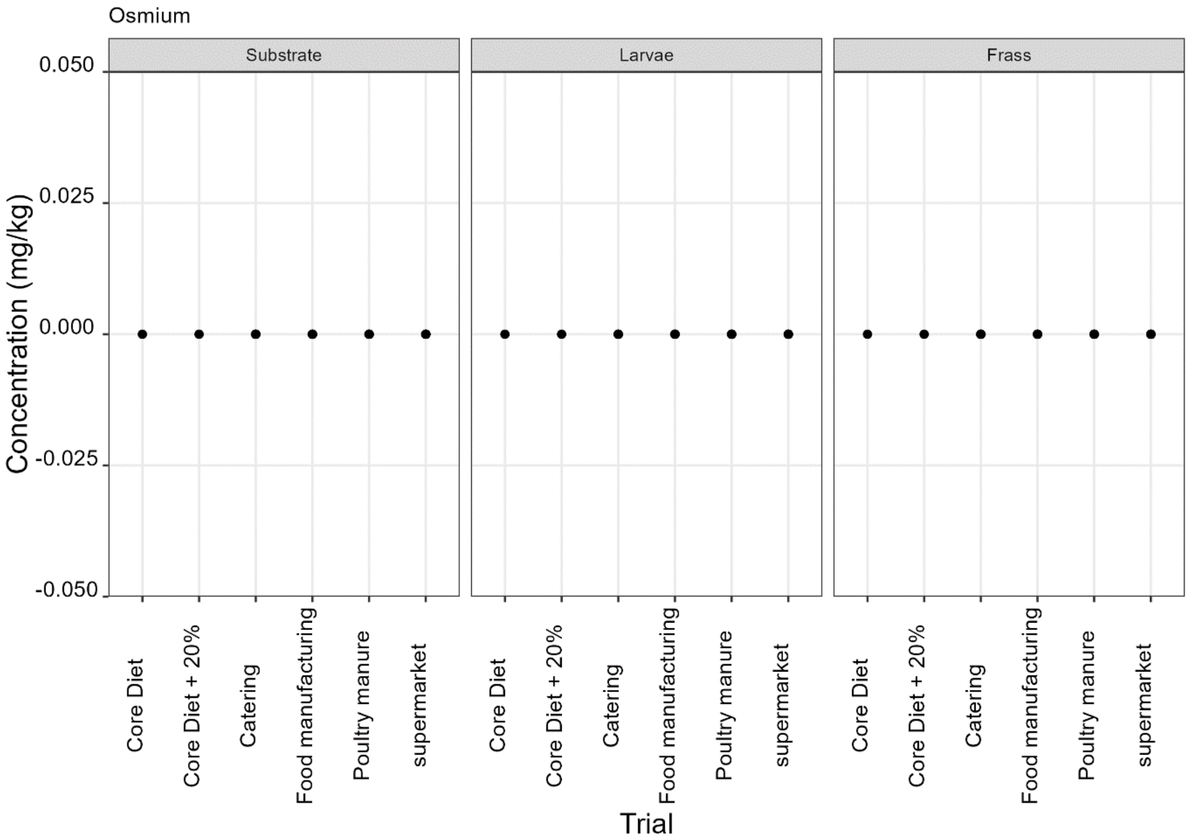
34.


Nickel
Substrate
Larvae
Frass
4
3
2
1
0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

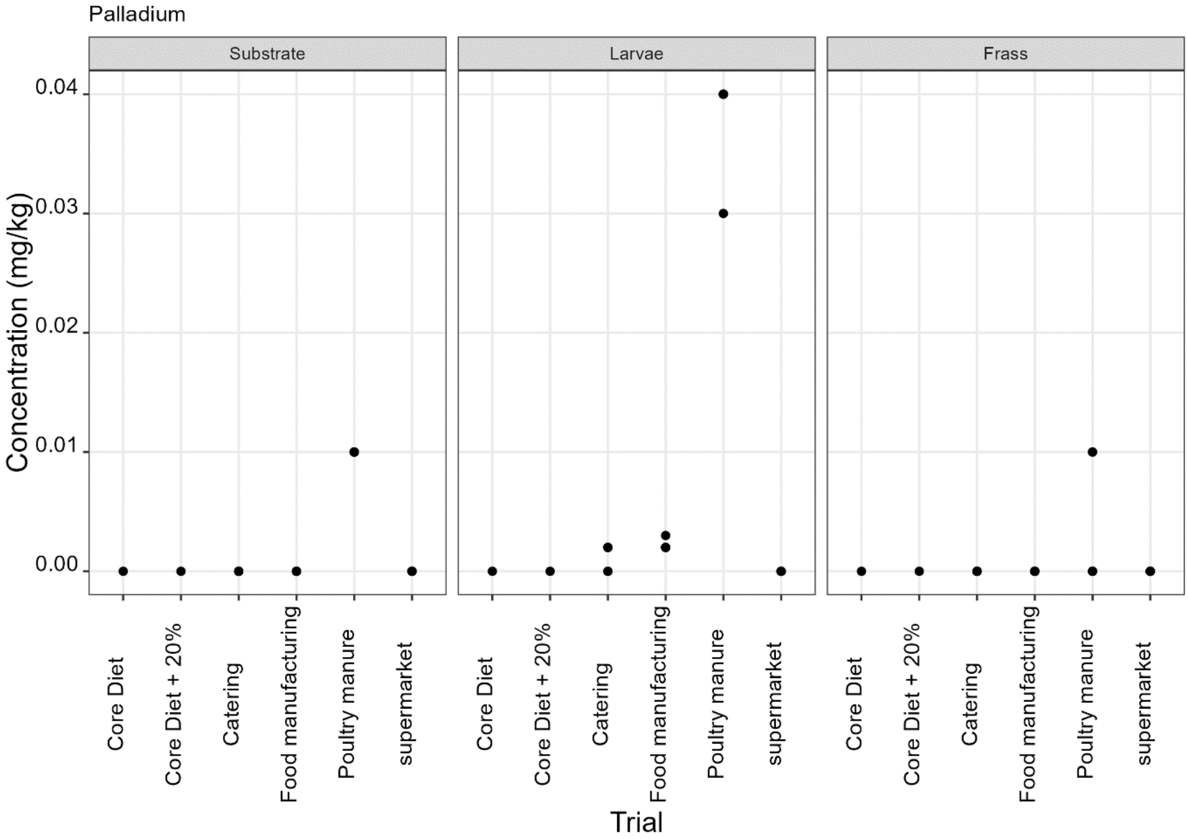
35.


Niobium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.03
0.02
0.01
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

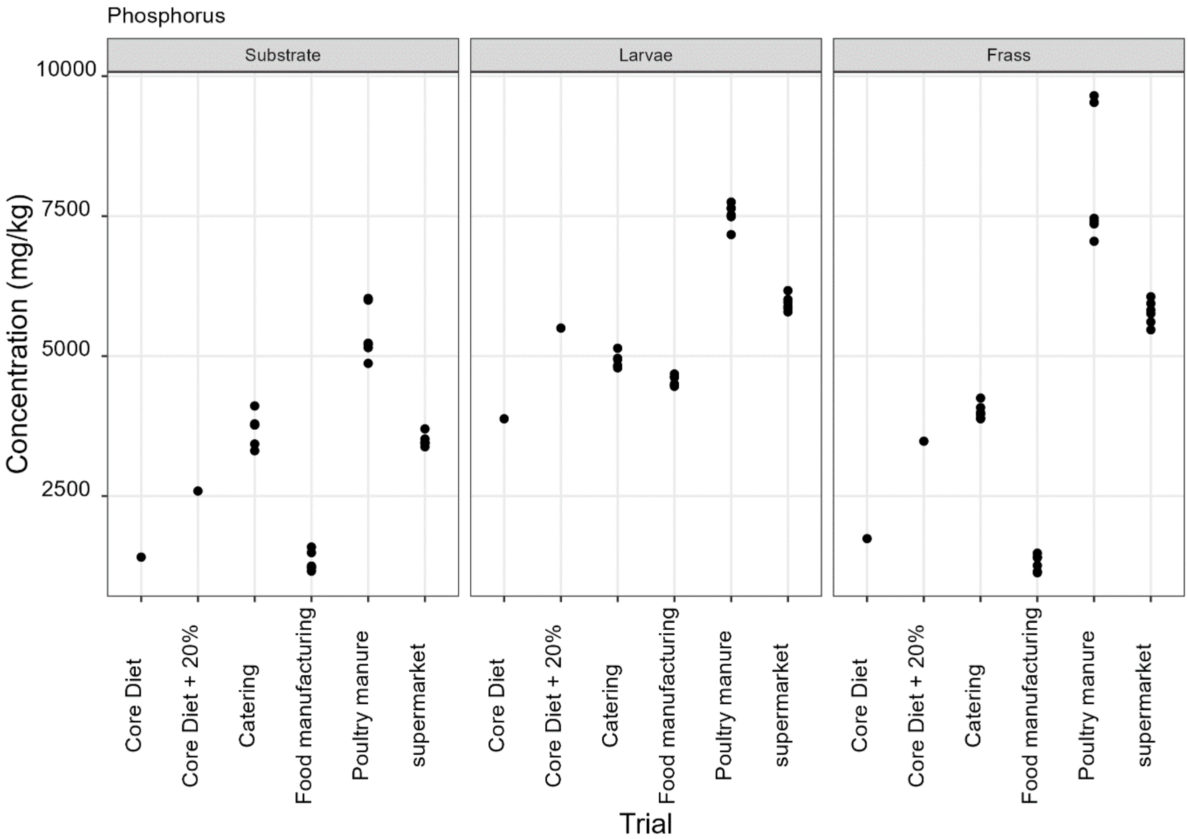
36.


Osmium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.050
0.025
0.000
-0.025
-0.050
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

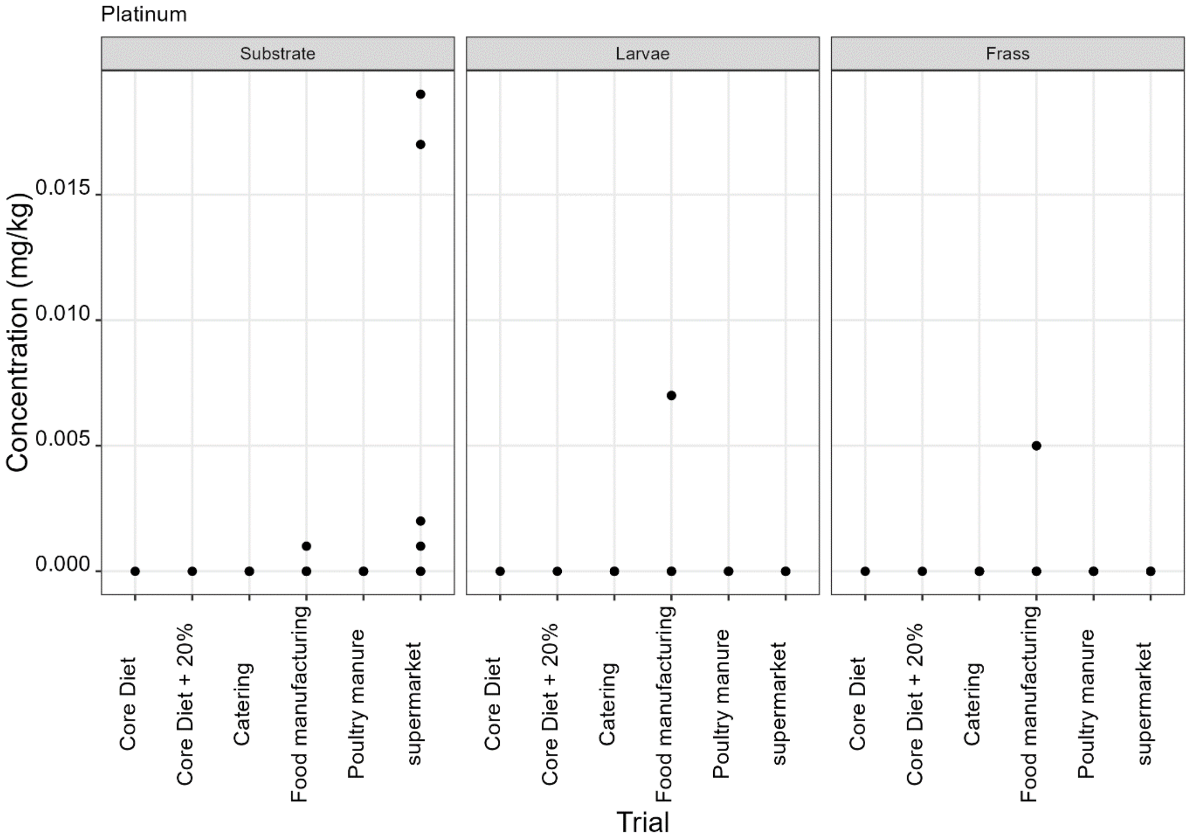
37.


Palladium
Substrate
Larvae
Frass
0.04
0.03
0.02
0.01
0.00
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

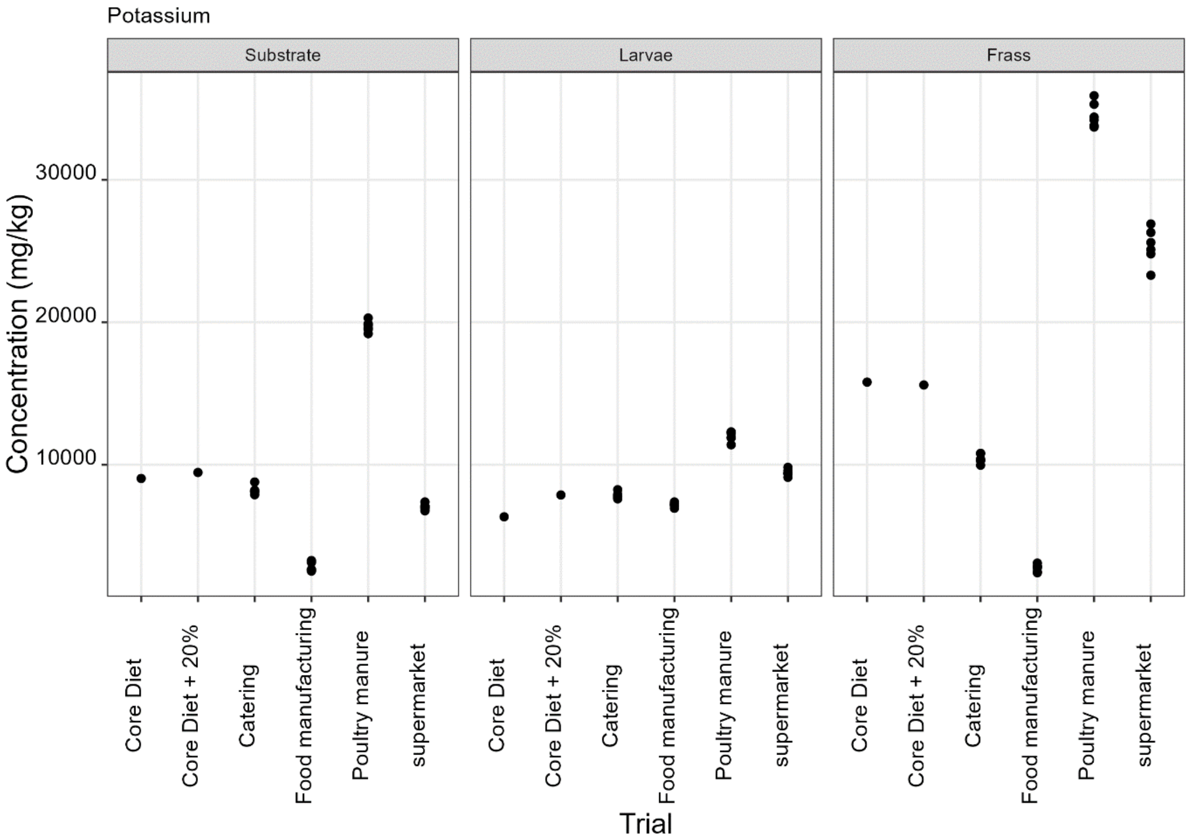
38.


Phosphorus
Substrate
Larvae
Frass
10000
7500
5000
2500
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

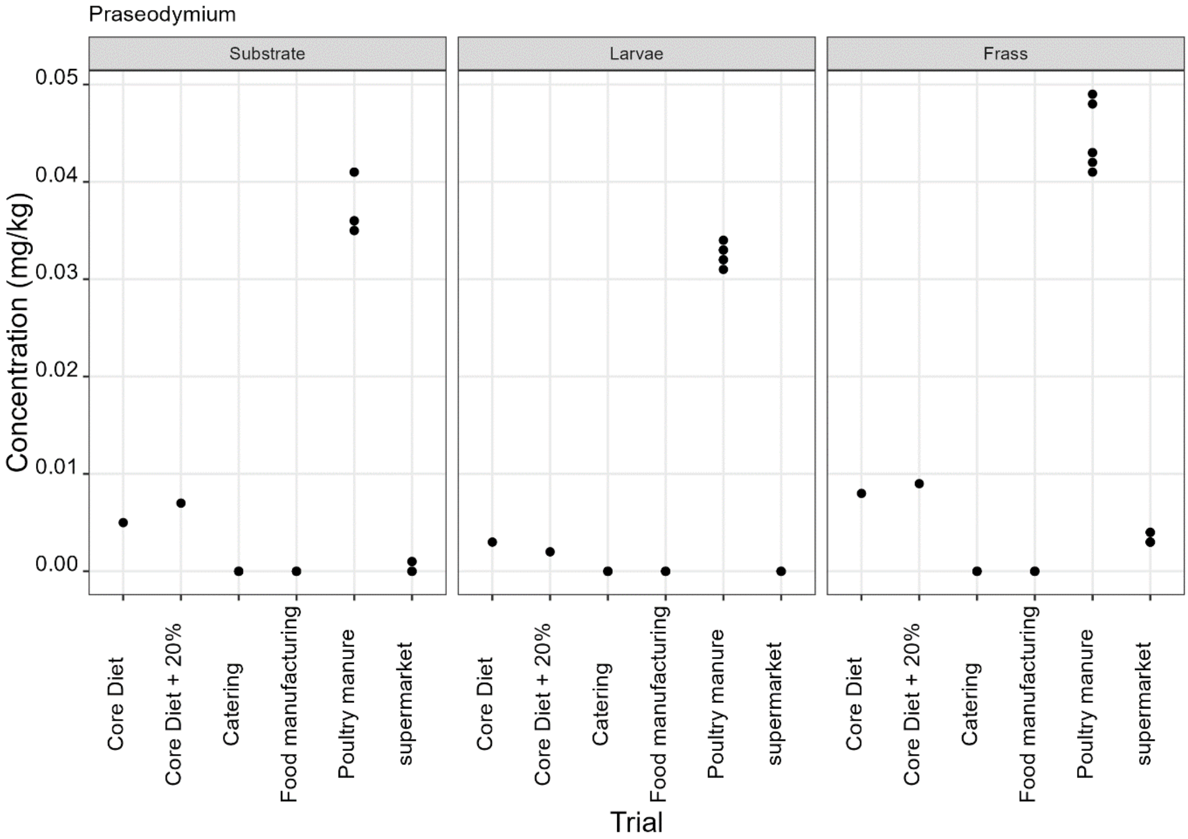
39.


Platinum
Substrate
Larvae
Frass
Concentration (mg/kg)
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

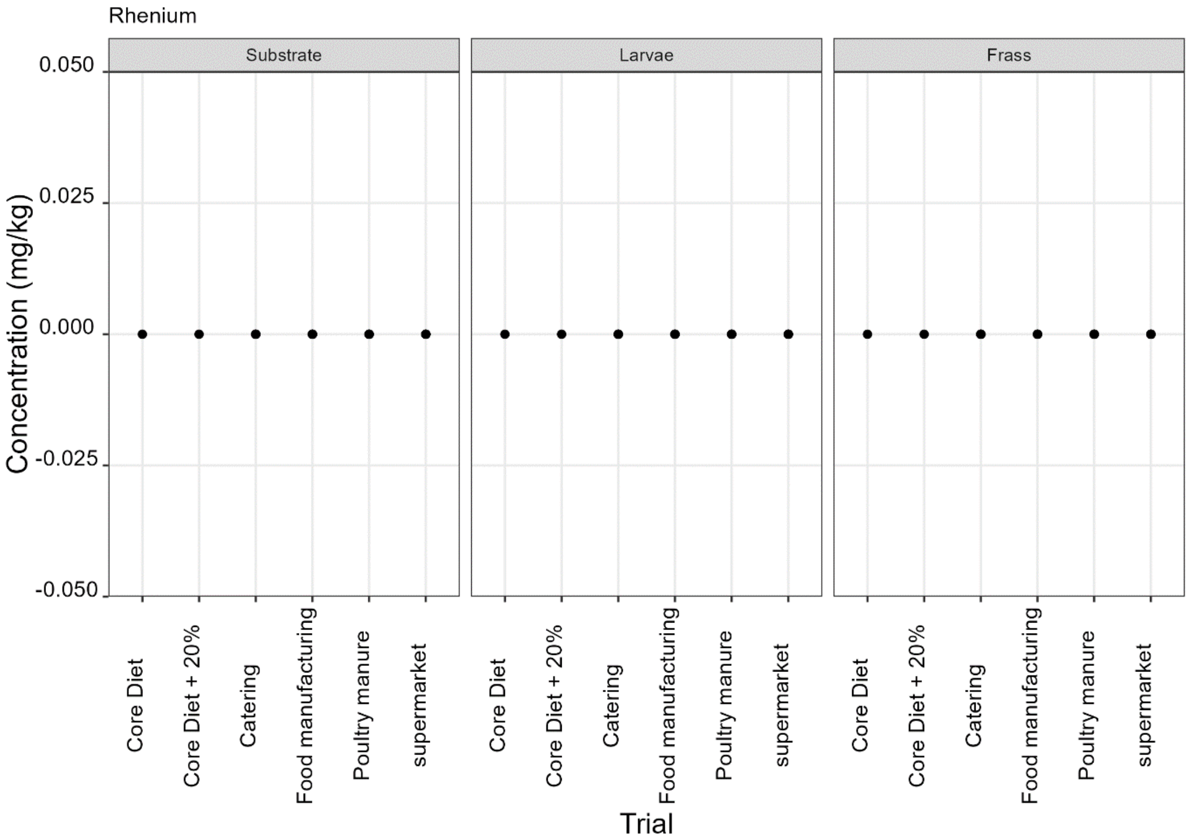
40.


Potassium
Substrate
Larvae
Frass
Concentration (mg/kg)
30000
20000
10000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

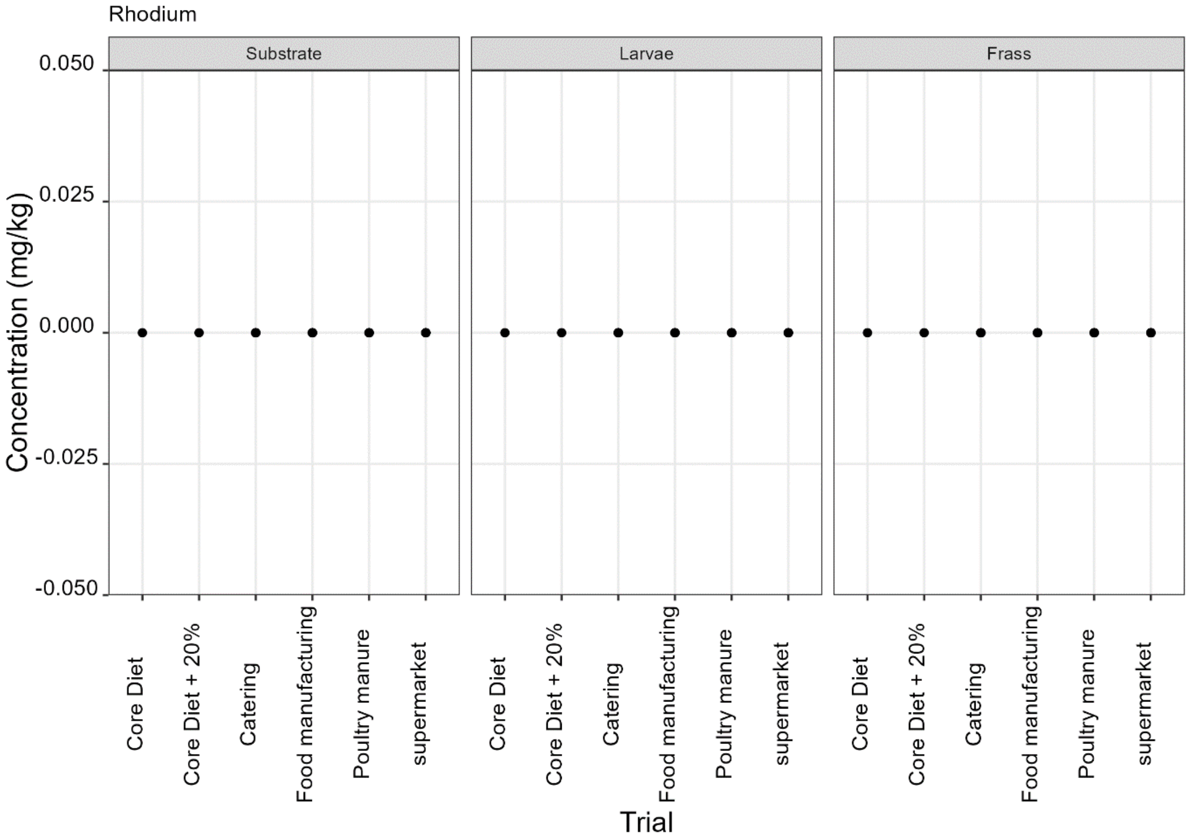
41.


Praseodymium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.05
0.04
0.03
0.02
0.01
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

42.

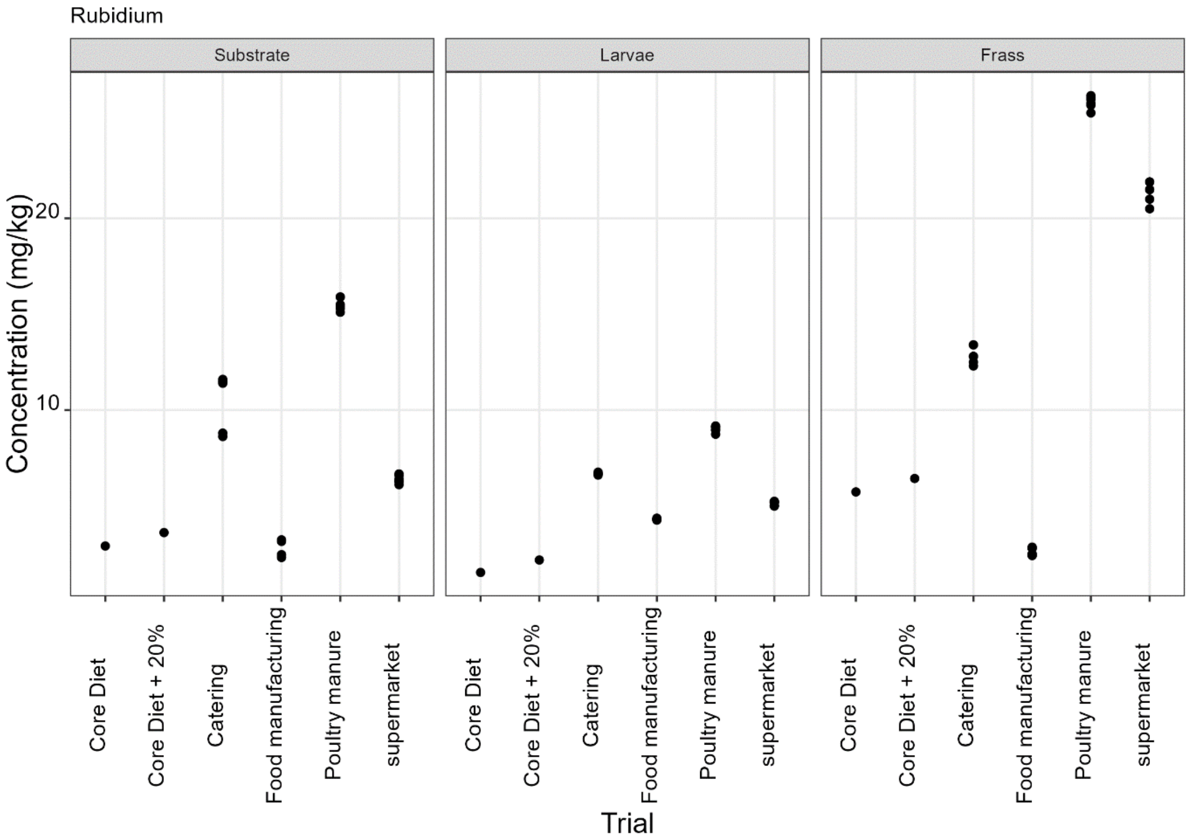

Rhenium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.050
0.025
0.000
-0.025
-0.050
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

43.



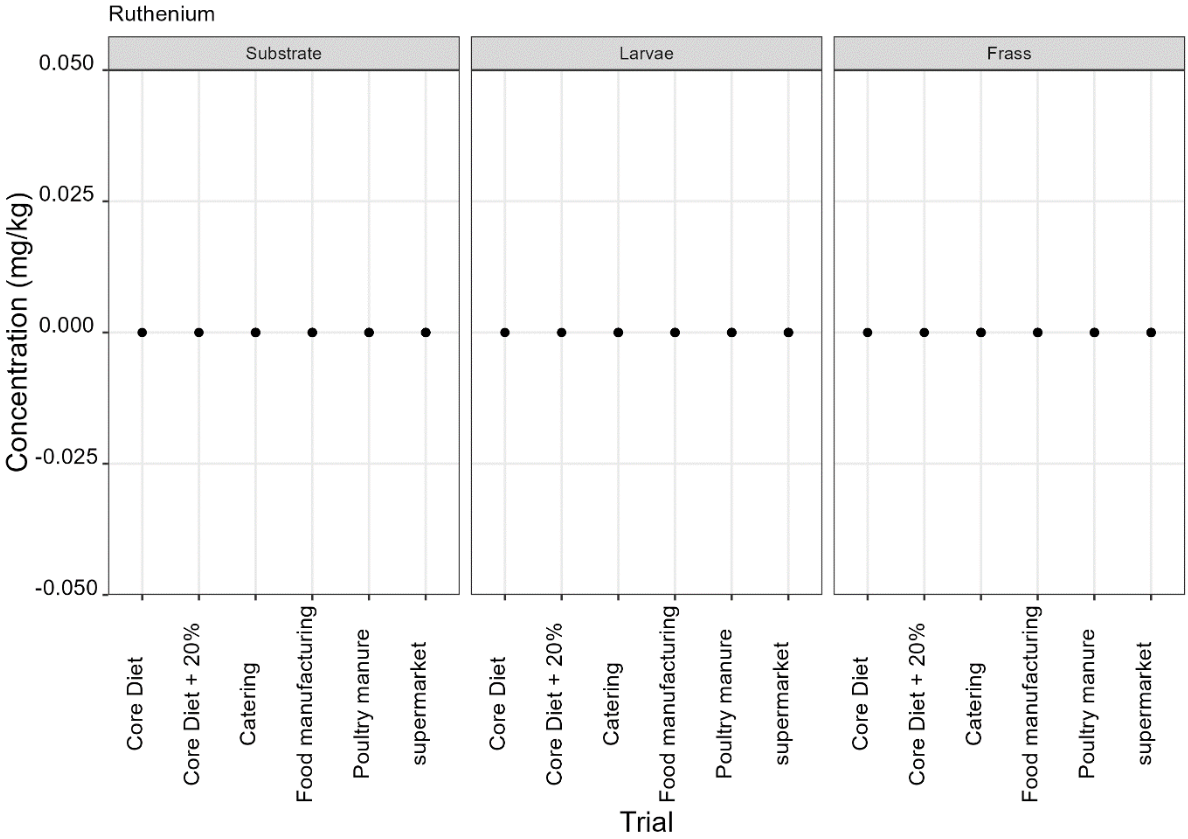
Rhodium
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

44.

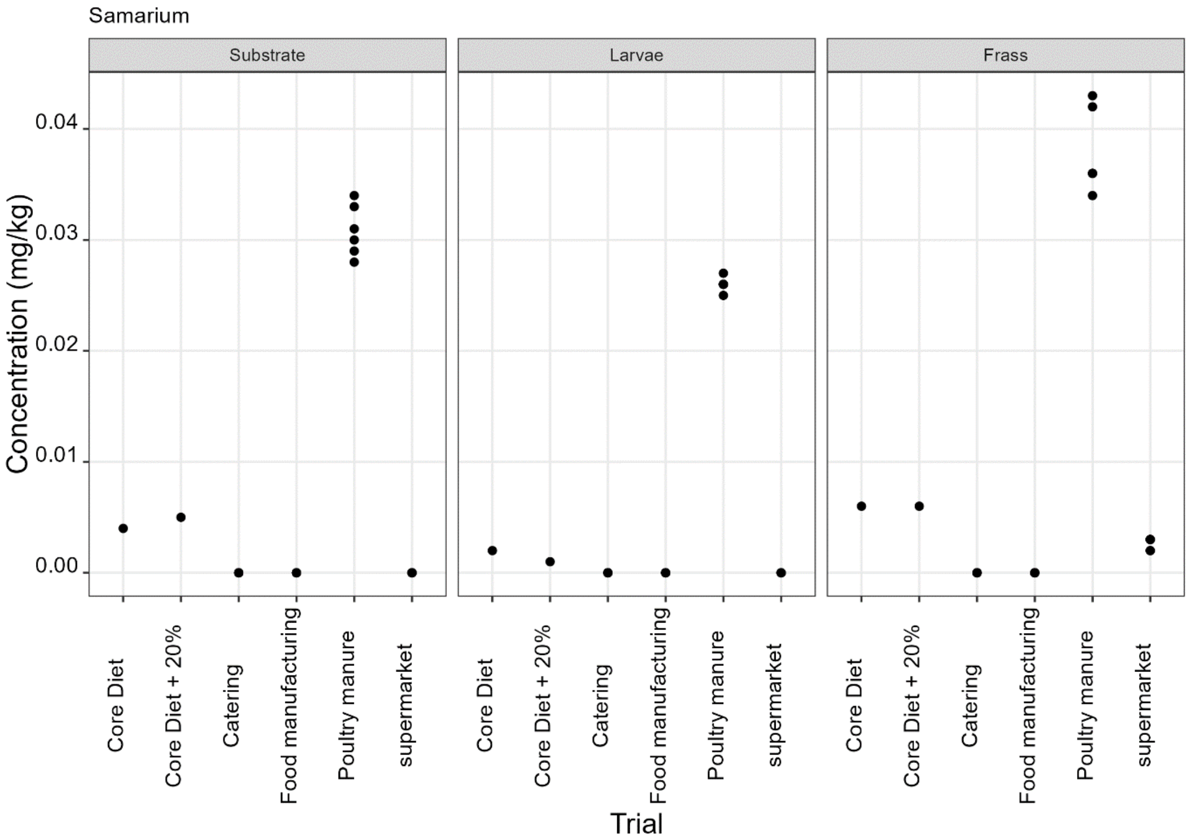


Rubidium
Substrate
Larvae
Frass
20
10
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

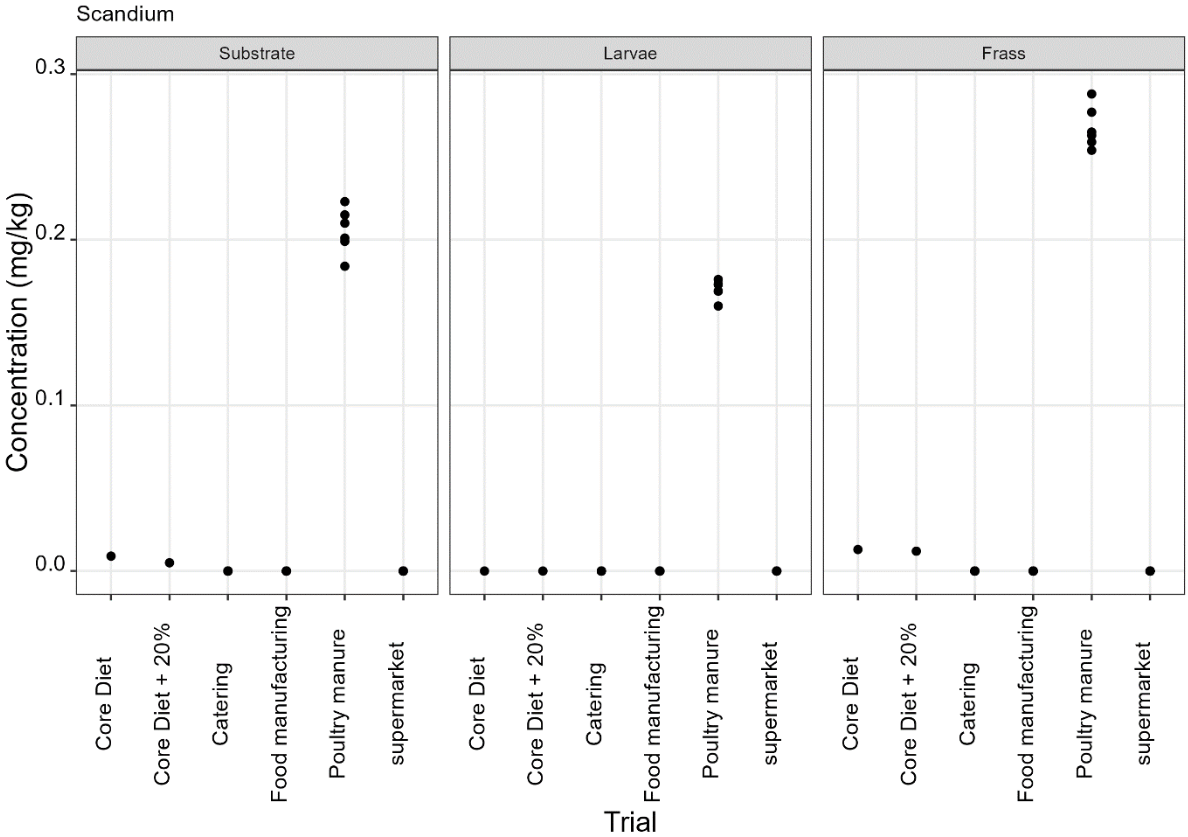
45.


Ruthenium
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

46.

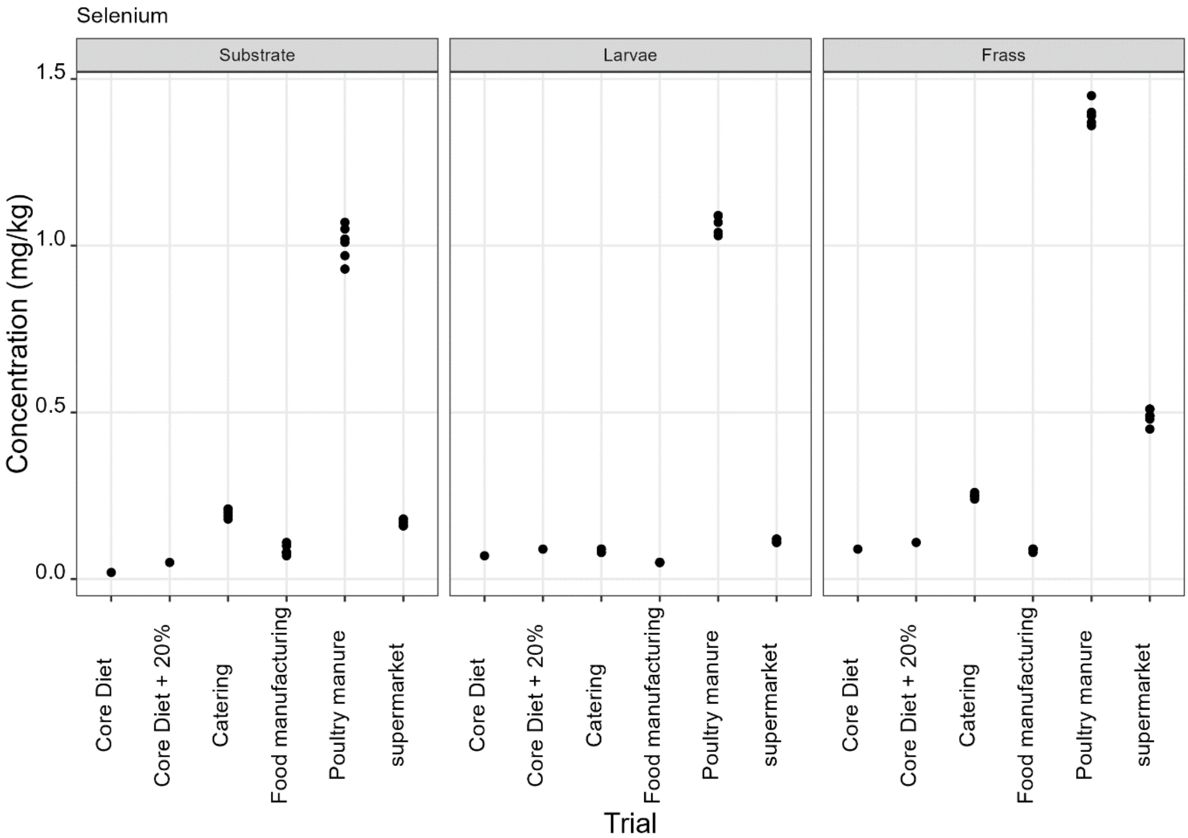

Samarium
Substrate
Larvae
Frass
0.04
0.03
0.02
0.01
0.00
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

47.



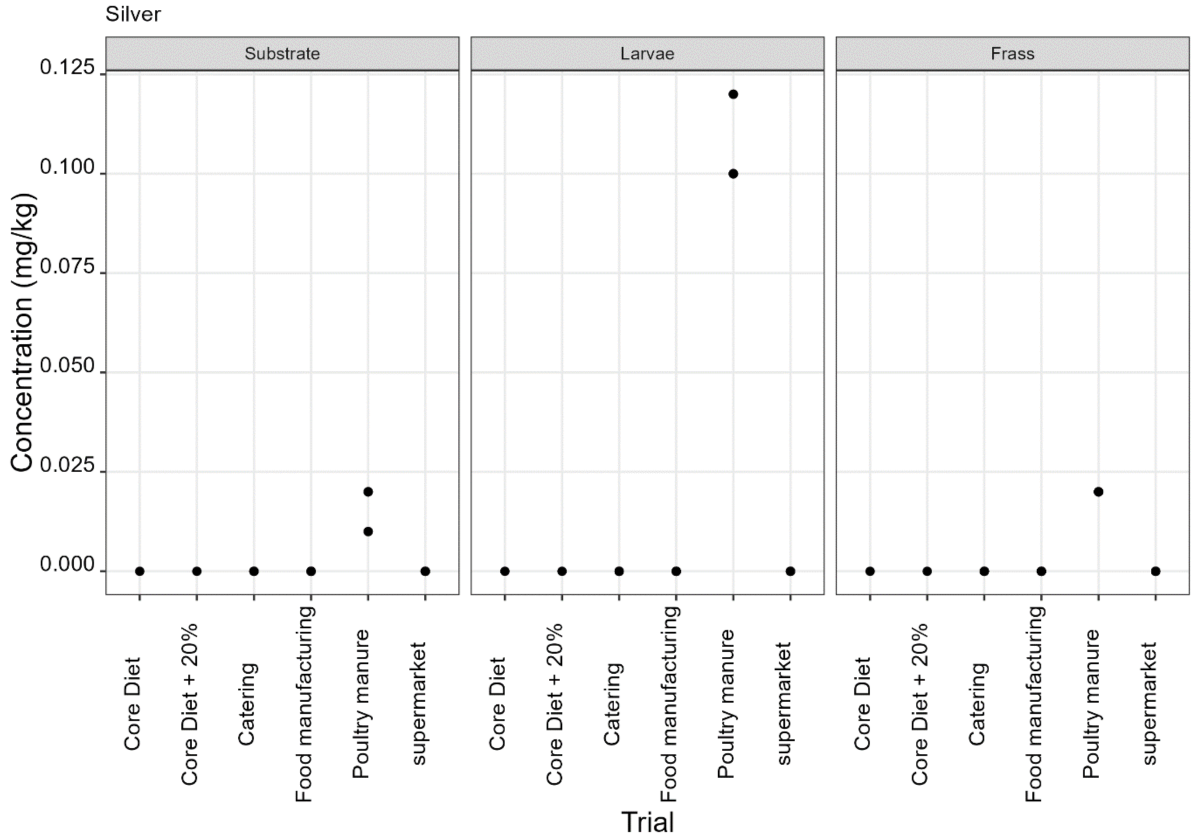
Scandium
Substrate
Larvae
Frass
0.3
0.2
0.1
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

48.

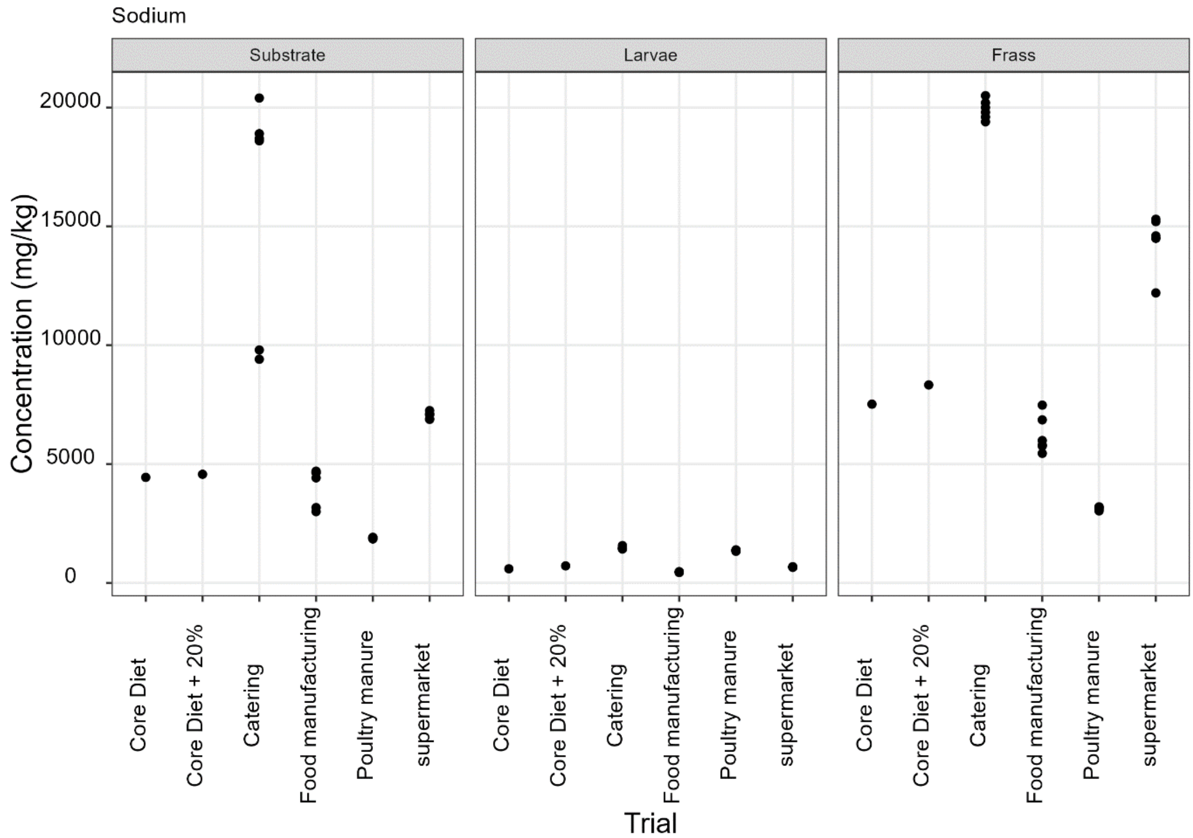


Selenium
Substrate
Larvae
Frass
1.5
1.0
0.5
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

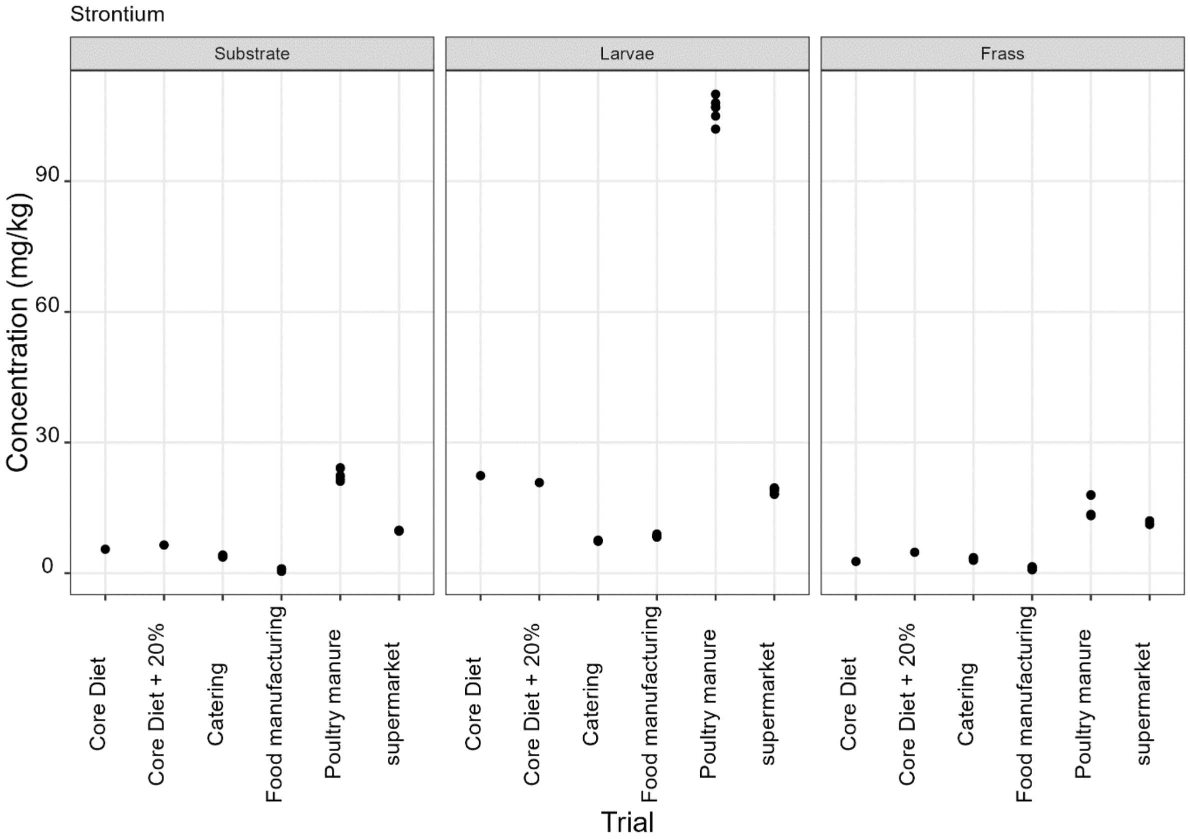
49.


Silver
Substrate
Larvae
Frass
Concentration (mg/kg)
0.125
0.100
0.075
0.050
0.025
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

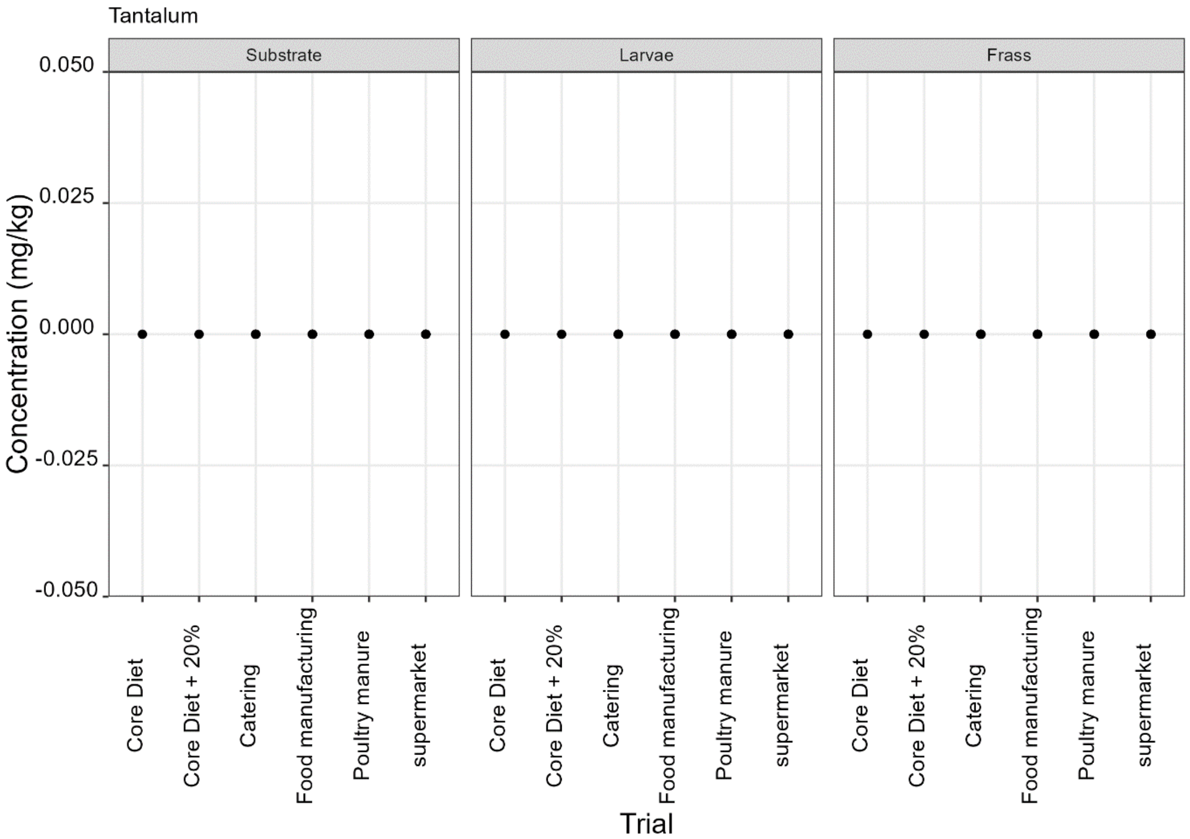
50.


Sodium
Substrate
Larvae
Frass
Concentration (mg/kg)
20000
15000
10000
5000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

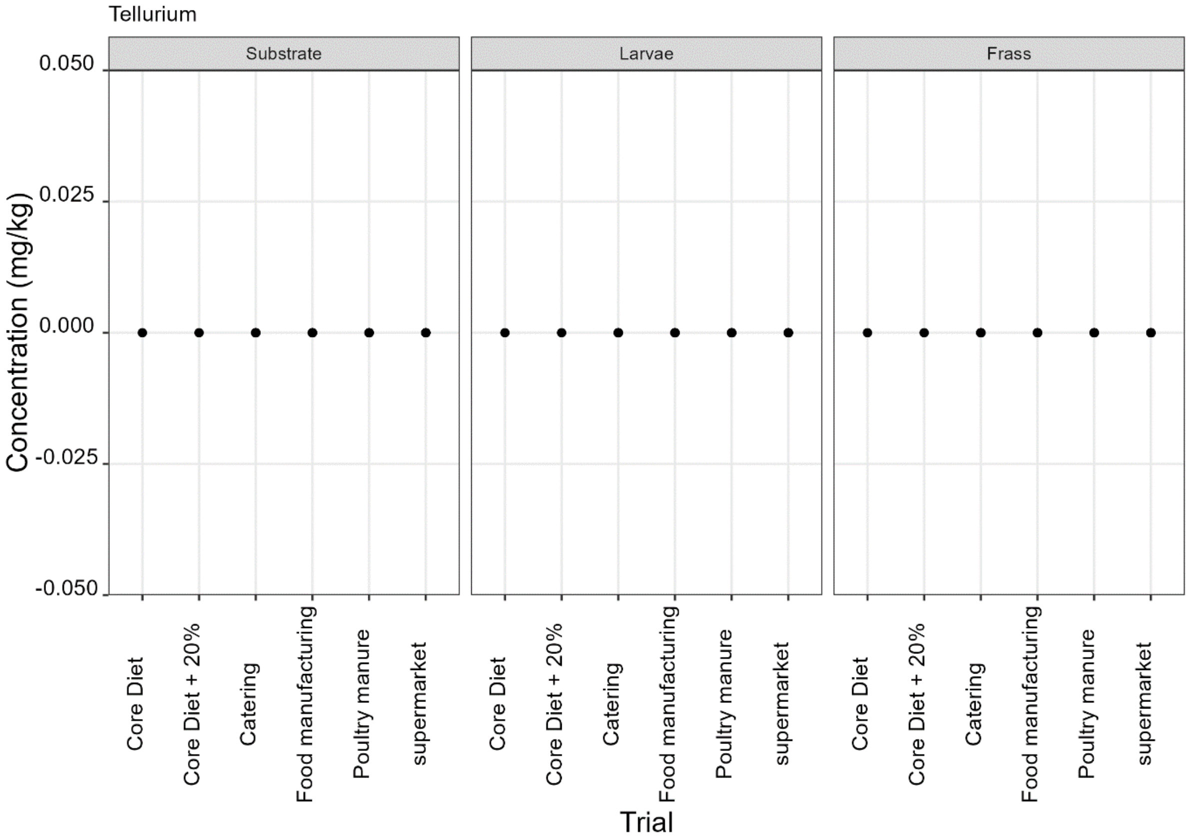
51.


Strontium
Substrate
Larvae
Frass
Concentration (mg/kg)
90
60
30
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

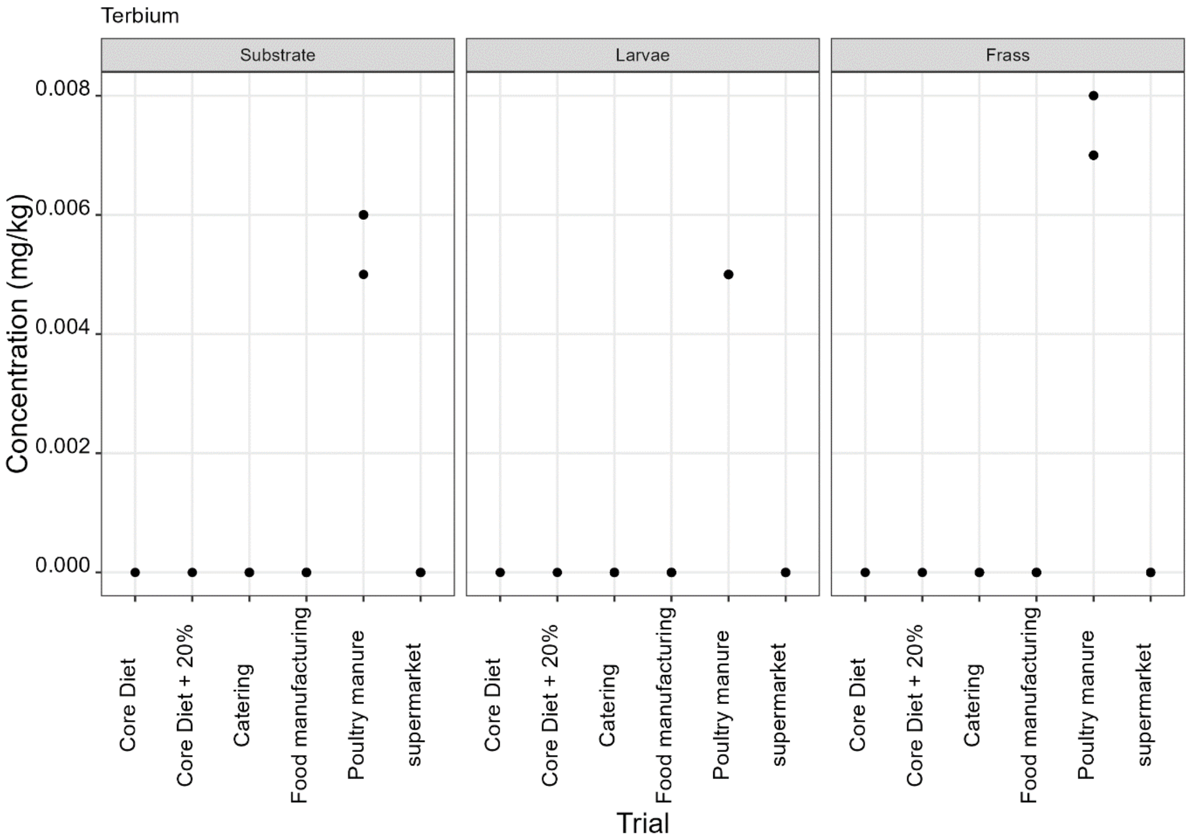
52.


Tantalum
Substrate
Larvae
Frass
Concentration (mg/kg)
0.050
0.025
0.000
-0.025
-0.050
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

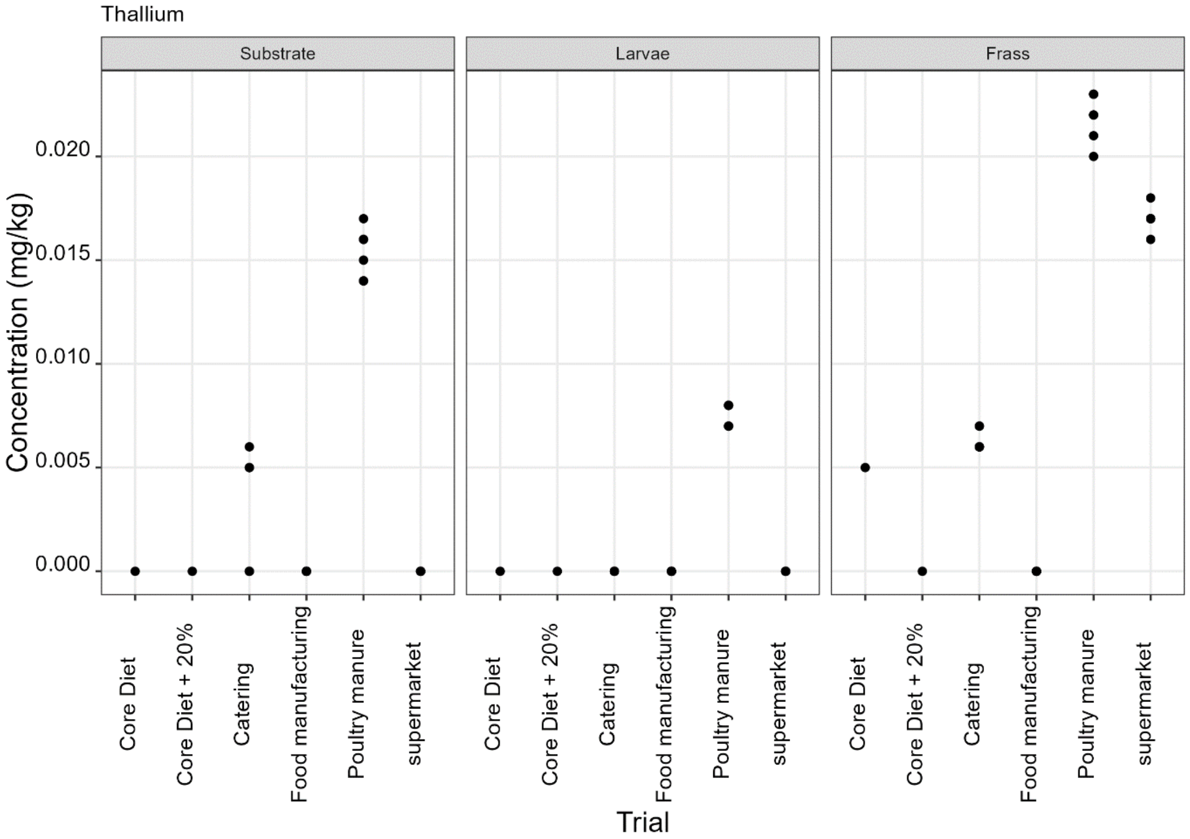
53.


Tellurium
Substrate
Larvae
Frass
0.050
0.025
0.000
-0.025
-0.050
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

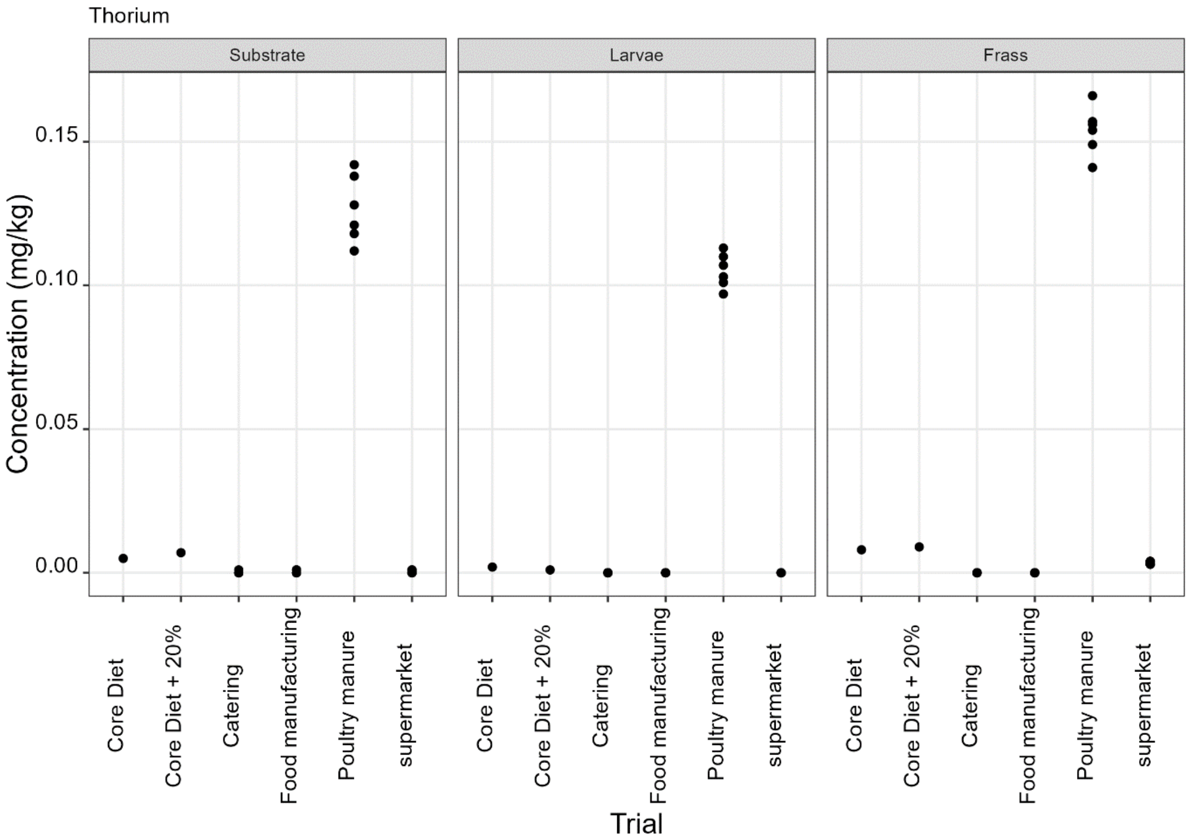
54.


Terbium
Substrate
Larvae
Frass
0.008
0.006
0.004
0.002
0.000
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

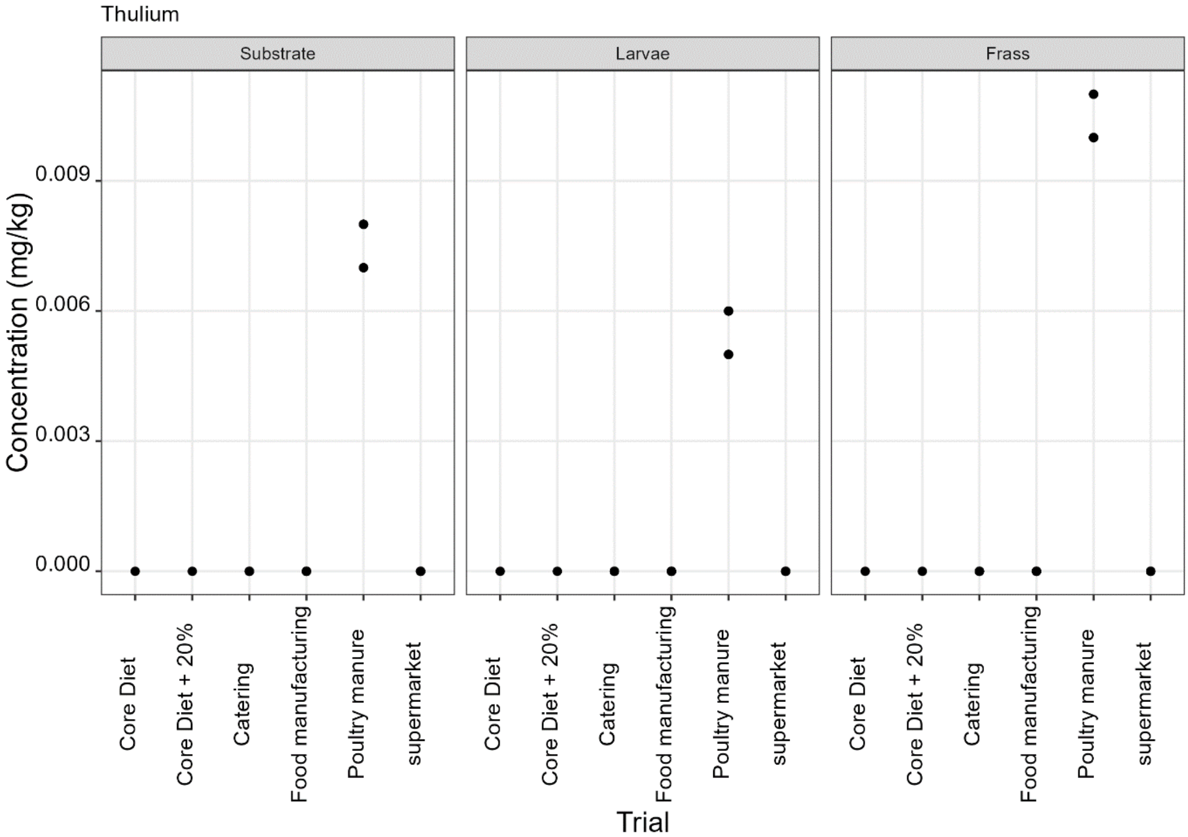
55.


Thallium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.020
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

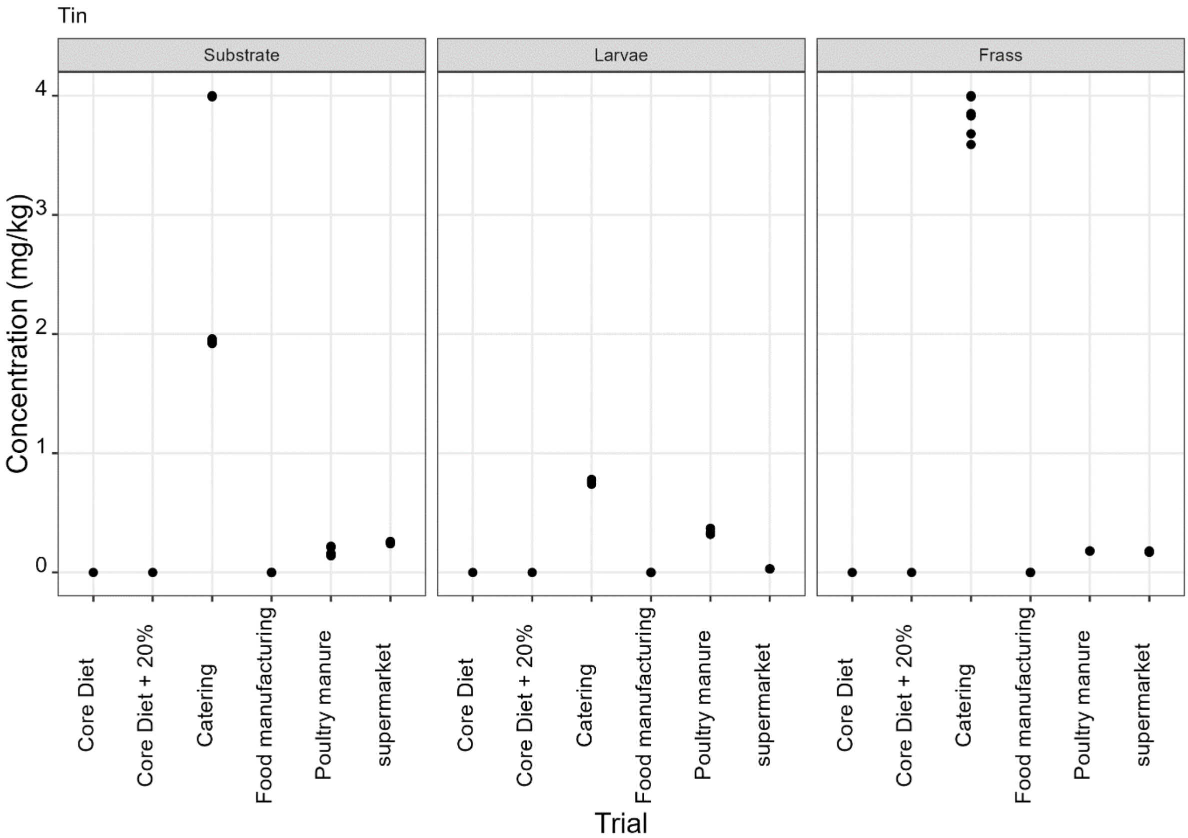
56.


Thorium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

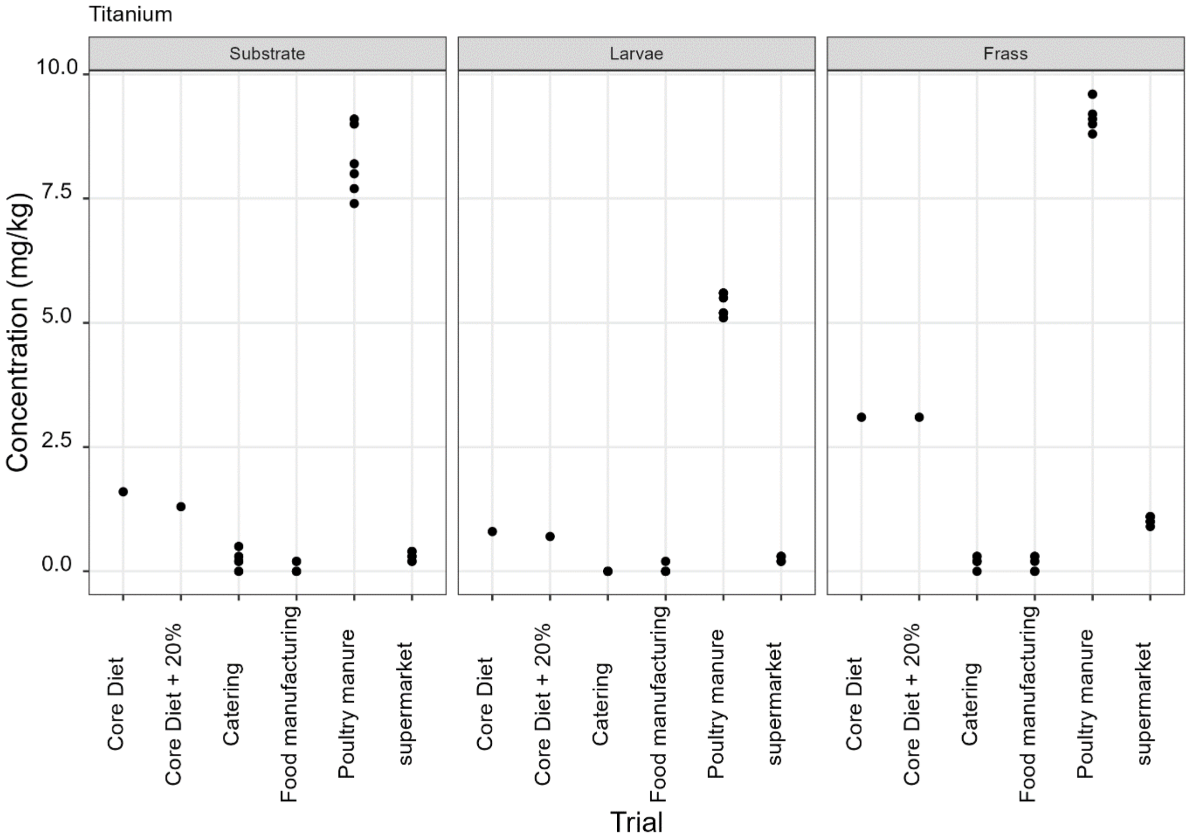
57.


Thulium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.000
0.003
0.006
0.009
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

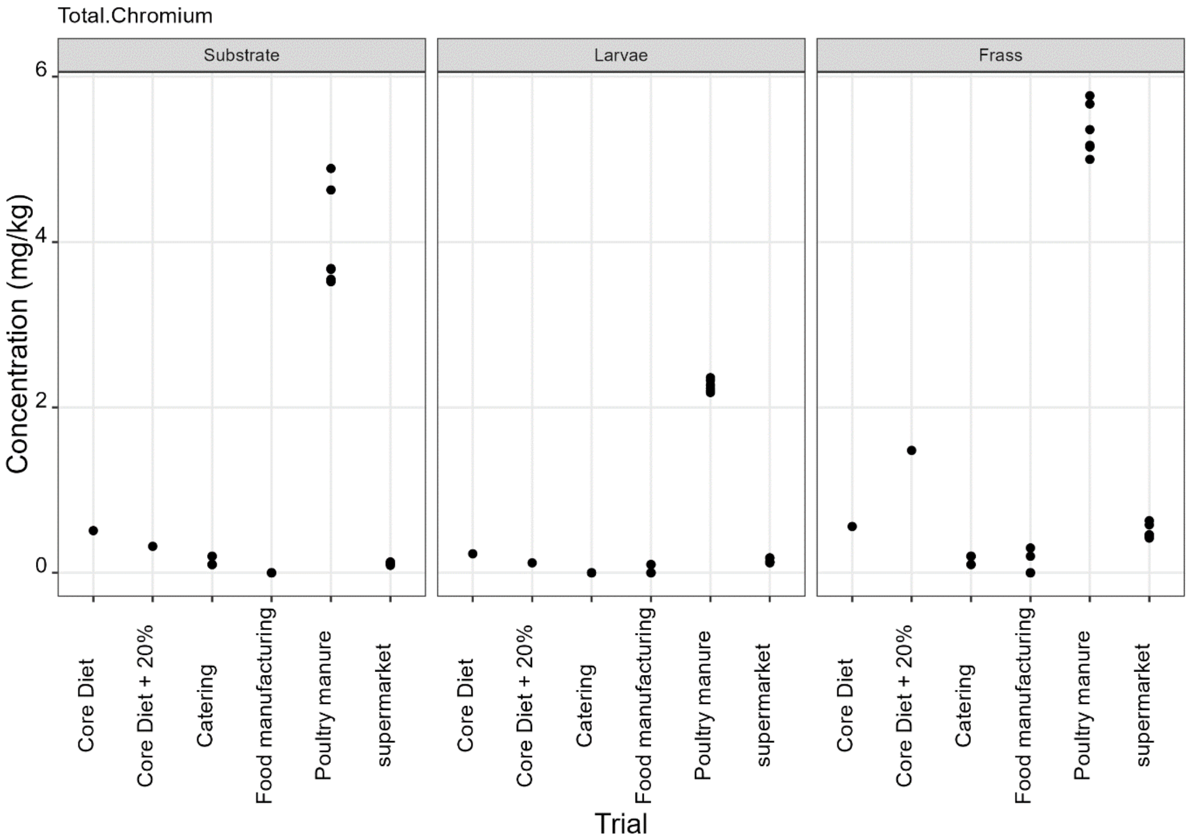
58.


Tin
Substrate
Larvae
Frass
Concentration (mg/kg)
0
1
2
3
4
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

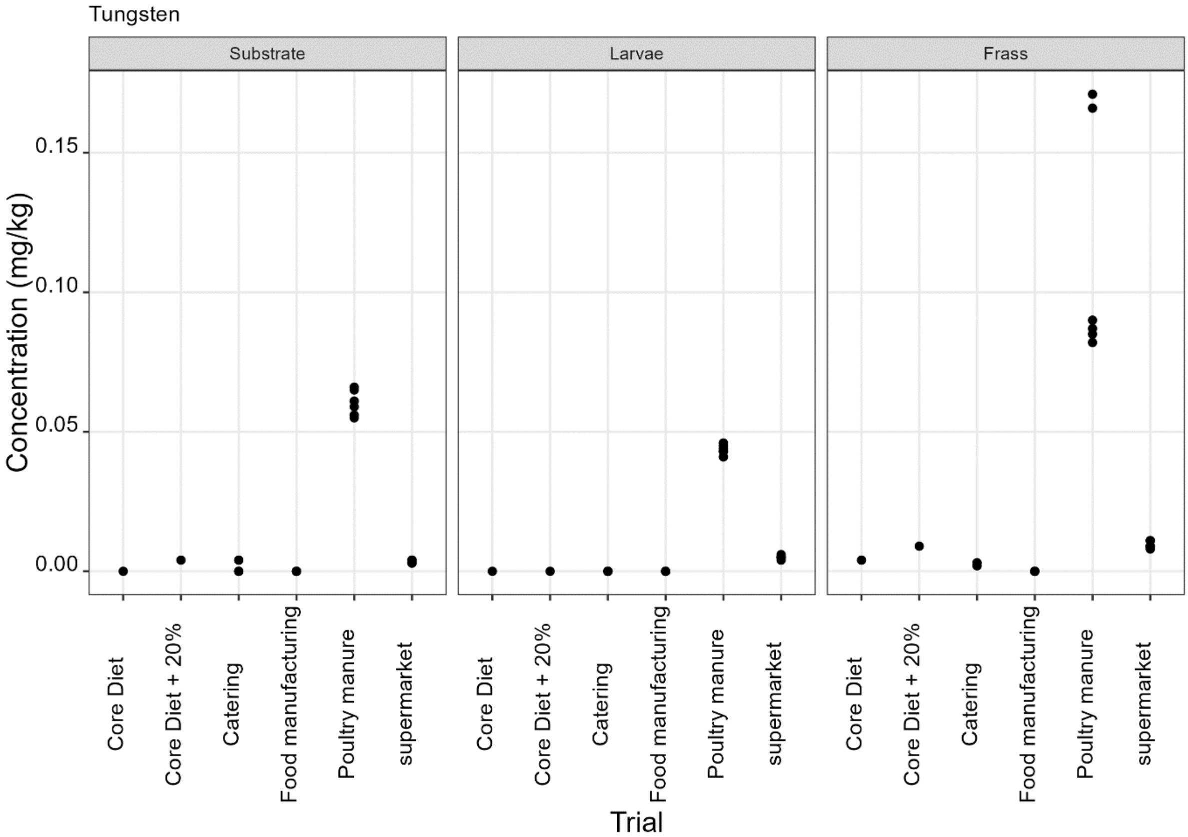
59.


Titanium
Substrate
Larvae
Frass
10.0
7.5
5.0
2.5
0.0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

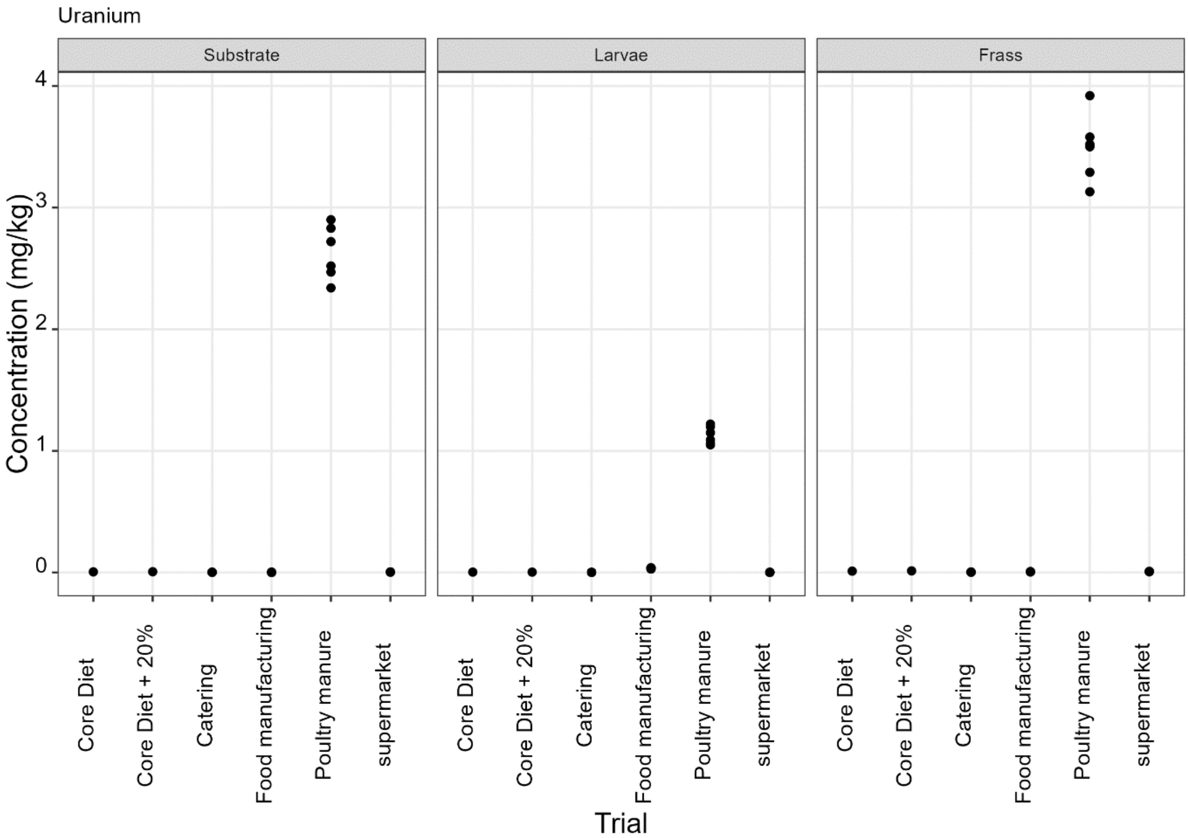
60.


Total.Chromium
Substrate
Larvae
Frass
6
4
2
0
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

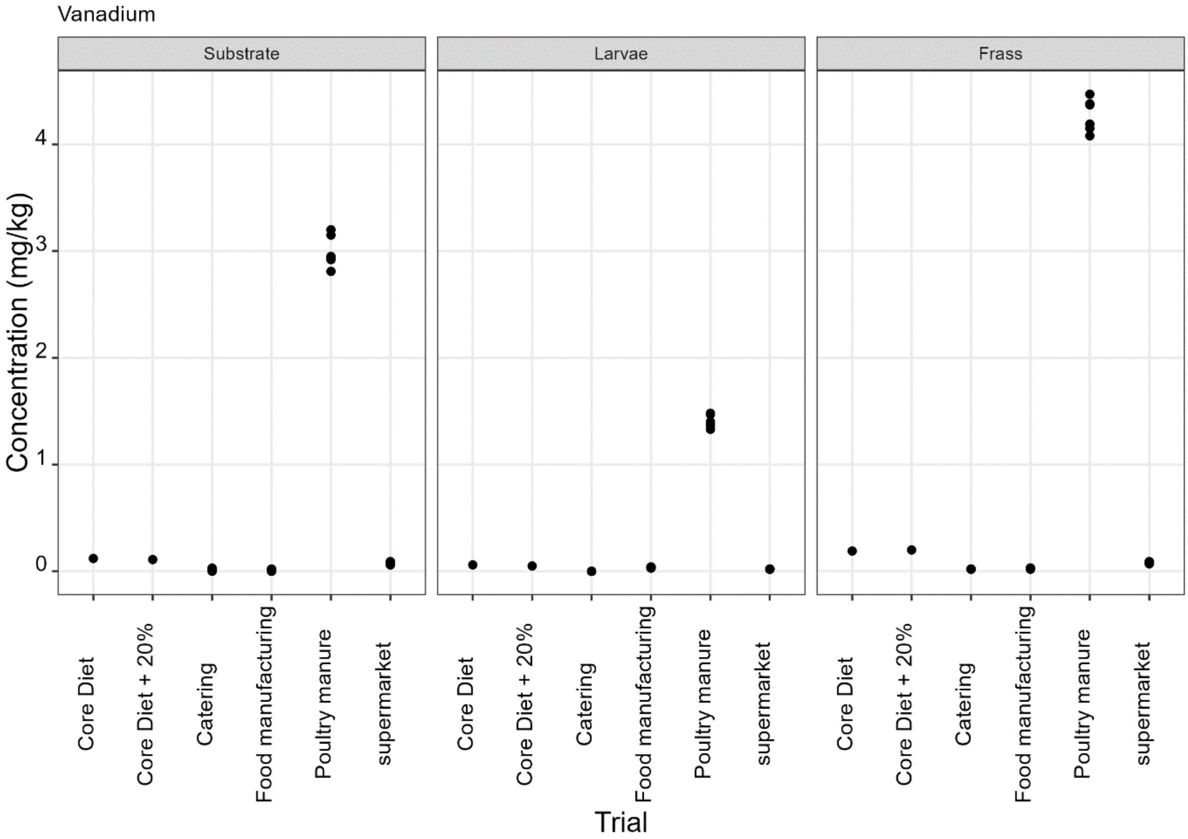
61.


Tungsten
Substrate
Larvae
Frass
Concentration (mg/kg)
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

62.

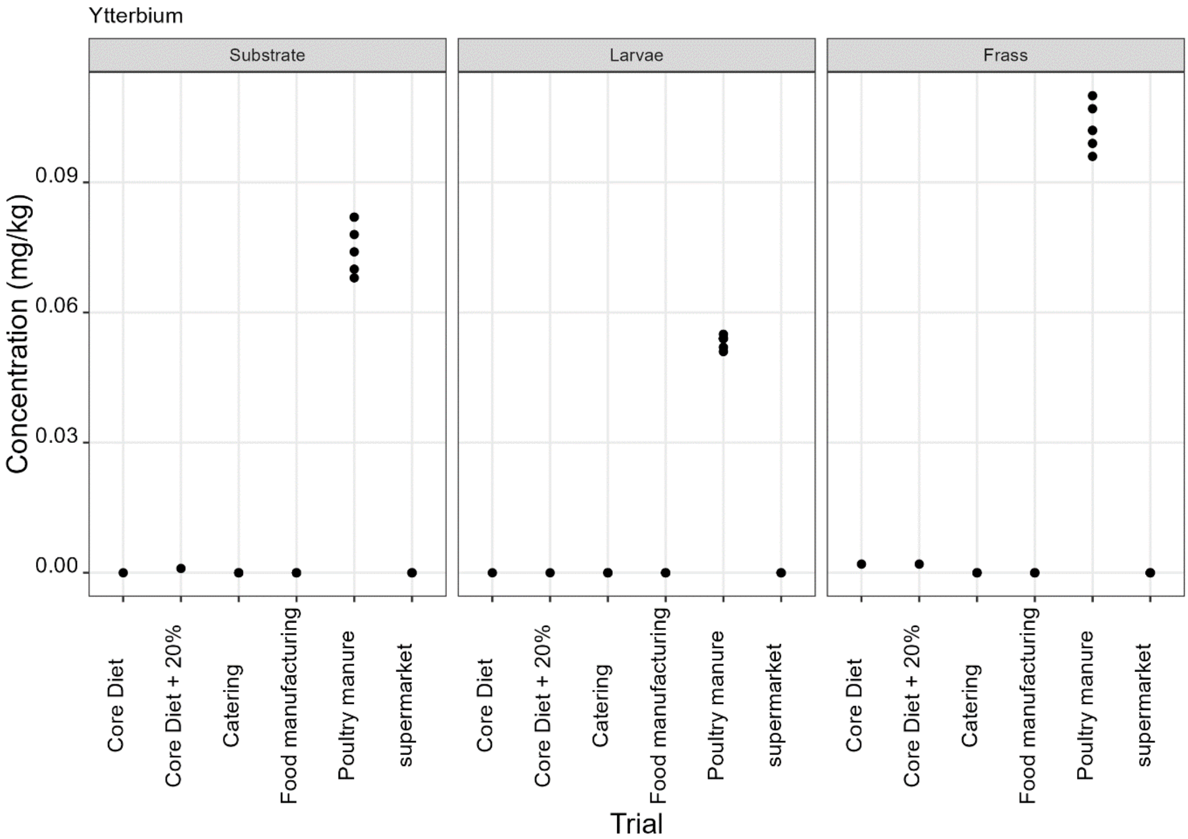

Uranium
Substrate
Larvae
Frass
Concentration (mg/kg)
4
3
2
1
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

63.



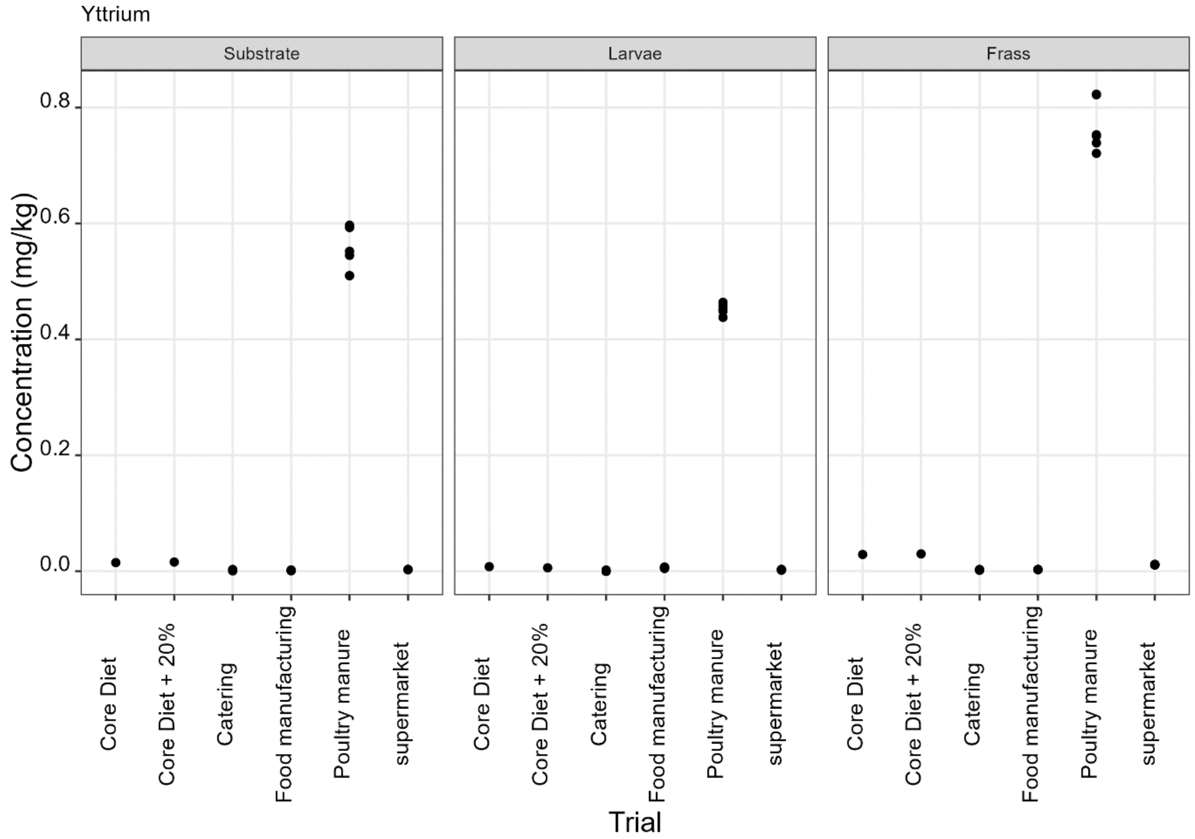
Vanadium
Substrate
Larvae
Frass
Concentration (mg/kg)
0
1
2
3
4
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

64.

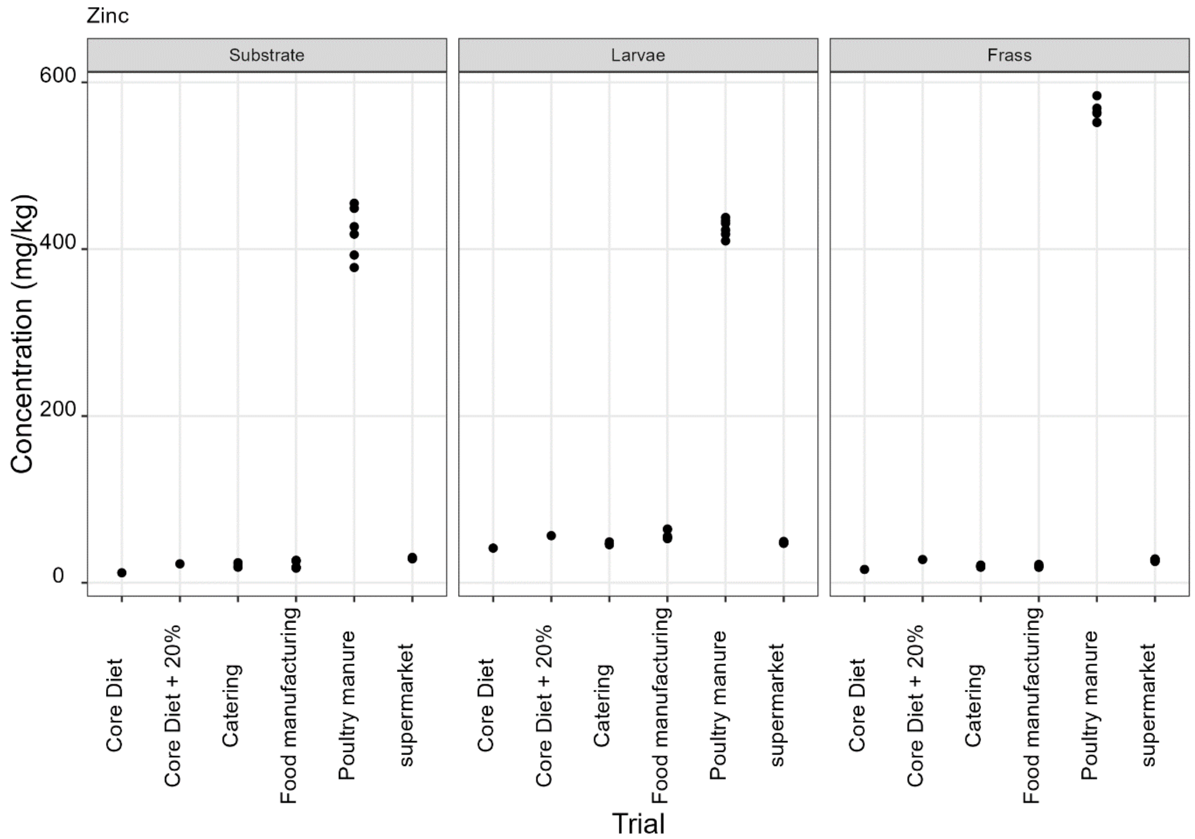


Ytterbium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.00
0.03
0.06
0.09
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

65.


Yttrium
Substrate
Larvae
Frass
Concentration (mg/kg)
0.8
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

66.


Zinc
Substrate
Larvae
Frass
Concentration (mg/kg)
600
400
200
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

67.

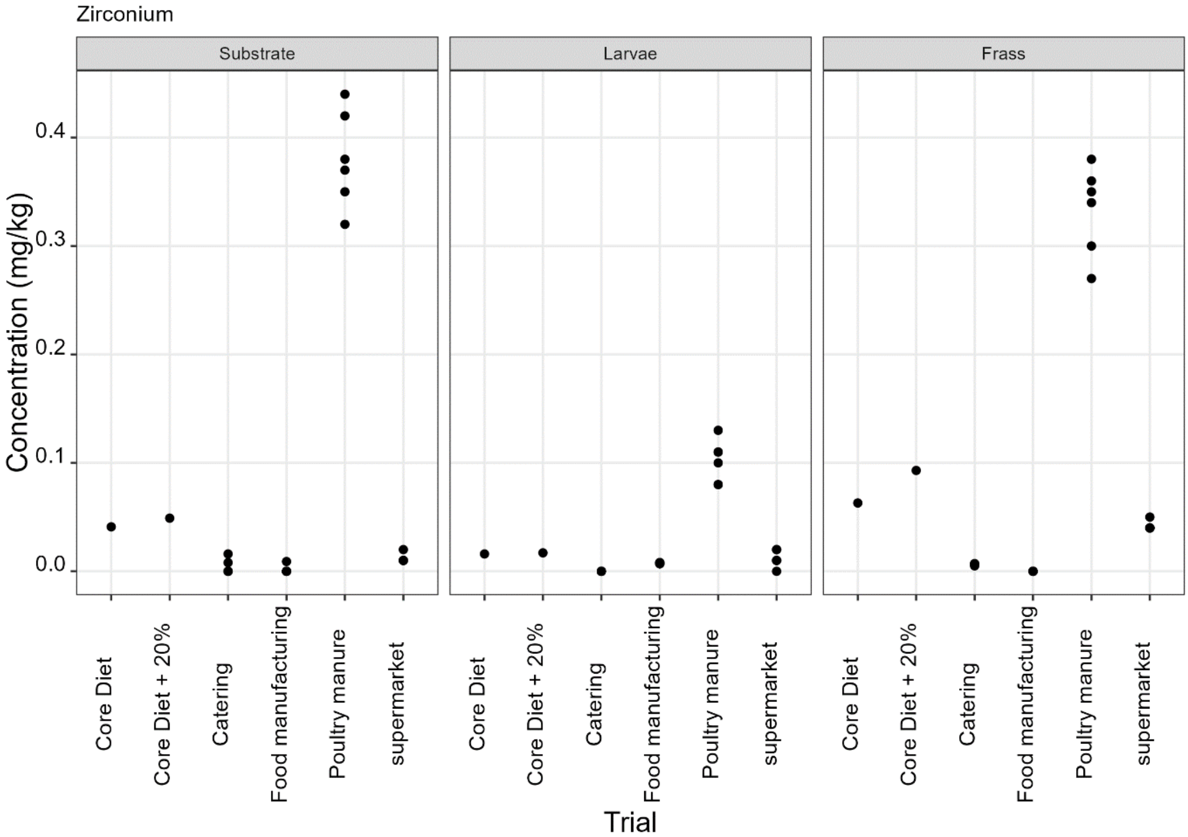


Figure I (1 – 67). Concentration (mg/kg) of metals analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table II. Calculated quantities of metals.

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Lithium	1	1	0.043	0.043	NA	NA	NA
core diet	Larvae	Lithium	1	1	0.025	0.025	0.581	NA	NA
core diet	Frass	Lithium	1	1	0.082	0.082	1.907	NA	NA
core diet	Substrate	Beryllium	1	1	0.001	0.001	NA	NA	NA
core diet	Larvae	Beryllium	1	0	0	0	0.000	NA	NA
core diet	Frass	Beryllium	1	1	0.003	0.003	3.000	NA	NA
core diet	Substrate	Boron	1	1	3	3	NA	NA	NA
core diet	Larvae	Boron	1	0	0	0	0.000	NA	NA
core diet	Frass	Boron	1	1	7	7	2.333	NA	NA
core diet	Substrate	Sodium	1	1	4440	4440	NA	NA	NA
core diet	Larvae	Sodium	1	1	594	594	0.134	NA	NA
core diet	Frass	Sodium	1	1	7520	7520	1.694	NA	NA
core diet	Substrate	Magnesium	1	1	470	470	NA	NA	NA
core diet	Larvae	Magnesium	1	1	1430	1430	3.043	NA	NA
core diet	Frass	Magnesium	1	1	576	576	1.226	NA	NA
core diet	Substrate	Aluminium	1	1	40.5	40.5	NA	NA	NA
core diet	Larvae	Aluminium	1	1	18.7	18.7	0.462	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Frass	Aluminium	1	1	70.3	70.3	1.736	NA	NA
core diet	Substrate	Phosphorus	1	1	1410	1410	NA	NA	NA
core diet	Larvae	Phosphorus	1	1	3880	3880	2.752	NA	NA
core diet	Frass	Phosphorus	1	1	1740	1740	1.234	NA	NA
core diet	Substrate	Potassium	1	1	9040	9040	NA	NA	NA
core diet	Larvae	Potassium	1	1	6350	6350	0.702	NA	NA
core diet	Frass	Potassium	1	1	15800	15800	1.748	NA	NA
core diet	Substrate	Calcium	1	1	2420	2420	NA	NA	NA
core diet	Larvae	Calcium	1	1	10400	10400	4.298	NA	NA
core diet	Frass	Calcium	1	1	634	634	0.262	NA	NA
core diet	Substrate	Scandium	1	1	0.009	0.009	NA	NA	NA
core diet	Larvae	Scandium	1	0	0	0	0.000	NA	NA
core diet	Frass	Scandium	1	1	0.013	0.013	1.444	NA	NA
core diet	Substrate	Titanium	1	1	1.6	1.6	NA	NA	NA
core diet	Larvae	Titanium	1	1	0.8	0.8	0.500	NA	NA
core diet	Frass	Titanium	1	1	3.1	3.1	1.938	NA	NA
core diet	Substrate	Vanadium	1	1	0.12	0.12	NA	NA	NA
core diet	Larvae	Vanadium	1	1	0.06	0.06	0.500	NA	NA
core diet	Frass	Vanadium	1	1	0.19	0.19	1.583	NA	NA
core diet	Substrate	Total.Chromium	1	1	0.51	0.51	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Larvae	Total.Chromium	1	1	0.23	0.23	0.451	NA	NA
core diet	Frass	Total.Chromium	1	1	0.56	0.56	1.098	NA	NA
core diet	Substrate	Manganese	1	1	6.92	6.92	NA	NA	NA
core diet	Larvae	Manganese	1	1	54.4	54.4	7.861	NA	NA
core diet	Frass	Manganese	1	1	3.4	3.4	0.491	NA	NA
core diet	Substrate	Iron	1	1	53.5	53.5	NA	NA	NA
core diet	Larvae	Iron	1	1	58	58	1.084	NA	NA
core diet	Frass	Iron	1	1	85	85	1.589	NA	NA
core diet	Substrate	Cobalt	1	1	0.033	0.033	NA	NA	NA
core diet	Larvae	Cobalt	1	1	0.016	0.016	0.485	NA	NA
core diet	Frass	Cobalt	1	1	0.054	0.054	1.636	NA	NA
core diet	Substrate	Nickel	1	1	0.25	0.25	NA	NA	NA
core diet	Larvae	Nickel	1	1	0.15	0.15	0.600	NA	NA
core diet	Frass	Nickel	1	1	0.37	0.37	1.480	NA	NA
core diet	Substrate	Copper	1	1	2.96	2.96	NA	NA	NA
core diet	Larvae	Copper	1	1	5.12	5.12	1.730	NA	NA
core diet	Frass	Copper	1	1	4.03	4.03	1.361	NA	NA
core diet	Substrate	Zinc	1	1	12	12	NA	NA	NA
core diet	Larvae	Zinc	1	1	41.6	41.6	3.467	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Frass	Zinc	1	1	16	16	1.333	NA	NA
core diet	Substrate	Gallium	1	1	0.01	0.01	NA	NA	NA
core diet	Larvae	Gallium	1	0	0	0	0.000	NA	NA
core diet	Frass	Gallium	1	1	0.03	0.03	3.000	NA	NA
core diet	Substrate	Germanium	1	0	0	0	NA	NA	NA
core diet	Larvae	Germanium	1	0	0	0	NA	NA	NA
core diet	Frass	Germanium	1	1	0.006	0.006	Inf	NA	NA
core diet	Substrate	Arsenic	1	1	0.023	0.023	NA	NA	NA
core diet	Larvae	Arsenic	1	1	0.015	0.015	0.652	NA	NA
core diet	Frass	Arsenic	1	1	0.042	0.042	1.826	NA	NA
core diet	Substrate	Selenium	1	1	0.02	0.02	NA	NA	NA
core diet	Larvae	Selenium	1	1	0.07	0.07	3.500	NA	NA
core diet	Frass	Selenium	1	1	0.09	0.09	4.500	NA	NA
core diet	Substrate	Rubidium	1	1	2.91	2.91	NA	NA	NA
core diet	Larvae	Rubidium	1	1	1.53	1.53	0.526	NA	NA
core diet	Frass	Rubidium	1	1	5.73	5.73	1.969	NA	NA
core diet	Substrate	Strontium	1	1	5.5	5.5	NA	NA	NA
core diet	Larvae	Strontium	1	1	22.4	22.4	4.073	NA	NA
core diet	Frass	Strontium	1	1	2.69	2.69	0.489	NA	NA
core diet	Substrate	Yttrium	1	1	0.015	0.015	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Larvae	Yttrium	1	1	0.008	0.008	0.533	NA	NA
core diet	Frass	Yttrium	1	1	0.029	0.029	1.933	NA	NA
core diet	Substrate	Zirconium	1	1	0.041	0.041	NA	NA	NA
core diet	Larvae	Zirconium	1	1	0.016	0.016	0.390	NA	NA
core diet	Frass	Zirconium	1	1	0.063	0.063	1.537	NA	NA
core diet	Substrate	Niobium	1	1	0.005	0.005	NA	NA	NA
core diet	Larvae	Niobium	1	1	0.002	0.002	0.400	NA	NA
core diet	Frass	Niobium	1	1	0.009	0.009	1.800	NA	NA
core diet	Substrate	Molybdenum	1	1	0.262	0.262	NA	NA	NA
core diet	Larvae	Molybdenum	1	1	0.265	0.265	1.011	NA	NA
core diet	Frass	Molybdenum	1	1	0.529	0.529	2.019	NA	NA
core diet	Substrate	Ruthenium	1	0	0	0	NA	NA	NA
core diet	Larvae	Ruthenium	1	0	0	0	NA	NA	NA
core diet	Frass	Ruthenium	1	0	0	0	NA	NA	NA
core diet	Substrate	Rhodium	1	0	0	0	NA	NA	NA
core diet	Larvae	Rhodium	1	0	0	0	NA	NA	NA
core diet	Frass	Rhodium	1	0	0	0	NA	NA	NA
core diet	Substrate	Palladium	1	0	0	0	NA	NA	NA
core diet	Larvae	Palladium	1	0	0	0	NA	NA	NA
core diet	Frass	Palladium	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Silver	1	0	0	0	NA	NA	NA
core diet	Larvae	Silver	1	0	0	0	NA	NA	NA
core diet	Frass	Silver	1	0	0	0	NA	NA	NA
core diet	Substrate	Cadmium	1	1	0.058	0.058	NA	NA	NA
core diet	Larvae	Cadmium	1	1	0.266	0.266	4.586	NA	NA
core diet	Frass	Cadmium	1	1	0.01	0.01	0.172	NA	NA
core diet	Substrate	Tin	1	0	0	0	NA	NA	NA
core diet	Larvae	Tin	1	0	0	0	NA	NA	NA
core diet	Frass	Tin	1	0	0	0	NA	NA	NA
core diet	Substrate	Antimony	1	1	0.003	0.003	NA	NA	NA
core diet	Larvae	Antimony	1	1	0.002	0.002	0.667	NA	NA
core diet	Frass	Antimony	1	1	0.005	0.005	1.667	NA	NA
core diet	Substrate	Tellurium	1	0	0	0	NA	NA	NA
core diet	Larvae	Tellurium	1	0	0	0	NA	NA	NA
core diet	Frass	Tellurium	1	0	0	0	NA	NA	NA
core diet	Substrate	Caesium	1	1	0.006	0.006	NA	NA	NA
core diet	Larvae	Caesium	1	1	0.003	0.003	0.500	NA	NA
core diet	Frass	Caesium	1	1	0.011	0.011	1.833	NA	NA
core diet	Substrate	Barium	1	1	1.77	1.77	NA	NA	NA
core diet	Larvae	Barium	1	1	6.36	6.36	3.593	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Frass	Barium	1	1	1.9	1.9	1.073	NA	NA
core diet	Substrate	Lanthanum	1	1	0.022	0.022	NA	NA	NA
core diet	Larvae	Lanthanum	1	1	0.014	0.014	0.636	NA	NA
core diet	Frass	Lanthanum	1	1	0.037	0.037	1.682	NA	NA
core diet	Substrate	Cerium	1	1	0.043	0.043	NA	NA	NA
core diet	Larvae	Cerium	1	1	0.027	0.027	0.628	NA	NA
core diet	Frass	Cerium	1	1	0.07	0.07	1.628	NA	NA
core diet	Substrate	Praseodymium	1	1	0.005	0.005	NA	NA	NA
core diet	Larvae	Praseodymium	1	1	0.003	0.003	0.600	NA	NA
core diet	Frass	Praseodymium	1	1	0.008	0.008	1.600	NA	NA
core diet	Substrate	Neodymium	1	1	0.019	0.019	NA	NA	NA
core diet	Larvae	Neodymium	1	1	0.012	0.012	0.632	NA	NA
core diet	Frass	Neodymium	1	1	0.031	0.031	1.632	NA	NA
core diet	Substrate	Samarium	1	1	0.004	0.004	NA	NA	NA
core diet	Larvae	Samarium	1	1	0.002	0.002	0.500	NA	NA
core diet	Frass	Samarium	1	1	0.006	0.006	1.500	NA	NA
core diet	Substrate	Europium	1	0	0	0	NA	NA	NA
core diet	Larvae	Europium	1	0	0	0	NA	NA	NA
core diet	Frass	Europium	1	1	0.002	0.002	Inf	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Gadolinium	1	1	0.003	0.003	NA	NA	NA
core diet	Larvae	Gadolinium	1	1	0.002	0.002	0.667	NA	NA
core diet	Frass	Gadolinium	1	1	0.006	0.006	2.000	NA	NA
core diet	Substrate	Terbium	1	0	0	0	NA	NA	NA
core diet	Larvae	Terbium	1	0	0	0	NA	NA	NA
core diet	Frass	Terbium	1	0	0	0	NA	NA	NA
core diet	Substrate	Dysprosium	1	1	0.002	0.002	NA	NA	NA
core diet	Larvae	Dysprosium	1	1	0.001	0.001	0.500	NA	NA
core diet	Frass	Dysprosium	1	1	0.005	0.005	2.500	NA	NA
core diet	Substrate	Holmium	1	0	0	0	NA	NA	NA
core diet	Larvae	Holmium	1	0	0	0	NA	NA	NA
core diet	Frass	Holmium	1	0	0	0	NA	NA	NA
core diet	Substrate	Erbium	1	1	0.001	0.001	NA	NA	NA
core diet	Larvae	Erbium	1	0	0	0	0.000	NA	NA
core diet	Frass	Erbium	1	1	0.003	0.003	3.000	NA	NA
core diet	Substrate	Thulium	1	0	0	0	NA	NA	NA
core diet	Larvae	Thulium	1	0	0	0	NA	NA	NA
core diet	Frass	Thulium	1	0	0	0	NA	NA	NA
core diet	Substrate	Ytterbium	1	0	0	0	NA	NA	NA
core diet	Larvae	Ytterbium	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Frass	Ytterbium	1	1	0.002	0.002	Inf	NA	NA
core diet	Substrate	Lutetium	1	0	0	0	NA	NA	NA
core diet	Larvae	Lutetium	1	0	0	0	NA	NA	NA
core diet	Frass	Lutetium	1	0	0	0	NA	NA	NA
core diet	Substrate	Hafnium	1	1	0.001	0.001	NA	NA	NA
core diet	Larvae	Hafnium	1	0	0	0	0.000	NA	NA
core diet	Frass	Hafnium	1	1	0.002	0.002	2.000	NA	NA
core diet	Substrate	Tantalum	1	0	0	0	NA	NA	NA
core diet	Larvae	Tantalum	1	0	0	0	NA	NA	NA
core diet	Frass	Tantalum	1	0	0	0	NA	NA	NA
core diet	Substrate	Tungsten	1	0	0	0	NA	NA	NA
core diet	Larvae	Tungsten	1	0	0	0	NA	NA	NA
core diet	Frass	Tungsten	1	1	0.004	0.004	Inf	NA	NA
core diet	Substrate	Rhenium	1	0	0	0	NA	NA	NA
core diet	Larvae	Rhenium	1	0	0	0	NA	NA	NA
core diet	Frass	Rhenium	1	0	0	0	NA	NA	NA
core diet	Substrate	Osmium	1	0	0	0	NA	NA	NA
core diet	Larvae	Osmium	1	0	0	0	NA	NA	NA
core diet	Frass	Osmium	1	0	0	0	NA	NA	NA
core diet	Substrate	Iridium	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Larvae	Iridium	1	0	0	0	NA	NA	NA
core diet	Frass	Iridium	1	0	0	0	NA	NA	NA
core diet	Substrate	Platinum	1	0	0	0	NA	NA	NA
core diet	Larvae	Platinum	1	0	0	0	NA	NA	NA
core diet	Frass	Platinum	1	0	0	0	NA	NA	NA
core diet	Substrate	Gold	1	0	0	0	NA	NA	NA
core diet	Larvae	Gold	1	0	0	0	NA	NA	NA
core diet	Frass	Gold	1	0	0	0	NA	NA	NA
core diet	Substrate	Mercury	1	0	0	0	NA	NA	NA
core diet	Larvae	Mercury	1	0	0	0	NA	NA	NA
core diet	Frass	Mercury	1	0	0	0	NA	NA	NA
core diet	Substrate	Thallium	1	0	0	0	NA	NA	NA
core diet	Larvae	Thallium	1	0	0	0	NA	NA	NA
core diet	Frass	Thallium	1	1	0.005	0.005	Inf	NA	NA
core diet	Substrate	Lead	1	1	0.259	0.259	NA	NA	NA
core diet	Larvae	Lead	1	1	0.044	0.044	0.170	NA	NA
core diet	Frass	Lead	1	1	0.062	0.062	0.239	NA	NA
core diet	Substrate	Bismuth	1	0	0	0	NA	NA	NA
core diet	Larvae	Bismuth	1	0	0	0	NA	NA	NA
core diet	Frass	Bismuth	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Thorium	1	1	0.005	0.005	NA	NA	NA
core diet	Larvae	Thorium	1	1	0.002	0.002	0.400	NA	NA
core diet	Frass	Thorium	1	1	0.008	0.008	1.600	NA	NA
core diet	Substrate	Uranium	1	1	0.006	0.006	NA	NA	NA
core diet	Larvae	Uranium	1	1	0.004	0.004	0.667	NA	NA
core diet	Frass	Uranium	1	1	0.012	0.012	2.000	NA	NA
core diet + 20%	Substrate	Lithium	1	1	0.055	0.055	NA	NA	NA
core diet + 20%	Larvae	Lithium	1	1	0.019	0.019	0.346	NA	NA
core diet + 20%	Frass	Lithium	1	1	0.103	0.103	1.873	NA	NA
core diet + 20%	Substrate	Beryllium	1	1	0.001	0.001	NA	NA	NA
core diet + 20%	Larvae	Beryllium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Beryllium	1	1	0.002	0.002	2.000	NA	NA
core diet + 20%	Substrate	Boron	1	1	3	3	NA	NA	NA
core diet + 20%	Larvae	Boron	1	1	1	1	0.333	NA	NA
core diet + 20%	Frass	Boron	1	1	6	6	2.000	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Sodium	1	1	4570	4570	NA	NA	NA
core diet + 20%	Larvae	Sodium	1	1	717	717	0.157	NA	NA
core diet + 20%	Frass	Sodium	1	1	8330	8330	1.823	NA	NA
core diet + 20%	Substrate	Magnesium	1	1	700	700	NA	NA	NA
core diet + 20%	Larvae	Magnesium	1	1	1790	1790	2.557	NA	NA
core diet + 20%	Frass	Magnesium	1	1	796	796	1.137	NA	NA
core diet + 20%	Substrate	Aluminium	1	1	30.8	30.8	NA	NA	NA
core diet + 20%	Larvae	Aluminium	1	1	12.3	12.3	0.399	NA	NA
core diet + 20%	Frass	Aluminium	1	1	62.5	62.5	2.029	NA	NA
core diet + 20%	Substrate	Phosphorus	1	1	2590	2590	NA	NA	NA
core diet + 20%	Larvae	Phosphorus	1	1	5500	5500	2.124	NA	NA
core diet + 20%	Frass	Phosphorus	1	1	3480	3480	1.344	NA	NA
core diet + 20%	Substrate	Potassium	1	1	9460	9460	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Potassium	1	1	7880	7880	0.833	NA	NA
core diet + 20%	Frass	Potassium	1	1	15600	15600	1.649	NA	NA
core diet + 20%	Substrate	Calcium	1	1	2660	2660	NA	NA	NA
core diet + 20%	Larvae	Calcium	1	1	11400	11400	4.286	NA	NA
core diet + 20%	Frass	Calcium	1	1	872	872	0.328	NA	NA
core diet + 20%	Substrate	Scandium	1	1	0.005	0.005	NA	NA	NA
core diet + 20%	Larvae	Scandium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Scandium	1	1	0.012	0.012	2.400	NA	NA
core diet + 20%	Substrate	Titanium	1	1	1.3	1.3	NA	NA	NA
core diet + 20%	Larvae	Titanium	1	1	0.7	0.7	0.539	NA	NA
core diet + 20%	Frass	Titanium	1	1	3.1	3.1	2.385	NA	NA
core diet + 20%	Substrate	Vanadium	1	1	0.11	0.11	NA	NA	NA
core diet + 20%	Larvae	Vanadium	1	1	0.05	0.05	0.455	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	Vanadium	1	1	0.2	0.2	1.818	NA	NA
core diet + 20%	Substrate	Total.Chromium	1	1	0.32	0.32	NA	NA	NA
core diet + 20%	Larvae	Total.Chromium	1	1	0.12	0.12	0.375	NA	NA
core diet + 20%	Frass	Total.Chromium	1	1	1.48	1.48	4.625	NA	NA
core diet + 20%	Substrate	Manganese	1	1	9.23	9.23	NA	NA	NA
core diet + 20%	Larvae	Manganese	1	1	58.7	58.7	6.360	NA	NA
core diet + 20%	Frass	Manganese	1	1	2.96	2.96	0.321	NA	NA
core diet + 20%	Substrate	Iron	1	1	61.7	61.7	NA	NA	NA
core diet + 20%	Larvae	Iron	1	1	71.5	71.5	1.159	NA	NA
core diet + 20%	Frass	Iron	1	1	106	106	1.718	NA	NA
core diet + 20%	Substrate	Cobalt	1	1	0.038	0.038	NA	NA	NA
core diet + 20%	Larvae	Cobalt	1	1	0.016	0.016	0.421	NA	NA
core diet + 20%	Frass	Cobalt	1	1	0.076	0.076	2.000	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Nickel	1	1	0.32	0.32	NA	NA	NA
core diet + 20%	Larvae	Nickel	1	1	0.15	0.15	0.469	NA	NA
core diet + 20%	Frass	Nickel	1	1	0.85	0.85	2.656	NA	NA
core diet + 20%	Substrate	Copper	1	1	3.62	3.62	NA	NA	NA
core diet + 20%	Larvae	Copper	1	1	6.18	6.18	1.707	NA	NA
core diet + 20%	Frass	Copper	1	1	5.43	5.43	1.500	NA	NA
core diet + 20%	Substrate	Zinc	1	1	22.7	22.7	NA	NA	NA
core diet + 20%	Larvae	Zinc	1	1	56.5	56.5	2.489	NA	NA
core diet + 20%	Frass	Zinc	1	1	27.9	27.9	1.229	NA	NA
core diet + 20%	Substrate	Gallium	1	1	0.01	0.01	NA	NA	NA
core diet + 20%	Larvae	Gallium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Gallium	1	1	0.02	0.02	2.000	NA	NA
core diet + 20%	Substrate	Germanium	1	1	0.006	0.006	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Germanium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Germanium	1	1	0.007	0.007	1.167	NA	NA
core diet + 20%	Substrate	Arsenic	1	1	0.027	0.027	NA	NA	NA
core diet + 20%	Larvae	Arsenic	1	1	0.021	0.021	0.778	NA	NA
core diet + 20%	Frass	Arsenic	1	1	0.06	0.06	2.222	NA	NA
core diet + 20%	Substrate	Selenium	1	1	0.05	0.05	NA	NA	NA
core diet + 20%	Larvae	Selenium	1	1	0.09	0.09	1.800	NA	NA
core diet + 20%	Frass	Selenium	1	1	0.11	0.11	2.200	NA	NA
core diet + 20%	Substrate	Rubidium	1	1	3.61	3.61	NA	NA	NA
core diet + 20%	Larvae	Rubidium	1	1	2.18	2.18	0.604	NA	NA
core diet + 20%	Frass	Rubidium	1	1	6.43	6.43	1.781	NA	NA
core diet + 20%	Substrate	Strontium	1	1	6.46	6.46	NA	NA	NA
core diet + 20%	Larvae	Strontium	1	1	20.8	20.8	3.220	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	Strontium	1	1	4.8	4.8	0.743	NA	NA
core diet + 20%	Substrate	Yttrium	1	1	0.016	0.016	NA	NA	NA
core diet + 20%	Larvae	Yttrium	1	1	0.006	0.006	0.375	NA	NA
core diet + 20%	Frass	Yttrium	1	1	0.03	0.03	1.875	NA	NA
core diet + 20%	Substrate	Zirconium	1	1	0.049	0.049	NA	NA	NA
core diet + 20%	Larvae	Zirconium	1	1	0.017	0.017	0.347	NA	NA
core diet + 20%	Frass	Zirconium	1	1	0.093	0.093	1.898	NA	NA
core diet + 20%	Substrate	Niobium	1	1	0.004	0.004	NA	NA	NA
core diet + 20%	Larvae	Niobium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Niobium	1	1	0.009	0.009	2.250	NA	NA
core diet + 20%	Substrate	Molybdenum	1	1	0.394	0.394	NA	NA	NA
core diet + 20%	Larvae	Molybdenum	1	1	0.342	0.342	0.868	NA	NA
core diet + 20%	Frass	Molybdenum	1	1	0.767	0.767	1.947	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Ruthenium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Ruthenium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Ruthenium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Rhodium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Rhodium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Rhodium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Palladium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Palladium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Palladium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Silver	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Silver	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Silver	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Cadmium	1	1	0.047	0.047	NA	NA	NA

Trial	Sample Type	Metal	Number of injection s	Detecte d	Mean (mg/kg)	Max (mg/kg)	Accumulatio n	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Cadmium	1	1	0.226	0.226	4.809	NA	NA
core diet + 20%	Frass	Cadmium	1	1	0.007	0.007	0.149	NA	NA
core diet + 20%	Substrat e	Tin	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Tin	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Tin	1	0	0	0	NA	NA	NA
core diet + 20%	Substrat e	Antimony	1	1	0.003	0.003	NA	NA	NA
core diet + 20%	Larvae	Antimony	1	1	0.001	0.001	0.333	NA	NA
core diet + 20%	Frass	Antimony	1	1	0.005	0.005	1.667	NA	NA
core diet + 20%	Substrat e	Tellurium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Tellurium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Tellurium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrat e	Caesium	1	1	0.006	0.006	NA	NA	NA
core diet + 20%	Larvae	Caesium	1	1	0.003	0.003	0.500	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	Caesium	1	1	0.01	0.01	1.667	NA	NA
core diet + 20%	Substrate	Barium	1	1	1.92	1.92	NA	NA	NA
core diet + 20%	Larvae	Barium	1	1	5.9	5.9	3.073	NA	NA
core diet + 20%	Frass	Barium	1	1	1.8	1.8	0.938	NA	NA
core diet + 20%	Substrate	Lanthanum	1	1	0.032	0.032	NA	NA	NA
core diet + 20%	Larvae	Lanthanum	1	1	0.007	0.007	0.219	NA	NA
core diet + 20%	Frass	Lanthanum	1	1	0.038	0.038	1.188	NA	NA
core diet + 20%	Substrate	Cerium	1	1	0.065	0.065	NA	NA	NA
core diet + 20%	Larvae	Cerium	1	1	0.013	0.013	0.200	NA	NA
core diet + 20%	Frass	Cerium	1	1	0.076	0.076	1.169	NA	NA
core diet + 20%	Substrate	Praseodymium	1	1	0.007	0.007	NA	NA	NA
core diet + 20%	Larvae	Praseodymium	1	1	0.002	0.002	0.286	NA	NA
core diet + 20%	Frass	Praseodymium	1	1	0.009	0.009	1.286	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Neodymium	1	1	0.029	0.029	NA	NA	NA
core diet + 20%	Larvae	Neodymium	1	1	0.006	0.006	0.207	NA	NA
core diet + 20%	Frass	Neodymium	1	1	0.034	0.034	1.172	NA	NA
core diet + 20%	Substrate	Samarium	1	1	0.005	0.005	NA	NA	NA
core diet + 20%	Larvae	Samarium	1	1	0.001	0.001	0.200	NA	NA
core diet + 20%	Frass	Samarium	1	1	0.006	0.006	1.200	NA	NA
core diet + 20%	Substrate	Europium	1	1	0.001	0.001	NA	NA	NA
core diet + 20%	Larvae	Europium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Europium	1	1	0.001	0.001	1.000	NA	NA
core diet + 20%	Substrate	Gadolinium	1	1	0.004	0.004	NA	NA	NA
core diet + 20%	Larvae	Gadolinium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Gadolinium	1	1	0.006	0.006	1.500	NA	NA
core diet + 20%	Substrate	Terbium	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Terbium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Terbium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Dysprosium	1	1	0.003	0.003	NA	NA	NA
core diet + 20%	Larvae	Dysprosium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Dysprosium	1	1	0.005	0.005	1.667	NA	NA
core diet + 20%	Substrate	Holmium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Holmium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Holmium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Erbium	1	1	0.001	0.001	NA	NA	NA
core diet + 20%	Larvae	Erbium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Erbium	1	1	0.003	0.003	3.000	NA	NA
core diet + 20%	Substrate	Thulium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Thulium	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	Thulium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Ytterbium	1	1	0.001	0.001	NA	NA	NA
core diet + 20%	Larvae	Ytterbium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Ytterbium	1	1	0.002	0.002	2.000	NA	NA
core diet + 20%	Substrate	Lutetium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Lutetium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Lutetium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Hafnium	1	1	0.001	0.001	NA	NA	NA
core diet + 20%	Larvae	Hafnium	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Hafnium	1	1	0.003	0.003	3.000	NA	NA
core diet + 20%	Substrate	Tantalum	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Tantalum	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Tantalum	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Tungsten	1	1	0.004	0.004	NA	NA	NA
core diet + 20%	Larvae	Tungsten	1	0	0	0	0.000	NA	NA
core diet + 20%	Frass	Tungsten	1	1	0.009	0.009	2.250	NA	NA
core diet + 20%	Substrate	Rhenium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Rhenium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Rhenium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Osmium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Osmium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Osmium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Iridium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Iridium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Iridium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Platinum	1	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Platinum	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Platinum	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Gold	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Gold	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Gold	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Mercury	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Mercury	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Mercury	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Thallium	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Thallium	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Thallium	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Lead	1	1	0.089	0.089	NA	NA	NA
core diet + 20%	Larvae	Lead	1	1	0.107	0.107	1.202	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	Lead	1	1	0.12	0.12	1.348	NA	NA
core diet + 20%	Substrate	Bismuth	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Bismuth	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Bismuth	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Thorium	1	1	0.007	0.007	NA	NA	NA
core diet + 20%	Larvae	Thorium	1	1	0.001	0.001	0.143	NA	NA
core diet + 20%	Frass	Thorium	1	1	0.009	0.009	1.286	NA	NA
core diet + 20%	Substrate	Uranium	1	1	0.007	0.007	NA	NA	NA
core diet + 20%	Larvae	Uranium	1	1	0.004	0.004	0.571	NA	NA
core diet + 20%	Frass	Uranium	1	1	0.014	0.014	2.000	NA	NA
Catering	Substrate	Lithium	6	6	0.0465	0.055	NA	0	0.3441
Catering	Larvae	Lithium	6	6	0.00775	0.009	0.167	-0.129	-0.06452
Catering	Frass	Lithium	6	6	0.056	0.058	1.204	0.05357	0
Catering	Substrate	Beryllium	6	0	0	0	NA	NA	NA
Catering	Larvae	Beryllium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Beryllium	6	0	0	0	NA	NA	NA
Catering	Substrate	Boron	6	6	2.625	3	NA	-0.1905	0.2857
Catering	Larvae	Boron	6	0	0	0	0.000	NA	NA
Catering	Frass	Boron	6	6	3	3	1.143	0	0
Catering	Substrate	Sodium	6	6	14380	20400	NA	-0.04868	-0.6638
Catering	Larvae	Sodium	6	6	1464	1570	0.102	-0.03757	-0.03245
Catering	Frass	Sodium	6	6	19960	20500	1.388	0.03257	0.01378
Catering	Substrate	Magnesium	6	6	688.8	720	NA	-0.0363	0.01815
Catering	Larvae	Magnesium	6	6	1649	1760	2.394	-0.03942	-0.02881
Catering	Frass	Magnesium	6	6	621.2	640	0.902	-0.00805	0.04427
Catering	Substrate	Aluminium	6	6	4.762	6.9	NA	-0.1155	0.8767
Catering	Larvae	Aluminium	6	6	0.8375	1.2	0.176	-0.4179	-0.3284
Catering	Frass	Aluminium	6	6	3.988	4.3	0.838	-0.163	0.1316
Catering	Substrate	Phosphorus	6	6	3615	4110	NA	-0.04426	-0.1355
Catering	Larvae	Phosphorus	6	6	4909	5140	1.358	-0.02343	-0.0056
Catering	Frass	Phosphorus	6	6	4000	4250	1.107	0.07	-0.0125
Catering	Substrate	Potassium	6	6	8172	8790	NA	-0.04161	-0.02876
Catering	Larvae	Potassium	6	6	7814	8250	0.956	-0.02623	-0.02272
Catering	Frass	Potassium	6	6	10520	10800	1.287	-0.02044	0.05299
Catering	Substrate	Calcium	6	6	2140	2330	NA	-0.02804	0.1449

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Calcium	6	6	5242	5370	2.450	-0.00763	0.02003
Catering	Frass	Calcium	6	6	1721	2050	0.804	0.1482	-0.1874
Catering	Substrate	Scandium	6	0	0	0	NA	NA	NA
Catering	Larvae	Scandium	6	0	0	0	NA	NA	NA
Catering	Frass	Scandium	6	0	0	0	NA	NA	NA
Catering	Substrate	Titanium	6	4	0.25	0.5	NA	0	1.2
Catering	Larvae	Titanium	6	0	0	0	0.000	NA	NA
Catering	Frass	Titanium	6	5	0.2125	0.3	0.850	-0.7059	0.3529
Catering	Substrate	Vanadium	6	5	0.01625	0.03	NA	-0.3077	1.077
Catering	Larvae	Vanadium	6	0	0	0	0.000	NA	NA
Catering	Frass	Vanadium	6	6	0.02	0.02	1.231	0	0
Catering	Substrate	Total.Chromium	6	6	0.15	0.2	NA	0	0.6667
Catering	Larvae	Total.Chromium	6	0	0	0	0.000	NA	NA
Catering	Frass	Total.Chromium	6	6	0.15	0.2	1.000	0	-0.6667
Catering	Substrate	Manganese	6	6	4.938	6.3	NA	-0.03038	0.5113
Catering	Larvae	Manganese	6	6	13.02	14	2.637	-0.02304	-0.06528
Catering	Frass	Manganese	6	6	3.375	3.5	0.684	-0.1185	0.04444
Catering	Substrate	Iron	6	6	22.26	26.3	NA	-0.00674	0.3223
Catering	Larvae	Iron	6	6	39.48	41.3	1.774	-0.04813	0.01393

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Iron	6	6	22.45	22.8	1.009	-0.01782	0.01782
Catering	Substrate	Cobalt	6	6	0.01387	0.019	NA	-0.03605	0.6669
Catering	Larvae	Cobalt	6	6	0.00975	0.011	0.703	-1.78E-16	-0.05128
Catering	Frass	Cobalt	6	6	0.01388	0.015	1.001	0.03602	0.1621
Catering	Substrate	Nickel	6	6	0.14	0.17	NA	-0.1429	0.3571
Catering	Larvae	Nickel	6	3	0.03	0.06	0.214	-2	0
Catering	Frass	Nickel	6	6	0.16	0.16	1.143	0	0
Catering	Substrate	Copper	6	6	2.25	2.7	NA	0	0.3111
Catering	Larvae	Copper	6	6	4.425	4.7	1.967	-0.0452	-0.0339
Catering	Frass	Copper	6	6	2	2.1	0.889	-0.05	0.1
Catering	Substrate	Zinc	6	6	21.49	24.1	NA	-0.02559	0.2106
Catering	Larvae	Zinc	6	6	46.96	49	2.185	-0.02449	-0.00905
Catering	Frass	Zinc	6	6	19.59	21.2	0.912	-0.06891	-0.0804
Catering	Substrate	Gallium	6	0	0	0	NA	NA	NA
Catering	Larvae	Gallium	6	0	0	0	NA	NA	NA
Catering	Frass	Gallium	6	0	0	0	NA	NA	NA
Catering	Substrate	Germanium	6	0	0	0	NA	NA	NA
Catering	Larvae	Germanium	6	0	0	0	NA	NA	NA
Catering	Frass	Germanium	6	0	0	0	NA	NA	NA
Catering	Substrate	Arsenic	6	6	0.07475	0.139	NA	-0.02676	1.679

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Arsenic	6	6	0.01188	0.013	0.159	-0.04209	0.02104
Catering	Frass	Arsenic	6	6	0.01688	0.018	0.226	0.02962	-0.04443
Catering	Substrate	Selenium	6	6	0.1962	0.21	NA	-0.02548	-0.1147
Catering	Larvae	Selenium	6	6	0.08375	0.09	0.427	0.0597	-0.08955
Catering	Frass	Selenium	6	6	0.25	0.26	1.274	0.04	0
Catering	Substrate	Rubidium	6	6	10.1	11.6	NA	0	-0.2762
Catering	Larvae	Rubidium	6	6	6.671	6.76	0.661	-0.00375	-0.00787
Catering	Frass	Rubidium	6	6	12.75	13.4	1.262	0.04706	-0.0549
Catering	Substrate	Strontium	6	6	3.97	4.14	NA	0.03023	0.08564
Catering	Larvae	Strontium	6	6	7.448	7.58	1.876	0.02014	-0.00067
Catering	Frass	Strontium	6	6	3.226	3.57	0.813	0.05425	-0.1527
Catering	Substrate	Yttrium	6	6	0.002125	0.003	NA	-0.2353	0.8235
Catering	Larvae	Yttrium	6	2	5.00E-04	0.002	0.235	-4	-2
Catering	Frass	Yttrium	6	6	0.00225	0.003	1.059	-0.4444	-0.2222
Catering	Substrate	Zirconium	6	2	0.006	0.016	NA	0	2
Catering	Larvae	Zirconium	6	0	0	0	0.000	NA	NA
Catering	Frass	Zirconium	6	6	0.0065	0.007	1.083	0	0.1538
Catering	Substrate	Niobium	6	0	0	0	NA	NA	NA
Catering	Larvae	Niobium	6	3	0.00075	0.002	Inf	-1.333	-2
Catering	Frass	Niobium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Substrate	Molybdenum	6	6	0.2886	0.324	NA	-0.01906	0.2244
Catering	Larvae	Molybdenum	6	6	0.3474	0.372	1.204	-0.0475	-0.07988
Catering	Frass	Molybdenum	6	6	0.3051	0.313	1.057	0.01147	0.02868
Catering	Substrate	Ruthenium	6	0	0	0	NA	NA	NA
Catering	Larvae	Ruthenium	6	0	0	0	NA	NA	NA
Catering	Frass	Ruthenium	6	0	0	0	NA	NA	NA
Catering	Substrate	Rhodium	6	0	0	0	NA	NA	NA
Catering	Larvae	Rhodium	6	0	0	0	NA	NA	NA
Catering	Frass	Rhodium	6	0	0	0	NA	NA	NA
Catering	Substrate	Palladium	6	0	0	0	NA	NA	NA
Catering	Larvae	Palladium	6	4	0.00125	0.002	Inf	0.8	-0.4
Catering	Frass	Palladium	6	0	0	0	NA	NA	NA
Catering	Substrate	Silver	6	0	0	0	NA	NA	NA
Catering	Larvae	Silver	6	0	0	0	NA	NA	NA
Catering	Frass	Silver	6	0	0	0	NA	NA	NA
Catering	Substrate	Cadmium	6	6	0.0255	0.029	NA	0.03922	0.2353
Catering	Larvae	Cadmium	6	6	0.0965	0.098	3.784	0.03109	0
Catering	Frass	Cadmium	6	6	0.03312	0.036	1.299	-0.07548	0.08303
Catering	Substrate	Tin	6	6	2.968	4	NA	0.01011	0.6924
Catering	Larvae	Tin	6	6	0.76	0.78	0.256	-0.01316	-0.03947

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Tin	6	6	3.776	4	1.272	0.04105	-0.07481
Catering	Substrate	Antimony	6	5	0.00425	0.006	NA	-0.4706	0.5882
Catering	Larvae	Antimony	6	0	0	0	0.000	NA	NA
Catering	Frass	Antimony	6	0	0	0	0.000	NA	NA
Catering	Substrate	Tellurium	6	0	0	0	NA	NA	NA
Catering	Larvae	Tellurium	6	0	0	0	NA	NA	NA
Catering	Frass	Tellurium	6	0	0	0	NA	NA	NA
Catering	Substrate	Caesium	6	6	0.03988	0.047	NA	-0.01254	-0.3448
Catering	Larvae	Caesium	6	6	0.018	0.018	0.451	0	0
Catering	Frass	Caesium	6	6	0.05512	0.06	1.382	0.0635	-0.0771
Catering	Substrate	Barium	6	6	1.311	1.47	NA	0.04958	0.2193
Catering	Larvae	Barium	6	6	2.196	2.29	1.675	0.0296	0.07172
Catering	Frass	Barium	6	6	1.37	1.58	1.045	0.05109	-0.2409
Catering	Substrate	Lanthanum	6	6	0.003	0.004	NA	0	0.6667
Catering	Larvae	Lanthanum	6	2	5.00E-04	0.002	0.167	-4	-2
Catering	Frass	Lanthanum	6	6	0.003	0.003	1.000	0	0
Catering	Substrate	Cerium	6	6	0.005	0.007	NA	0	0.8
Catering	Larvae	Cerium	6	6	0.001875	0.004	0.375	-0.8	-0.9333
Catering	Frass	Cerium	6	6	0.005	0.006	1.000	0.2	-0.2
Catering	Substrate	Praseodymium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Praseodymium	6	0	0	0	NA	NA	NA
Catering	Frass	Praseodymium	6	0	0	0	NA	NA	NA
Catering	Substrate	Neodymium	6	6	0.002	0.003	NA	0	1
Catering	Larvae	Neodymium	6	2	0.00025	0.001	0.125	-4	-2
Catering	Frass	Neodymium	6	6	0.002	0.002	1.000	0	0
Catering	Substrate	Samarium	6	0	0	0	NA	NA	NA
Catering	Larvae	Samarium	6	0	0	0	NA	NA	NA
Catering	Frass	Samarium	6	0	0	0	NA	NA	NA
Catering	Substrate	Europium	6	0	0	0	NA	NA	NA
Catering	Larvae	Europium	6	0	0	0	NA	NA	NA
Catering	Frass	Europium	6	0	0	0	NA	NA	NA
Catering	Substrate	Gadolinium	6	0	0	0	NA	NA	NA
Catering	Larvae	Gadolinium	6	0	0	0	NA	NA	NA
Catering	Frass	Gadolinium	6	0	0	0	NA	NA	NA
Catering	Substrate	Terbium	6	0	0	0	NA	NA	NA
Catering	Larvae	Terbium	6	0	0	0	NA	NA	NA
Catering	Frass	Terbium	6	0	0	0	NA	NA	NA
Catering	Substrate	Dysprosium	6	0	0	0	NA	NA	NA
Catering	Larvae	Dysprosium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Dysprosium	6	0	0	0	NA	NA	NA
Catering	Substrate	Holmium	6	0	0	0	NA	NA	NA
Catering	Larvae	Holmium	6	0	0	0	NA	NA	NA
Catering	Frass	Holmium	6	0	0	0	NA	NA	NA
Catering	Substrate	Erbium	6	0	0	0	NA	NA	NA
Catering	Larvae	Erbium	6	0	0	0	NA	NA	NA
Catering	Frass	Erbium	6	0	0	0	NA	NA	NA
Catering	Substrate	Thulium	6	0	0	0	NA	NA	NA
Catering	Larvae	Thulium	6	0	0	0	NA	NA	NA
Catering	Frass	Thulium	6	0	0	0	NA	NA	NA
Catering	Substrate	Ytterbium	6	0	0	0	NA	NA	NA
Catering	Larvae	Ytterbium	6	0	0	0	NA	NA	NA
Catering	Frass	Ytterbium	6	0	0	0	NA	NA	NA
Catering	Substrate	Lutetium	6	0	0	0	NA	NA	NA
Catering	Larvae	Lutetium	6	0	0	0	NA	NA	NA
Catering	Frass	Lutetium	6	0	0	0	NA	NA	NA
Catering	Substrate	Hafnium	6	0	0	0	NA	NA	NA
Catering	Larvae	Hafnium	6	0	0	0	NA	NA	NA
Catering	Frass	Hafnium	6	0	0	0	NA	NA	NA
Catering	Substrate	Tantalum	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Tantalum	6	0	0	0	NA	NA	NA
Catering	Frass	Tantalum	6	0	0	0	NA	NA	NA
Catering	Substrate	Tungsten	6	2	0.002	0.004	NA	0	2
Catering	Larvae	Tungsten	6	0	0	0	0.000	NA	NA
Catering	Frass	Tungsten	6	6	0.0025	0.003	1.250	0	-0.4
Catering	Substrate	Rhenium	6	0	0	0	NA	NA	NA
Catering	Larvae	Rhenium	6	0	0	0	NA	NA	NA
Catering	Frass	Rhenium	6	0	0	0	NA	NA	NA
Catering	Substrate	Osmium	6	0	0	0	NA	NA	NA
Catering	Larvae	Osmium	6	0	0	0	NA	NA	NA
Catering	Frass	Osmium	6	0	0	0	NA	NA	NA
Catering	Substrate	Iridium	6	0	0	0	NA	NA	NA
Catering	Larvae	Iridium	6	0	0	0	NA	NA	NA
Catering	Frass	Iridium	6	0	0	0	NA	NA	NA
Catering	Substrate	Platinum	6	0	0	0	NA	NA	NA
Catering	Larvae	Platinum	6	0	0	0	NA	NA	NA
Catering	Frass	Platinum	6	0	0	0	NA	NA	NA
Catering	Substrate	Gold	6	0	0	0	NA	NA	NA
Catering	Larvae	Gold	6	0	0	0	NA	NA	NA
Catering	Frass	Gold	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Substrate	Mercury	6	0	0	0	NA	NA	NA
Catering	Larvae	Mercury	6	0	0	0	NA	NA	NA
Catering	Frass	Mercury	6	0	0	0	NA	NA	NA
Catering	Substrate	Thallium	6	3	0.002	0.006	NA	1.5	-2
Catering	Larvae	Thallium	6	0	0	0	0.000	NA	NA
Catering	Frass	Thallium	6	6	0.00625	0.007	3.125	0.16	-0.08
Catering	Substrate	Lead	6	6	0.008375	0.011	NA	0.0597	0.6269
Catering	Larvae	Lead	6	6	0.0065	0.007	0.776	-0.1538	0
Catering	Frass	Lead	6	6	0.00925	0.01	1.104	0	-0.05405
Catering	Substrate	Bismuth	6	6	0.00625	0.01	NA	0.48	1.2
Catering	Larvae	Bismuth	6	0	0	0	0.000	NA	NA
Catering	Frass	Bismuth	6	6	0.001	0.001	0.160	0	0
Catering	Substrate	Thorium	6	1	0.00025	0.001	NA	0	2
Catering	Larvae	Thorium	6	0	0	0	0.000	NA	NA
Catering	Frass	Thorium	6	0	0	0	0.000	NA	NA
Catering	Substrate	Uranium	6	6	0.00275	0.003	NA	0	0.1818
Catering	Larvae	Uranium	6	6	0.002	0.002	0.727	0	0
Catering	Frass	Uranium	6	6	0.00375	0.005	1.364	-0.2667	-0.4
Manufacturing	Substrate	Lithium	6	6	0.0185	0.022	NA	-0.05405	0.2703

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Lithium	6	5	0.004	0.006	0.216	0	-0.75
Manufacturing	Frass	Lithium	6	6	0.02787	0.03	1.506	-0.01794	0.1166
Manufacturing	Substrate	Beryllium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Beryllium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Beryllium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Boron	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Boron	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Boron	6	6	4	6	Inf	-1	0
Manufacturing	Substrate	Sodium	6	6	3842	4700	NA	-0.02082	-0.3943
Manufacturing	Larvae	Sodium	6	6	455	470	0.118	-0.04396	-0.02198
Manufacturing	Frass	Sodium	6	6	6460	7480	1.681	0.04489	0.2198
Manufacturing	Substrate	Magnesium	6	6	216.2	220	NA	-0.02313	-0.01156
Manufacturing	Larvae	Magnesium	6	6	1849	1890	8.552	-0.01352	0.01758

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Magnesium	6	6	205	310	0.948	-0.6829	-0.2927
Manufacturing	Substrate	Aluminium	6	6	3.162	4	NA	0.3321	-0.1344
Manufacturing	Larvae	Aluminium	6	6	2.262	2.4	0.715	0.0221	-0.05526
Manufacturing	Frass	Aluminium	6	6	10.9	13.9	3.447	-0.1743	0.4587
Manufacturing	Substrate	Phosphorus	6	6	1378	1590	NA	-0.02903	0.2358
Manufacturing	Larvae	Phosphorus	6	6	4566	4680	3.313	-0.00548	0.006023
Manufacturing	Frass	Phosphorus	6	6	1312	1480	0.952	-0.2287	0.0343
Manufacturing	Substrate	Potassium	6	6	2914	3280	NA	-0.01201	0.2068
Manufacturing	Larvae	Potassium	6	6	7212	7390	2.475	-0.0208	0.01317
Manufacturing	Frass	Potassium	6	6	2809	3100	0.964	-0.1299	0.1219
Manufacturing	Substrate	Calcium	6	6	826.2	1210	NA	-0.03026	-0.8745
Manufacturing	Larvae	Calcium	6	6	10640	11000	12.880	-0.0047	-0.03524
Manufacturing	Frass	Calcium	6	6	1224	1830	1.481	-0.625	-0.3084

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Scandium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Scandium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Scandium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Titanium	6	2	0.05	0.2	NA	-4	-2
Manufacturing	Larvae	Titanium	6	1	0.025	0.2	0.500	4	-2
Manufacturing	Frass	Titanium	6	3	0.175	0.3	3.500	-0.5714	1.429
Manufacturing	Substrate	Vanadium	6	4	0.01	0.02	NA	0	-2
Manufacturing	Larvae	Vanadium	6	6	0.035	0.04	3.500	0	-0.2857
Manufacturing	Frass	Vanadium	6	6	0.0275	0.03	2.750	0	0.1818
Manufacturing	Substrate	Total.Chromium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Total.Chromium	6	2	0.05	0.1	Inf	0	2
Manufacturing	Frass	Total.Chromium	6	2	0.125	0.3	Inf	0	2
Manufacturing	Substrate	Manganese	6	6	2.088	2.7	NA	0.02395	-0.5148

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Manganese	6	6	27.78	29.3	13.300	0.0324	-0.0558
Manufacturing	Frass	Manganese	6	6	2.55	4.1	1.221	-0.8235	-0.3529
Manufacturing	Substrate	Iron	6	6	26.79	30.9	NA	-0.00187	0.2622
Manufacturing	Larvae	Iron	6	6	71.24	73.6	2.659	-0.03439	0.03264
Manufacturing	Frass	Iron	6	6	25.49	27.9	0.952	-0.1628	0.05983
Manufacturing	Substrate	Cobalt	6	1	0.000625	0.005	NA	4	-2
Manufacturing	Larvae	Cobalt	6	0	0	0	0.000	NA	NA
Manufacturing	Frass	Cobalt	6	5	0.005375	0.007	8.600	0.4651	0.4186
Manufacturing	Substrate	Nickel	6	4	0.02625	0.06	NA	0.1905	-2
Manufacturing	Larvae	Nickel	6	0	0	0	0.000	NA	NA
Manufacturing	Frass	Nickel	6	6	0.06125	0.07	2.333	-0.08163	0.1224
Manufacturing	Substrate	Copper	6	6	1.138	1.2	NA	-0.04394	-0.06591
Manufacturing	Larvae	Copper	6	6	5.738	5.9	5.042	-0.04357	0.02178

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Copper	6	6	1.425	1.7	1.252	-0.3509	0.03509
Manufacturing	Substrate	Zinc	6	6	22.38	27	NA	-0.01787	0.3686
Manufacturing	Larvae	Zinc	6	6	57	64.4	2.547	-0.186	-0.06842
Manufacturing	Frass	Zinc	6	6	20.36	22	0.910	-0.06139	0.08718
Manufacturing	Substrate	Gallium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Gallium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Gallium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Germanium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Germanium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Germanium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Arsenic	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Arsenic	6	3	0.002125	0.006	Inf	-1.647	-2
Manufacturing	Frass	Arsenic	6	1	0.00125	0.005	Inf	0	2

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Selenium	6	6	0.09125	0.11	NA	-0.05479	0.3014
Manufacturing	Larvae	Selenium	6	6	0.05	0.05	0.548	0	0
Manufacturing	Frass	Selenium	6	6	0.08875	0.09	0.973	0.05634	0.02817
Manufacturing	Substrate	Rubidium	6	6	2.79	3.23	NA	-0.04301	0.2867
Manufacturing	Larvae	Rubidium	6	6	4.302	4.36	1.542	0.004649	-0.00349
Manufacturing	Frass	Rubidium	6	6	2.629	2.85	0.942	-0.01712	0.1417
Manufacturing	Substrate	Strontium	6	6	0.7012	1	NA	-0.0927	-0.7594
Manufacturing	Larvae	Strontium	6	6	8.475	8.98	12.090	0.05546	-0.04366
Manufacturing	Frass	Strontium	6	6	0.98	1.48	1.398	-0.6327	-0.3265
Manufacturing	Substrate	Yttrium	6	6	0.0015	0.002	NA	0	-0.6667
Manufacturing	Larvae	Yttrium	6	6	0.006	0.007	4.000	0	-0.3333
Manufacturing	Frass	Yttrium	6	6	0.003	0.003	2.000	0	0
Manufacturing	Substrate	Zirconium	6	1	0.001125	0.009	NA	4	-2

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Zirconium	6	6	0.007375	0.008	6.556	0.0678	-0.1017
Manufacturing	Frass	Zirconium	6	0	0	0	0.000	NA	NA
Manufacturing	Substrate	Niobium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Niobium	6	2	5.00E-04	0.002	Inf	-4	-2
Manufacturing	Frass	Niobium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Molybdenum	6	6	0.07488	0.107	NA	-0.0601	-0.7579
Manufacturing	Larvae	Molybdenum	6	6	0.2884	0.296	3.851	0.008669	0.04248
Manufacturing	Frass	Molybdenum	6	6	0.1325	0.139	1.769	-0.1208	0.07547
Manufacturing	Substrate	Ruthenium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Ruthenium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Ruthenium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Rhodium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Rhodium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Rhodium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Palladium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Palladium	6	6	0.002125	0.003	Inf	0.2353	-0.1176
Manufacturing	Frass	Palladium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Silver	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Silver	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Silver	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Cadmium	6	6	0.01025	0.014	NA	0	-0.7317
Manufacturing	Larvae	Cadmium	6	6	0.1276	0.13	12.450	-0.0431	0.02155
Manufacturing	Frass	Cadmium	6	6	0.014	0.024	1.366	-0.8571	-0.3571
Manufacturing	Substrate	Tin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Tin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Tin	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Antimony	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Antimony	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Antimony	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Tellurium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Tellurium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Tellurium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Caesium	6	6	0.007875	0.009	NA	-0.06349	0.2857
Manufacturing	Larvae	Caesium	6	6	0.003	0.003	0.381	0	0
Manufacturing	Frass	Caesium	6	6	0.0085	0.009	1.079	0	0.1176
Manufacturing	Substrate	Barium	6	6	0.4063	0.56	NA	-0.1108	-0.6461
Manufacturing	Larvae	Barium	6	6	3.281	3.51	8.075	0.08077	-0.05258
Manufacturing	Frass	Barium	6	6	0.6213	0.8	1.529	-0.3943	-0.165
Manufacturing	Substrate	Lanthanum	6	6	0.00175	0.003	NA	0.5714	-0.8571

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Lanthanum	6	6	0.00175	0.003	1.000	0.5714	-0.8571
Manufacturing	Frass	Lanthanum	6	6	0.002625	0.003	1.500	-0.1905	-0.09524
Manufacturing	Substrate	Cerium	6	6	0.00325	0.006	NA	0.9231	-0.7692
Manufacturing	Larvae	Cerium	6	6	0.003	0.005	0.923	0.6667	-0.6667
Manufacturing	Frass	Cerium	6	6	0.00475	0.005	1.462	0	-0.1053
Manufacturing	Substrate	Praseodymium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Praseodymium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Praseodymium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Neodymium	6	5	0.00125	0.003	NA	1.6	-1.2
Manufacturing	Larvae	Neodymium	6	6	0.0015	0.002	1.200	0	-0.6667
Manufacturing	Frass	Neodymium	6	6	0.00225	0.003	1.800	-0.4444	-0.2222
Manufacturing	Substrate	Samarium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Samarium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Samarium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Europium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Europium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Europium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Gadolinium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Gadolinium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Gadolinium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Terbium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Terbium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Terbium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Dysprosium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Dysprosium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Dysprosium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Holmium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Holmium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Holmium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Erbium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Erbium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Erbium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Thulium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Thulium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Thulium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Ytterbium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Ytterbium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Ytterbium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Lutetium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Lutetium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Lutetium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Hafnium	6	1	5.00E-04	0.002	NA	0	2
Manufacturing	Larvae	Hafnium	6	0	0	0	0.000	NA	NA
Manufacturing	Frass	Hafnium	6	0	0	0	0.000	NA	NA
Manufacturing	Substrate	Tantalum	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Tantalum	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Tantalum	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Tungsten	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Tungsten	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Tungsten	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Rhenium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Rhenium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Rhenium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Osmium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Osmium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Osmium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Iridium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Iridium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Iridium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Platinum	6	1	0.00025	0.001	NA	0	2
Manufacturing	Larvae	Platinum	6	2	0.00175	0.007	7.000	4	-2
Manufacturing	Frass	Platinum	6	2	0.00125	0.005	5.000	-4	-2
Manufacturing	Substrate	Gold	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Gold	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Gold	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Mercury	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Mercury	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Mercury	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Thallium	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Thallium	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Thallium	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Lead	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Lead	6	6	0.009125	0.01	Inf	0.05479	-0.0274
Manufacturing	Frass	Lead	6	6	0.007125	0.008	Inf	0.07018	0.1053
Manufacturing	Substrate	Bismuth	6	6	0.00275	0.003	NA	0.3636	0.1818
Manufacturing	Larvae	Bismuth	6	0	0	0	0.000	NA	NA
Manufacturing	Frass	Bismuth	6	6	0.002875	0.004	1.045	-0.1739	0.4348
Manufacturing	Substrate	Thorium	6	2	0.00025	0.001	NA	4	-2

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Thorium	6	0	0	0	0.000	NA	NA
Manufacturing	Frass	Thorium	6	0	0	0	0.000	NA	NA
Manufacturing	Substrate	Uranium	6	6	0.002125	0.004	NA	-0.2353	-1.059
Manufacturing	Larvae	Uranium	6	6	0.034	0.039	16.000	0	-0.2647
Manufacturing	Frass	Uranium	6	6	0.006875	0.008	3.235	-0.2182	0.03636
Poultry manure	Substrate	Lithium	6	6	0.285	0.306	NA	-0.04211	-0.05614
Poultry manure	Larvae	Lithium	6	6	0.2351	0.24	0.825	0.01063	0.02446
Poultry manure	Frass	Lithium	6	6	0.4488	0.478	1.575	0.006684	0.1148
Poultry manure	Substrate	Beryllium	6	6	0.04612	0.052	NA	-0.05421	-0.1572
Poultry manure	Larvae	Beryllium	6	6	0.0325	0.034	0.705	0	0.03077
Poultry manure	Frass	Beryllium	6	6	0.06712	0.07	1.455	-0.00745	0.04097
Poultry manure	Substrate	Boron	6	6	39.75	42	NA	-0.05031	-0.03774
Poultry manure	Larvae	Boron	6	6	11.62	12	0.292	-0.1291	0.06454

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Frass	Boron	6	6	69.5	71	1.748	0.01439	0
Poultry manure	Substrate	Sodium	6	6	1889	1920	NA	-0.00794	-0.00397
Poultry manure	Larvae	Sodium	6	6	1369	1390	0.725	0.01826	0.02374
Poultry manure	Frass	Sodium	6	6	3140	3200	1.662	-0.00637	0.009554
Poultry manure	Substrate	Magnesium	6	6	3792	4290	NA	-0.1424	-0.1015
Poultry manure	Larvae	Magnesium	6	6	3415	3450	0.901	0.005857	0.01757
Poultry manure	Frass	Magnesium	6	6	6032	6290	1.591	-0.01492	0.06383
Poultry manure	Substrate	Aluminium	6	6	183.8	206	NA	-0.09793	-0.117
Poultry manure	Larvae	Aluminium	6	6	207	214	1.126	0.02899	0.05314
Poultry manure	Frass	Aluminium	6	6	218.2	226	1.187	-0.00458	0.04354
Poultry manure	Substrate	Phosphorus	6	6	5314	6030	NA	-0.1496	-0.1143
Poultry manure	Larvae	Phosphorus	6	6	7560	7750	1.423	-0.02116	0.01984
Poultry manure	Frass	Phosphorus	6	6	8455	9650	1.591	-0.01538	0.2685

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Potassium	6	6	19720	20300	NA	0	0.002535
Poultry manure	Larvae	Potassium	6	6	12020	12300	0.610	-0.01664	0.01248
Poultry manure	Frass	Potassium	6	6	34410	35900	1.745	-0.00145	-0.02398
Poultry manure	Substrate	Calcium	6	6	9180	9700	NA	-0.08061	0.06754
Poultry manure	Larvae	Calcium	6	6	38290	39500	4.171	-0.01436	0.04505
Poultry manure	Frass	Calcium	6	6	9442	12800	1.029	-0.04872	0.7112
Poultry manure	Substrate	Scandium	6	6	0.2019	0.223	NA	-0.0421	-0.1028
Poultry manure	Larvae	Scandium	6	6	0.1711	0.176	0.847	0.03799	0.03945
Poultry manure	Frass	Scandium	6	6	0.2714	0.288	1.344	-0.0129	0.08198
Poultry manure	Substrate	Titanium	6	6	8.062	9.1	NA	-0.1178	-0.1271
Poultry manure	Larvae	Titanium	6	6	5.425	5.6	0.673	0.03687	0.06452
Poultry manure	Frass	Titanium	6	6	9.312	9.6	1.155	-0.01611	0.06175
Poultry manure	Substrate	Vanadium	6	6	2.965	3.2	NA	-0.08432	-0.05734

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Vanadium	6	6	1.412	1.48	0.476	0.05666	0.03895
Poultry manure	Frass	Vanadium	6	6	4.31	4.47	1.454	0.01392	0.05104
Poultry manure	Substrate	Total.Chromium	6	6	4.182	4.89	NA	-0.03348	0.2762
Poultry manure	Larvae	Total.Chromium	6	6	2.275	2.36	0.544	0.02637	0.03516
Poultry manure	Frass	Total.Chromium	6	6	5.445	5.77	1.302	0.003673	0.101
Poultry manure	Substrate	Manganese	6	6	420.1	456	NA	-0.1297	-0.01726
Poultry manure	Larvae	Manganese	6	6	1369	1420	3.259	0.003652	0.05296
Poultry manure	Frass	Manganese	6	6	407.2	421	0.969	-0.00246	0.05526
Poultry manure	Substrate	Iron	6	6	2076	3830	NA	-0.02119	1.618
Poultry manure	Larvae	Iron	6	6	349.4	360	0.168	0.0186	0.04078
Poultry manure	Frass	Iron	6	6	519.9	534	0.250	0.01827	0.05049
Poultry manure	Substrate	Cobalt	6	6	0.264	0.323	NA	-0.04924	0.3636
Poultry manure	Larvae	Cobalt	6	6	0.1222	0.125	0.463	0	0.01227

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Frass	Cobalt	6	6	0.3372	0.35	1.277	0.002966	0.004448
Poultry manure	Substrate	Nickel	6	6	3.185	3.75	NA	-0.0314	0.2889
Poultry manure	Larvae	Nickel	6	6	1.554	1.6	0.488	0.04826	0.03378
Poultry manure	Frass	Nickel	6	6	4.466	4.55	1.402	0.003359	0.02631
Poultry manure	Substrate	Copper	6	6	96.32	104	NA	-0.05814	-0.09084
Poultry manure	Larvae	Copper	6	6	52.65	53.5	0.547	0.01709	0.007597
Poultry manure	Frass	Copper	6	6	143.8	147	1.493	-0.00695	0.03129
Poultry manure	Substrate	Zinc	6	6	411.4	455	NA	-0.07171	-0.1258
Poultry manure	Larvae	Zinc	6	6	426	438	1.035	-0.05164	0.004695
Poultry manure	Frass	Zinc	6	6	563.9	584	1.371	0.0133	-0.00133
Poultry manure	Substrate	Gallium	6	6	0.0975	0.11	NA	-0.1026	0.2564
Poultry manure	Larvae	Gallium	6	6	0.0775	0.09	0.795	0	0.1935
Poultry manure	Frass	Gallium	6	6	0.1025	0.11	1.051	0.09756	0.04878

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Germanium	6	6	0.1125	0.15	NA	-0.08889	0.5778
Poultry manure	Larvae	Germanium	6	6	0.0425	0.05	0.378	-0.2353	0.1176
Poultry manure	Frass	Germanium	6	6	0.1013	0.11	0.900	0.04936	-0.02468
Poultry manure	Substrate	Arsenic	6	6	0.26	0.331	NA	-0.06538	0.4692
Poultry manure	Larvae	Arsenic	6	6	0.2349	0.241	0.904	-0.02767	0.01809
Poultry manure	Frass	Arsenic	6	6	0.246	0.253	0.946	0.004065	-0.00407
Poultry manure	Substrate	Selenium	6	6	0.9938	1.07	NA	-0.04528	-0.08805
Poultry manure	Larvae	Selenium	6	6	1.066	1.09	1.073	-0.01407	-0.01173
Poultry manure	Frass	Selenium	6	6	1.4	1.45	1.409	-0.00714	0.02857
Poultry manure	Substrate	Rubidium	6	6	15.5	15.9	NA	-0.04516	-0.00645
Poultry manure	Larvae	Rubidium	6	6	9.02	9.17	0.582	-0.03215	0.005543
Poultry manure	Frass	Rubidium	6	6	26.06	26.4	1.681	0.01343	0.002878
Poultry manure	Substrate	Strontium	6	6	22.26	24.2	NA	-0.08311	-0.08648

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Strontium	6	6	107.1	110	4.811	-0.03268	0.03501
Poultry manure	Frass	Strontium	6	6	15.66	18	0.704	0.009579	0.2921
Poultry manure	Substrate	Yttrium	6	6	0.5409	0.597	NA	-0.08597	-0.1142
Poultry manure	Larvae	Yttrium	6	6	0.4542	0.464	0.840	-0.01321	0.02972
Poultry manure	Frass	Yttrium	6	6	0.7817	0.823	1.445	0.01023	0.1043
Poultry manure	Substrate	Zirconium	6	6	0.3925	0.44	NA	0.1019	0.1911
Poultry manure	Larvae	Zirconium	6	6	0.1062	0.13	0.271	0.2354	0.2589
Poultry manure	Frass	Zirconium	6	6	0.3288	0.38	0.838	0.1369	-0.08364
Poultry manure	Substrate	Niobium	6	6	0.02975	0.035	NA	-0.06723	0.3529
Poultry manure	Larvae	Niobium	6	6	0.01462	0.016	0.491	-0.0342	0.1881
Poultry manure	Frass	Niobium	6	6	0.02762	0.03	0.928	0.1267	0.1358
Poultry manure	Substrate	Molybdenum	6	6	6.578	7.13	NA	-0.05473	-0.09653
Poultry manure	Larvae	Molybdenum	6	6	4.256	4.31	0.647	0.01762	0.01116

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Frass	Molybdenum	6	6	8.992	9.34	1.367	0.006673	0.07284
Poultry manure	Substrate	Ruthenium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Ruthenium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Ruthenium	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Rhodium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Rhodium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Rhodium	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Palladium	6	6	0.01	0.01	NA	0	0
Poultry manure	Larvae	Palladium	6	6	0.03875	0.04	3.875	-0.129	0.06452
Poultry manure	Frass	Palladium	6	2	0.005	0.01	0.500	0	2
Poultry manure	Substrate	Silver	6	6	0.0175	0.02	NA	-0.5714	0.2857
Poultry manure	Larvae	Silver	6	6	0.11	0.12	6.286	0	0.1818
Poultry manure	Frass	Silver	6	6	0.02	0.02	1.143	0	0

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Cadmium	6	6	0.304	0.337	NA	-0.1151	-0.09211
Poultry manure	Larvae	Cadmium	6	6	0.6135	0.624	2.018	-0.03749	0.01793
Poultry manure	Frass	Cadmium	6	6	0.3314	0.343	1.090	0.01961	0.05205
Poultry manure	Substrate	Tin	6	6	0.1825	0.22	NA	-0.1096	0.3562
Poultry manure	Larvae	Tin	6	6	0.3488	0.37	1.911	-0.043	0.1218
Poultry manure	Frass	Tin	6	6	0.18	0.18	0.986	0	0
Poultry manure	Substrate	Antimony	6	6	0.083	0.098	NA	-0.07229	0.3373
Poultry manure	Larvae	Antimony	6	6	0.1858	0.223	2.239	0.01076	0.401
Poultry manure	Frass	Antimony	6	6	0.0915	0.095	1.102	0.01093	0.06557
Poultry manure	Substrate	Tellurium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Tellurium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Tellurium	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Caesium	6	6	0.08788	0.092	NA	-0.07396	-0.01991

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Caesium	6	6	0.0885	0.09	1.007	-0.0226	0.0113
Poultry manure	Frass	Caesium	6	6	0.133	0.136	1.513	0.02256	0.02256
Poultry manure	Substrate	Barium	6	6	24.84	28.4	NA	-0.155	-0.1238
Poultry manure	Larvae	Barium	6	6	61.34	63.2	2.469	-0.05298	0.03953
Poultry manure	Frass	Barium	6	6	25.16	28.7	1.013	0.009936	0.2415
Poultry manure	Substrate	Lanthanum	6	6	0.1825	0.202	NA	-0.1425	-0.07123
Poultry manure	Larvae	Lanthanum	6	6	0.1708	0.176	0.936	0	0.04391
Poultry manure	Frass	Lanthanum	6	6	0.2238	0.243	1.226	0.004468	0.1318
Poultry manure	Substrate	Cerium	6	6	0.2535	0.283	NA	-0.1578	-0.07495
Poultry manure	Larvae	Cerium	6	6	0.2312	0.238	0.912	-0.00433	0.04109
Poultry manure	Frass	Cerium	6	6	0.3056	0.329	1.206	0.01473	0.1301
Poultry manure	Substrate	Praseodymium	6	6	0.03688	0.041	NA	-0.1491	-0.07457
Poultry manure	Larvae	Praseodymium	6	6	0.03275	0.034	0.888	0	0.0458

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Frass	Praseodymium	6	6	0.04538	0.049	1.230	0.01102	0.1377
Poultry manure	Substrate	Neodymium	6	6	0.1482	0.165	NA	-0.1417	-0.0776
Poultry manure	Larvae	Neodymium	6	6	0.1321	0.136	0.891	0.01893	0.04353
Poultry manure	Frass	Neodymium	6	6	0.1866	0.202	1.259	0.008039	0.138
Poultry manure	Substrate	Samarium	6	6	0.03038	0.034	NA	-0.1152	-0.09052
Poultry manure	Larvae	Samarium	6	6	0.02613	0.027	0.860	0.01914	0.0287
Poultry manure	Frass	Samarium	6	6	0.039	0.043	1.284	0.02564	0.1795
Poultry manure	Substrate	Europium	6	6	0.00925	0.01	NA	-0.1081	-0.05405
Poultry manure	Larvae	Europium	6	6	0.01175	0.012	1.270	-0.08511	0.04255
Poultry manure	Frass	Europium	6	6	0.01175	0.013	1.270	0	0.1277
Poultry manure	Substrate	Gadolinium	6	6	0.03563	0.04	NA	-0.1263	-0.09122
Poultry manure	Larvae	Gadolinium	6	6	0.03138	0.032	0.881	0.01593	0.03983
Poultry manure	Frass	Gadolinium	6	6	0.0465	0.051	1.305	0.02151	0.172

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Terbium	6	6	0.00575	0.006	NA	0	-0.08696
Poultry manure	Larvae	Terbium	6	6	0.005	0.005	0.870	0	0
Poultry manure	Frass	Terbium	6	6	0.0075	0.008	1.304	0	0.1333
Poultry manure	Substrate	Dysprosium	6	6	0.03912	0.043	NA	-0.115	-0.08308
Poultry manure	Larvae	Dysprosium	6	6	0.03	0.031	0.767	0.03333	0.03333
Poultry manure	Frass	Dysprosium	6	6	0.05238	0.056	1.339	0.009546	0.1193
Poultry manure	Substrate	Holmium	6	6	0.009625	0.011	NA	-0.1558	-0.1299
Poultry manure	Larvae	Holmium	6	6	0.007125	0.008	0.740	0.07018	-0.03509
Poultry manure	Frass	Holmium	6	6	0.01325	0.014	1.377	0	0.1132
Poultry manure	Substrate	Erbium	6	6	0.03887	0.043	NA	-0.1158	-0.09648
Poultry manure	Larvae	Erbium	6	6	0.02862	0.03	0.736	0.01747	0.06115
Poultry manure	Frass	Erbium	6	6	0.05313	0.056	1.367	0.009411	0.1082
Poultry manure	Substrate	Thulium	6	6	0.0075	0.008	NA	0	-0.1333

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Thulium	6	6	0.005625	0.006	0.750	-0.08889	0.1333
Poultry manure	Frass	Thulium	6	6	0.0105	0.011	1.400	0	0.09524
Poultry manure	Substrate	Ytterbium	6	6	0.074	0.082	NA	-0.08108	-0.1351
Poultry manure	Larvae	Ytterbium	6	6	0.05362	0.055	0.725	-0.00933	0.03264
Poultry manure	Frass	Ytterbium	6	6	0.1041	0.11	1.407	0.01441	0.08405
Poultry manure	Substrate	Lutetium	6	6	0.0155	0.017	NA	-0.06452	-0.129
Poultry manure	Larvae	Lutetium	6	6	0.01125	0.012	0.726	0	0.04444
Poultry manure	Frass	Lutetium	6	6	0.02238	0.024	1.444	-0.02234	0.1005
Poultry manure	Substrate	Hafnium	6	6	0.004625	0.005	NA	0.1081	0.1622
Poultry manure	Larvae	Hafnium	6	6	0.002	0.003	0.432	0.5	0.5
Poultry manure	Frass	Hafnium	6	6	0.0035	0.004	0.757	0	-0.2857
Poultry manure	Substrate	Tantalum	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Tantalum	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Frass	Tantalum	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Tungsten	6	6	0.06025	0.066	NA	-0.166	-0.0083
Poultry manure	Larvae	Tungsten	6	6	0.04388	0.046	0.728	0.05697	0.02849
Poultry manure	Frass	Tungsten	6	6	0.107	0.171	1.776	0.785	-0.3645
Poultry manure	Substrate	Rhenium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Rhenium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Rhenium	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Osmium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Osmium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Osmium	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Iridium	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Iridium	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Iridium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Platinum	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Platinum	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Platinum	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Gold	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Gold	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Gold	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Mercury	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Mercury	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Mercury	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Thallium	6	6	0.01512	0.017	NA	-0.09921	-0.08267
Poultry manure	Larvae	Thallium	6	6	0.0075	0.008	0.496	0	0
Poultry manure	Frass	Thallium	6	6	0.02212	0.023	1.463	0.0226	0.07911
Poultry manure	Substrate	Lead	6	6	0.4999	0.569	NA	-0.159	-0.09952

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Lead	6	6	0.5846	0.614	1.169	-0.00257	0.03892
Poultry manure	Frass	Lead	6	6	0.6908	0.735	1.382	-0.06225	0.009409
Poultry manure	Substrate	Bismuth	6	6	0.0035	0.004	NA	0	-0.2857
Poultry manure	Larvae	Bismuth	6	6	0.00375	0.004	1.071	0.2667	0.1333
Poultry manure	Frass	Bismuth	6	6	0.004	0.004	1.143	0	0
Poultry manure	Substrate	Thorium	6	6	0.124	0.142	NA	-0.1371	-0.121
Poultry manure	Larvae	Thorium	6	6	0.1059	0.113	0.854	-0.00472	0.04013
Poultry manure	Frass	Thorium	6	6	0.1554	0.166	1.253	0.02252	0.05952
Poultry manure	Substrate	Uranium	6	6	2.58	2.9	NA	-0.1047	-0.1163
Poultry manure	Larvae	Uranium	6	6	1.132	1.22	0.439	0.0265	0.02208
Poultry manure	Frass	Uranium	6	6	3.555	3.92	1.378	0.01969	0.1097
Supermarket	Substrate	Lithium	6	6	0.0555	0.057	NA	-0.01802	-0.03604
Supermarket	Larvae	Lithium	6	6	0.02012	0.021	0.363	-0.02485	0.03728
Supermarket	Frass	Lithium	6	6	0.1415	0.159	2.550	0.04947	-0.1837

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Substrate	Beryllium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Beryllium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Beryllium	6	6	0.001	0.001	Inf	0	0
Supermarket	Substrate	Boron	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Boron	6	0	0	0	NA	NA	NA
Supermarket	Frass	Boron	6	6	22.88	25	Inf	0.02185	-0.1639
Supermarket	Substrate	Sodium	6	6	7011	7250	NA	0.01355	-0.03459
Supermarket	Larvae	Sodium	6	6	669.6	682	0.096	-0.00224	0.02203
Supermarket	Frass	Sodium	6	6	14120	15300	2.014	0.04958	-0.1098
Supermarket	Substrate	Magnesium	6	6	655.1	681	NA	0.02061	-0.03702
Supermarket	Larvae	Magnesium	6	6	1686	1730	2.574	0.008897	0.04597
Supermarket	Frass	Magnesium	6	6	726.5	771	1.109	0.03441	-0.08809
Supermarket	Substrate	Aluminium	6	6	6.225	6.6	NA	0.06426	-0.04016
Supermarket	Larvae	Aluminium	6	6	7.225	7.6	1.161	-0.1107	0.0346
Supermarket	Frass	Aluminium	6	6	31.69	33.4	5.091	0.07416	-0.0213
Supermarket	Substrate	Phosphorus	6	6	3472	3700	NA	0.04608	-0.03312
Supermarket	Larvae	Phosphorus	6	6	5981	6170	1.723	-0.01087	0.03637
Supermarket	Frass	Phosphorus	6	6	5718	6060	1.647	0.03673	-0.06208
Supermarket	Substrate	Potassium	6	6	6999	7390	NA	0.02643	-0.04251
Supermarket	Larvae	Potassium	6	6	9518	9820	1.360	-0.01891	0.0394

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Frass	Potassium	6	6	25010	26900	3.573	0.04998	-0.07697
Supermarket	Substrate	Calcium	6	6	2870	3030	NA	0.01394	-0.04878
Supermarket	Larvae	Calcium	6	6	5675	6020	1.977	-0.01938	0.09163
Supermarket	Frass	Calcium	6	6	790.4	823	0.275	-0.08413	0.02562
Supermarket	Substrate	Scandium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Scandium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Scandium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Titanium	6	6	0.275	0.4	NA	0.3636	-0.5455
Supermarket	Larvae	Titanium	6	6	0.25	0.3	0.909	-0.4	0
Supermarket	Frass	Titanium	6	6	1.05	1.1	3.818	0.09524	0.09524
Supermarket	Substrate	Vanadium	6	6	0.07	0.09	NA	-0.2857	-0.2857
Supermarket	Larvae	Vanadium	6	6	0.02	0.02	0.286	0	0
Supermarket	Frass	Vanadium	6	6	0.08	0.09	1.143	0.125	-0.125
Supermarket	Substrate	Total.Chromium	6	6	0.1075	0.13	NA	-0.186	-0.2326
Supermarket	Larvae	Total.Chromium	6	6	0.1538	0.18	1.431	0.03251	0.3414
Supermarket	Frass	Total.Chromium	6	6	0.525	0.63	4.884	0.05714	0.3048
Supermarket	Substrate	Manganese	6	6	6	6	NA	0	0
Supermarket	Larvae	Manganese	6	6	17	18	2.833	0	0.1176
Supermarket	Frass	Manganese	6	6	1	1	0.167	0	0

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Substrate	Iron	6	6	25.42	26.5	NA	0.01574	-0.02557
Supermarket	Larvae	Iron	6	6	46.4	47.6	1.825	-0.00647	0.03879
Supermarket	Frass	Iron	6	6	30.85	32.7	1.214	0.05835	-0.0389
Supermarket	Substrate	Cobalt	6	6	0.01713	0.019	NA	-0.08757	-0.07297
Supermarket	Larvae	Cobalt	6	6	0.01462	0.016	0.854	-0.0342	0.0513
Supermarket	Frass	Cobalt	6	6	0.06562	0.072	3.831	0.06858	-0.06477
Supermarket	Substrate	Nickel	6	6	0.1675	0.18	NA	0	-0.08955
Supermarket	Larvae	Nickel	6	6	0.1288	0.14	0.769	0.03882	0.1747
Supermarket	Frass	Nickel	6	6	0.5538	0.58	3.306	0.04514	0.02257
Supermarket	Substrate	Copper	6	6	2.954	3.13	NA	0.04909	-0.04316
Supermarket	Larvae	Copper	6	6	6.624	6.84	2.242	0.009813	-0.05246
Supermarket	Frass	Copper	6	6	2.088	2.22	0.707	-0.01437	-0.0886
Supermarket	Substrate	Zinc	6	6	29.25	30.4	NA	0.02735	-0.02735
Supermarket	Larvae	Zinc	6	6	48.45	49.6	1.656	0	0.02683
Supermarket	Frass	Zinc	6	6	27.08	28.6	0.926	0.02954	-0.06093
Supermarket	Substrate	Gallium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Gallium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Gallium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Germanium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Germanium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Frass	Germanium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Arsenic	6	6	0.012	0.014	NA	0	-0.08333
Supermarket	Larvae	Arsenic	6	6	0.0145	0.016	1.208	-0.06897	-0.06897
Supermarket	Frass	Arsenic	6	6	0.0285	0.031	2.375	0.03509	-0.07018
Supermarket	Substrate	Selenium	6	6	0.1675	0.18	NA	0.1194	-0.02985
Supermarket	Larvae	Selenium	6	6	0.115	0.12	0.687	0	0
Supermarket	Frass	Selenium	6	6	0.4825	0.51	2.881	0	-0.07254
Supermarket	Substrate	Rubidium	6	6	6.338	6.67	NA	0.04418	-0.04812
Supermarket	Larvae	Rubidium	6	6	5.15	5.24	0.813	-0.04466	0.01165
Supermarket	Frass	Rubidium	6	6	21.22	21.9	3.348	0.01885	-0.04477
Supermarket	Substrate	Strontium	6	6	9.731	9.85	NA	-0.00874	-0.01156
Supermarket	Larvae	Strontium	6	6	19.08	19.6	1.961	-0.04717	0.04979
Supermarket	Frass	Strontium	6	6	11.49	12	1.181	-0.06527	-0.02393
Supermarket	Substrate	Yttrium	6	6	0.003	0.003	NA	0	0
Supermarket	Larvae	Yttrium	6	6	0.00275	0.003	0.917	-0.3636	0.1818
Supermarket	Frass	Yttrium	6	6	0.01125	0.012	3.750	0.08889	-0.04444
Supermarket	Substrate	Zirconium	6	6	0.01125	0.02	NA	-0.4444	-0.2222
Supermarket	Larvae	Zirconium	6	5	0.01375	0.02	1.222	-0.3636	0.9091
Supermarket	Frass	Zirconium	6	6	0.04125	0.05	3.667	0.1212	-0.06061
Supermarket	Substrate	Niobium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Larvae	Niobium	6	2	5.00E-04	0.002	Inf	4	-2
Supermarket	Frass	Niobium	6	6	0.004	0.004	Inf	0	0
Supermarket	Substrate	Molybdenum	6	6	0.3062	0.32	NA	0.04899	-0.04082
Supermarket	Larvae	Molybdenum	6	6	0.395	0.4	1.290	-0.02532	0
Supermarket	Frass	Molybdenum	6	6	0.5063	0.53	1.653	0.02963	-0.04444
Supermarket	Substrate	Ruthenium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Ruthenium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Ruthenium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Rhodium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Rhodium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Rhodium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Palladium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Palladium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Palladium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Silver	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Silver	6	0	0	0	NA	NA	NA
Supermarket	Frass	Silver	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Cadmium	6	6	0.02938	0.031	NA	0.01702	-0.05956
Supermarket	Larvae	Cadmium	6	6	0.1255	0.128	4.272	0.01594	0.03187
Supermarket	Frass	Cadmium	6	6	0.00825	0.009	0.281	0	0.06061

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Substrate	Tin	6	6	0.2525	0.26	NA	0.0396	0.0198
Supermarket	Larvae	Tin	6	6	0.03	0.03	0.119	0	0
Supermarket	Frass	Tin	6	6	0.1725	0.18	0.683	0.05797	-0.02899
Supermarket	Substrate	Antimony	6	6	0.002	0.002	NA	0	0
Supermarket	Larvae	Antimony	6	6	0.001625	0.002	0.813	-0.3077	-0.1538
Supermarket	Frass	Antimony	6	6	0.009125	0.01	4.562	0.05479	-0.0274
Supermarket	Substrate	Tellurium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Tellurium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Tellurium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Caesium	6	6	0.02012	0.021	NA	0.07455	-0.01243
Supermarket	Larvae	Caesium	6	6	0.01188	0.013	0.591	-0.04209	-0.06313
Supermarket	Frass	Caesium	6	6	0.07412	0.076	3.684	0.02024	-0.00337
Supermarket	Substrate	Barium	6	6	1.649	1.72	NA	-0.0091	-0.06519
Supermarket	Larvae	Barium	6	6	3.682	3.76	2.233	-0.04074	0.0258
Supermarket	Frass	Barium	6	6	4.438	4.52	2.691	-0.00676	-0.0169
Supermarket	Substrate	Lanthanum	6	6	0.0045	0.006	NA	0.4444	-0.2222
Supermarket	Larvae	Lanthanum	6	6	0.005	0.005	1.111	0	0
Supermarket	Frass	Lanthanum	6	6	0.01638	0.018	3.640	0.09158	-0.04579
Supermarket	Substrate	Cerium	6	6	0.008	0.011	NA	0.5	-0.25
Supermarket	Larvae	Cerium	6	6	0.007375	0.008	0.922	-0.0678	-0.1017

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Frass	Cerium	6	6	0.0285	0.03	3.562	0.03509	-0.07018
Supermarket	Substrate	Praseodymium	6	2	0.00025	0.001	NA	4	-2
Supermarket	Larvae	Praseodymium	6	0	0	0	0.000	NA	NA
Supermarket	Frass	Praseodymium	6	6	0.00325	0.004	13.000	0.3077	-0.1538
Supermarket	Substrate	Neodymium	6	6	0.00375	0.005	NA	0.5333	-0.1333
Supermarket	Larvae	Neodymium	6	6	0.003125	0.004	0.833	-0.16	-0.08
Supermarket	Frass	Neodymium	6	6	0.01375	0.014	3.667	0	-0.03636
Supermarket	Substrate	Samarium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Samarium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Samarium	6	6	0.00275	0.003	Inf	0	0.1818
Supermarket	Substrate	Europium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Europium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Europium	6	1	0.000125	0.001	Inf	4	-2
Supermarket	Substrate	Gadolinium	6	2	0.001125	0.007	NA	-4	-2
Supermarket	Larvae	Gadolinium	6	0	0	0	0.000	NA	NA
Supermarket	Frass	Gadolinium	6	6	0.004	0.008	3.556	0.25	0.75
Supermarket	Substrate	Terbium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Terbium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Terbium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Substrate	Dysprosium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Dysprosium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Dysprosium	6	6	0.002	0.002	Inf	0	0
Supermarket	Substrate	Holmium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Holmium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Holmium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Erbium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Erbium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Erbium	6	2	0.00025	0.001	Inf	0	-2
Supermarket	Substrate	Thulium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Thulium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Thulium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Ytterbium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Ytterbium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Ytterbium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Lutetium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Lutetium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Lutetium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Hafnium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Hafnium	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Frass	Hafnium	6	6	0.001	0.001	Inf	0	0
Supermarket	Substrate	Tantalum	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Tantalum	6	0	0	0	NA	NA	NA
Supermarket	Frass	Tantalum	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Tungsten	6	6	0.00325	0.004	NA	0.3077	-0.1538
Supermarket	Larvae	Tungsten	6	6	0.005	0.006	1.538	-0.2	0
Supermarket	Frass	Tungsten	6	6	0.009875	0.011	3.038	0.05063	0.2278
Supermarket	Substrate	Rhenium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Rhenium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Rhenium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Osmium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Osmium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Osmium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Iridium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Iridium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Iridium	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Platinum	6	4	0.009375	0.019	NA	0.16	1.84
Supermarket	Larvae	Platinum	6	0	0	0	0.000	NA	NA
Supermarket	Frass	Platinum	6	0	0	0	0.000	NA	NA
Supermarket	Substrate	Gold	6	0	0	0	NA	NA	NA

Trial	Sample Type	Metal	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Supermarket	Larvae	Gold	6	0	0	0	NA	NA	NA
Supermarket	Frass	Gold	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Mercury	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Mercury	6	0	0	0	NA	NA	NA
Supermarket	Frass	Mercury	6	0	0	0	NA	NA	NA
Supermarket	Substrate	Thallium	6	0	0	0	NA	NA	NA
Supermarket	Larvae	Thallium	6	0	0	0	NA	NA	NA
Supermarket	Frass	Thallium	6	6	0.01713	0.018	Inf	-0.02919	0.04378
Supermarket	Substrate	Lead	6	6	0.01162	0.013	NA	-0.04303	-0.1076
Supermarket	Larvae	Lead	6	6	0.01675	0.017	1.441	0	-0.02985
Supermarket	Frass	Lead	6	6	0.03375	0.035	2.904	0	0.04444
Supermarket	Substrate	Bismuth	6	6	0.007	0.008	NA	0.1429	0
Supermarket	Larvae	Bismuth	6	6	0.002	0.002	0.286	0	0
Supermarket	Frass	Bismuth	6	6	0.00725	0.008	1.036	0	0.06897
Supermarket	Substrate	Thorium	6	2	0.00025	0.001	NA	4	-2
Supermarket	Larvae	Thorium	6	0	0	0	0.000	NA	NA
Supermarket	Frass	Thorium	6	6	0.00325	0.004	13.000	0.3077	-0.1538
Supermarket	Substrate	Uranium	6	6	0.00425	0.005	NA	0	-0.1176
Supermarket	Larvae	Uranium	6	6	0.002125	0.003	0.500	-0.2353	-0.1176
Supermarket	Frass	Uranium	6	6	0.0085	0.009	2.000	0.1176	0

Inf = inferred, NA = not applicable

Appendix C - Veterinary medicines

The aim of the analysis was to

- estimate the quantity of each substance in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of (bio)accumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

Individual results are shown in the figures and the shows the calculated quantities.

Substances that were not detected in any extracts from any samples are not reported here. These were: 2.4.6.Triamino.pyrimidine.5.carbonitrile, 3.O.acetyltylosin, acepromazine, albendazole, albendazole.amino.sulphone, albendazole.sulphone, albendazole.sulphoxide, aminoflubendazole, amoxicillin, ampicillin, azaperol, azaperone, carazolol, chlorpromazine, chlortetracycline, ciprofloxacin, clopidol, clorsulon, closantel, cloxacillin, cyromazine, danofloxacin, dapsone, decoquinate, derquantel, diclazuril, dicloxacillin, dicyclanil, difloxacin, diflubenzuron, doxycycline, enrofloxacin, epi.chlortetracycline, epi.oxytetracycline, epi.tetracycline, erythromycin, fenbendazole, fenbendazole.sulphone, firocoxib, fluazuron, flumequine, gamithromycin, halofuginone, haloperidol, hydroxy.mebendazole, imidiocarb, josamycin, levamisole, lincomycin, maduramycin, marbofloxacin, mebendazole, mebendazole.amine, monensin, monepantel.sulphone, nafcillin, nalidixic.acid, nitroxynil, norfloxacin, oxacillin, oxfendazole, oxibendazole, oxolinic.acid, oxyclozanide, oxytetracycline, penicillin.G, penicillin.V, pirlimycin, propionylpromazine, rafoxanide, rifampicin, rifaximin, robenidine, sarafloxacin, semduramicin, spiramycin, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfadimidine.sulfamethazine, sulfadoxine, sulfaguanadine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisoxazole, teflubenzuron, tetracycline, tildipirosin, tilmicosin, toltrazuril.sulfone, triclabendazole, triclabendazole.sulphone, trimethoprim, tulathromycin, tylosin, tylvalosin, valnemulin, virginiamycin, and xylazine.

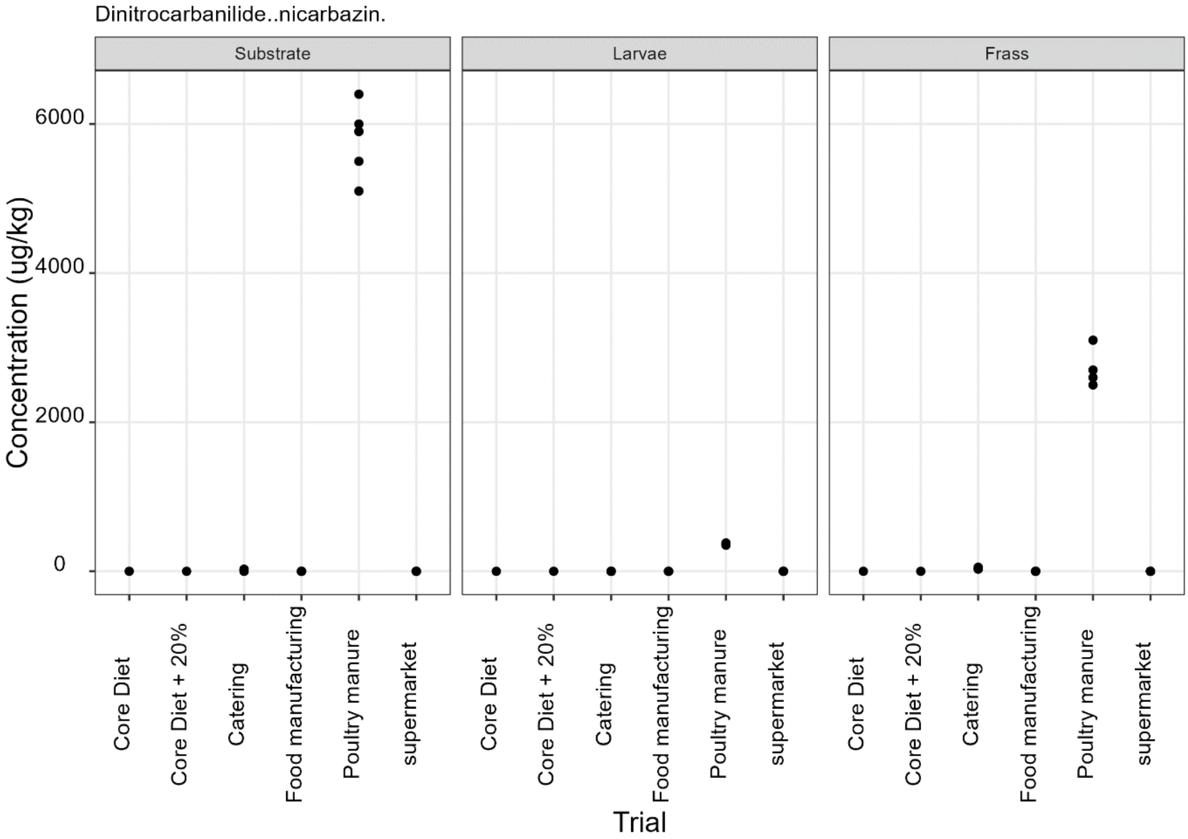
The reporting limits are shown below:

Table III. Reporting limits for veterinary medicines analysis.

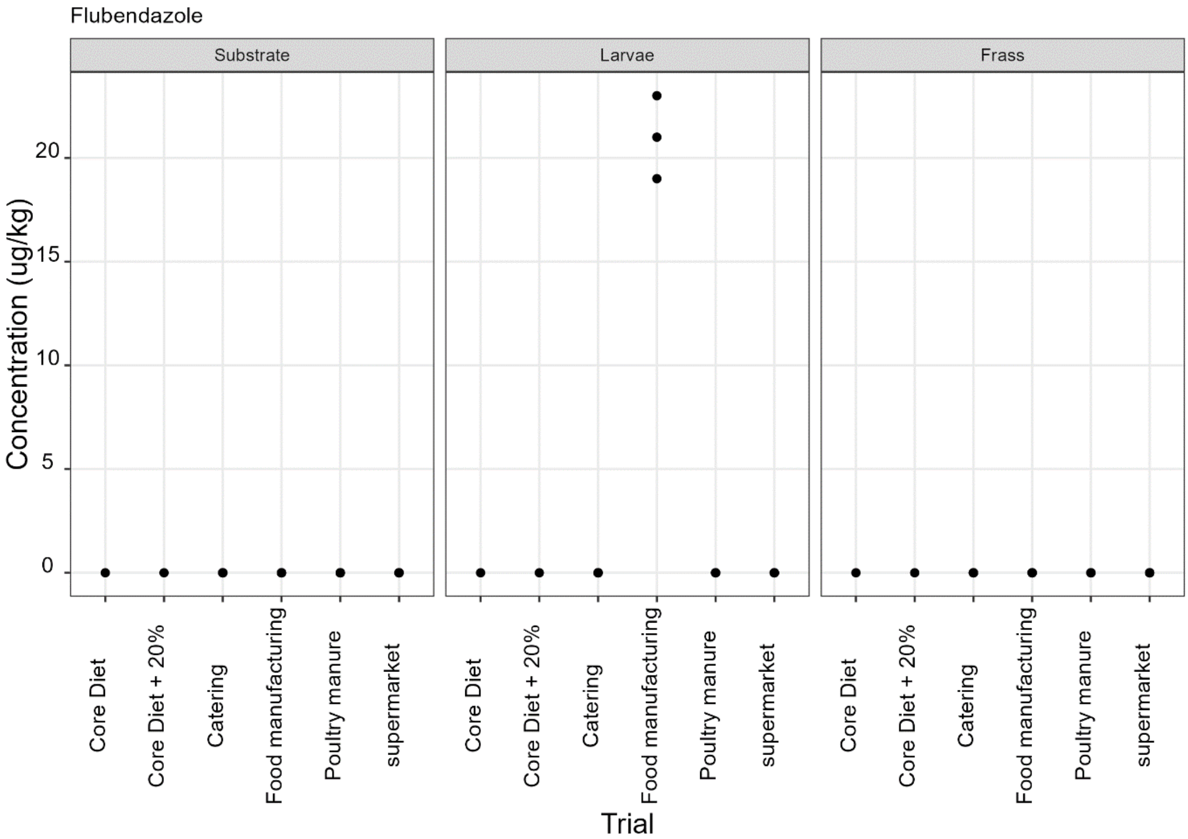
Veterinary medicine	µg/kg	Veterinary medicine	µg/kg
2,4,6-Triamino-pyrimidine-5-carbonitrile	100	Monepantel sulphone	150
3-O-Acetyltylosin	12.5	Nafcillin	75
5-hydroxythiabendazole	12.5	Nalidixic acid	25
Acepromazine	12.5	Narasin	1
Albendazole	12.5	Nitroxynil	10
Albendazole amino sulphone	12.5	Norfloxacin	25
Albendazole sulphone	12.5	Oxacillin	75
Albendazole sulphoxide	12.5	Oxfendazole	12.5
Aminoflubendazole	12.5	Oxibendazole	12.5
Amoxicillin	12.5	Oxolinic acid	25
Ampicillin	12.5	Oxyclozanide	5
Azaperol	6.25	Oxytetracycline	25
Azaperone	6.25	Penicillin G	12.5
Carazolol	2.5	Penicillin V	12.5
Chlorpromazine	2.5	Pirlimycin	50
Chlortetracycline	25	Propionylpromazine	12.5
Ciprofloxacin	12.5	Rafoxanide	5
Clopidol	5	Rifampicin	50
Clorsulon	17.5	Rifaximin	30
Closantel	500	Robenidine	2.5
Cloxacillin	150	Salinomycin	1
Cyromazine	150	Sarafloxacin	15

Veterinary medicine	µg/kg	Veterinary medicine	µg/kg
Danofloxacin	25	Semduramicin	1
Dapsone	2.5	Spiramycin	50
Decoquinate	10	Sulfachloropyridazine	50
Derquantel	1	Sulfadiazine	50
Diclazuril	1	Sulfadimethoxine	50
Dicloxacillin	75	Sulfadimidine/sulfamethazine	50
Dicyclanil	5	Sulfadoxine	50
Difloxacin	75	Sulfaguanadine	50
Diflubenzuron	5	Sulfamerazine	50
Dinitrocarbanilide [nicarbazin]	25	Sulfamethizole	50
Doxycycline	50	Sulfamethoxazole	50
Enrofloxacin	12.5	Sulfamethoxypyridazine	50
epi-Chlortetracycline	25	Sulfamonomethoxine	50
epi-Oxytetracycline	25	Sulfamoxole	50
epi-Tetracycline	25	Sulfanilamide	50
Erythromycin	75	Sulfapyridine	50
Fenbendazole	12.5	Sulfaquinoxaline	50
Fenbendazole sulphone	12.5	Sulfathiazole	50
Firocoxib	30	Sulfisoxazole	50
Fluazuron	100	Teflubenzuron	50
Flubendazole	12.5	Tetracycline	25
Flumequine	100	Thiabendazole	12.5
Gamithromycin	25	Tildipirosin	200
Halofuginone	1	Tilmicosin	25
Haloperidol	25	Toltrazuril sulfone	50
Hydroxy mebendazole	12.5	Triclabendazole	12.5
Imidiocarb	150	Triclabendazole sulphone	12.5
Josamycin	100	Triclabendazole sulphoxide	12.5
Lasalocid	2.5	Trimethoprim	25
Levamisole	5	Tulathromycin	150
Lincomycin	25	Tylosin	50
Maduramycin	1	Tylvalosin	12.5
Marbofloxacin	25	Valnemulin	25
Mebendazole	12.5	Virginiamycin	5
Mebendazole amine	12.5	Xylazine	12.5
Monensin	1		

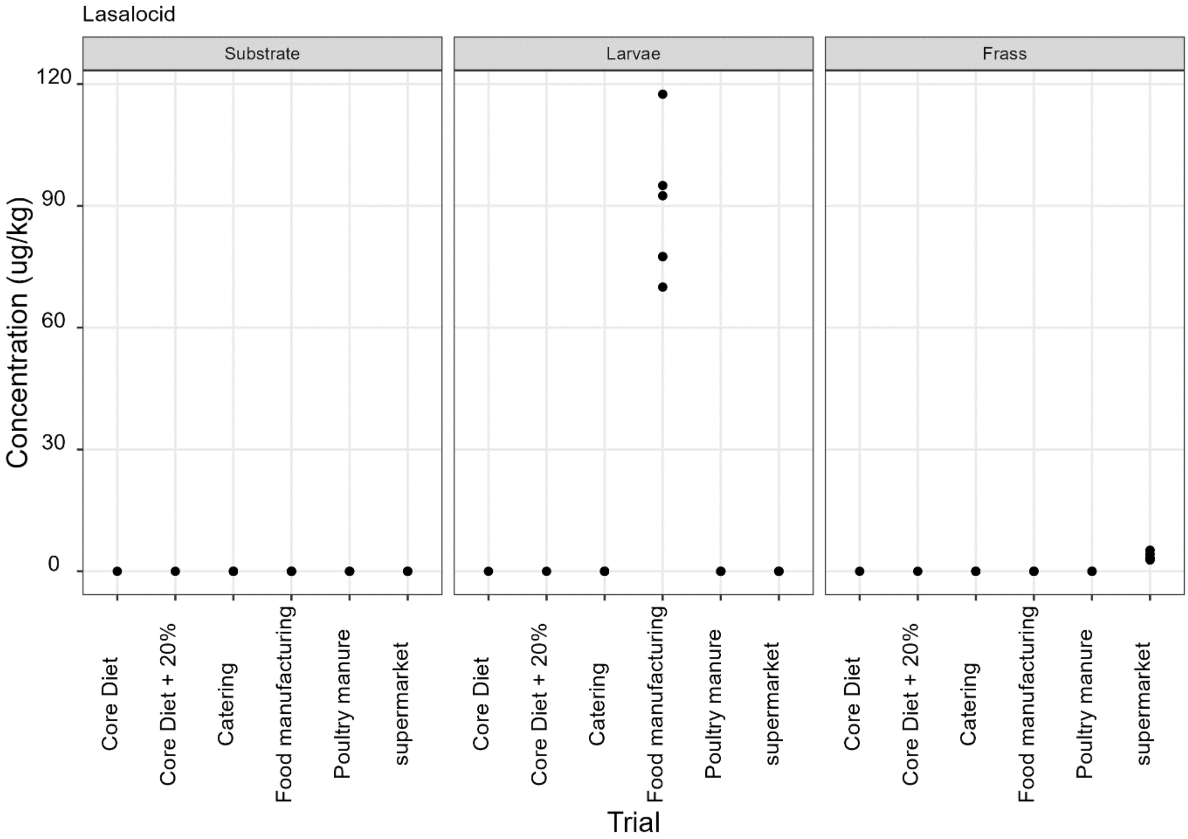
1.


Dinitrocarbanilide..nicarbazin.
Substrate
Larvae
Frass
Concentration (ug/kg)
6000
4000
2000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

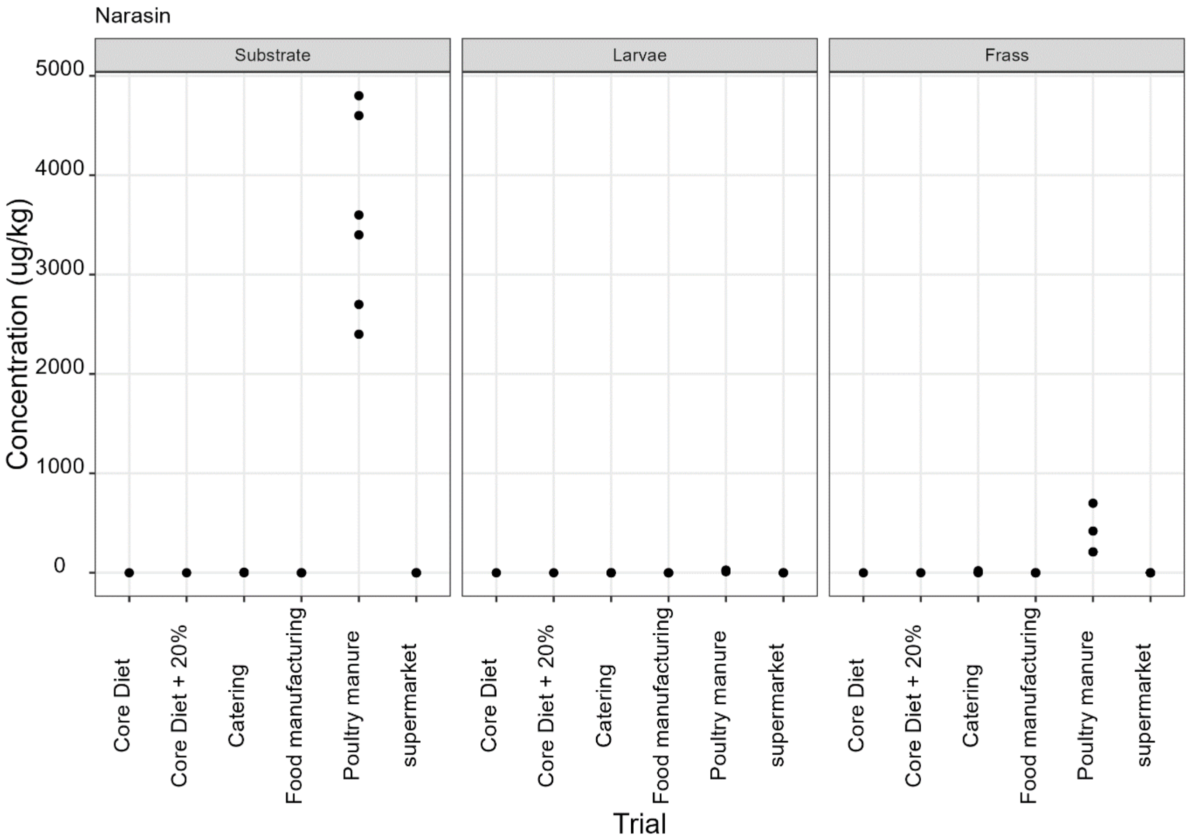
2.


Flubendazole
Substrate
Larvae
Frass
Concentration (ug/kg)
20
15
10
5
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

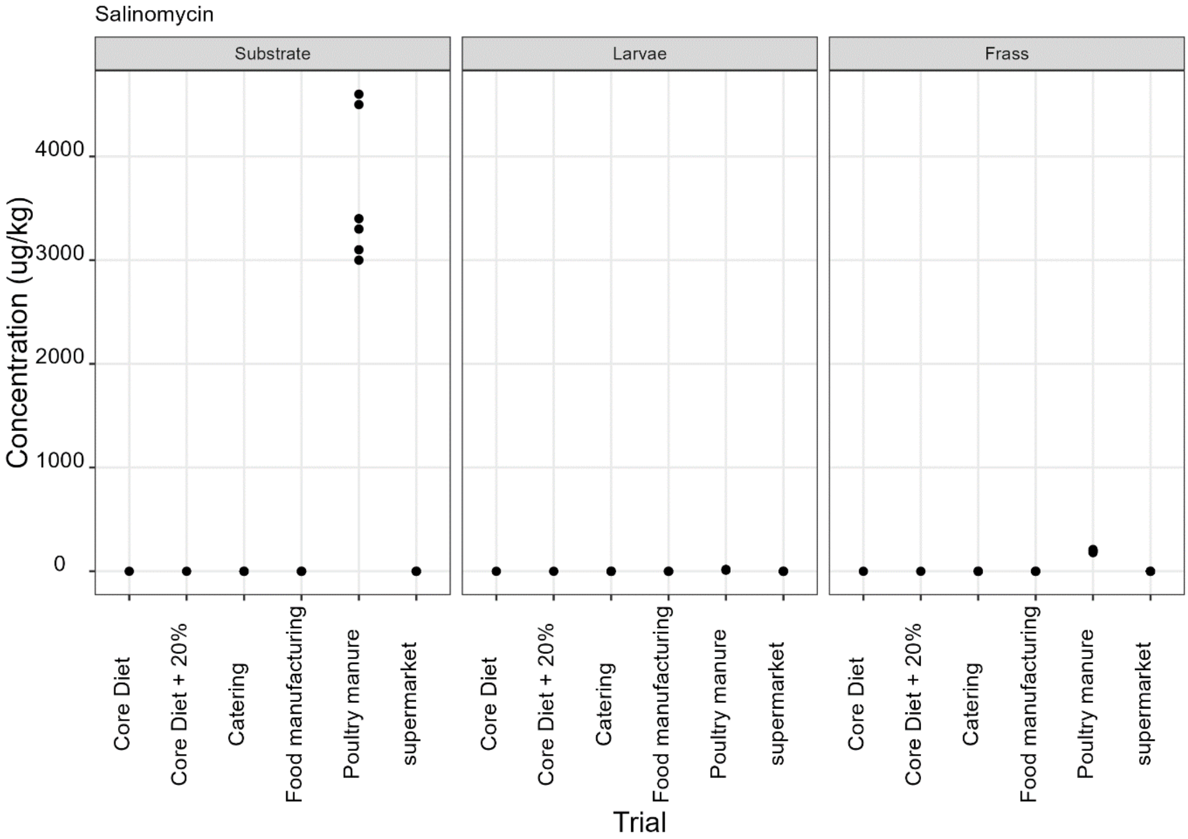
3.


Lasalocid
Substrate
Larvae
Frass
Concentration (ug/kg)
120
90
60
30
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

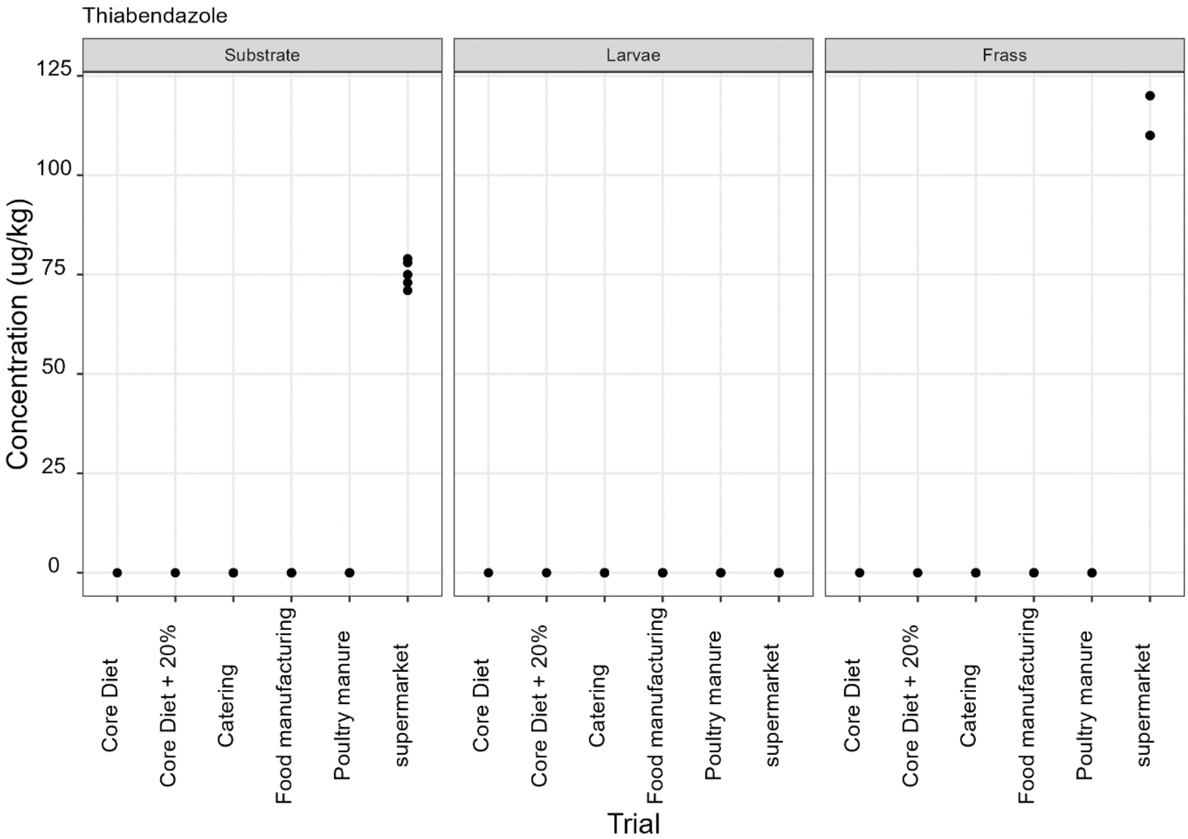
4.


Narasin
Substrate
Larvae
Frass
Concentration (ug/kg)
5000
4000
3000
2000
1000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

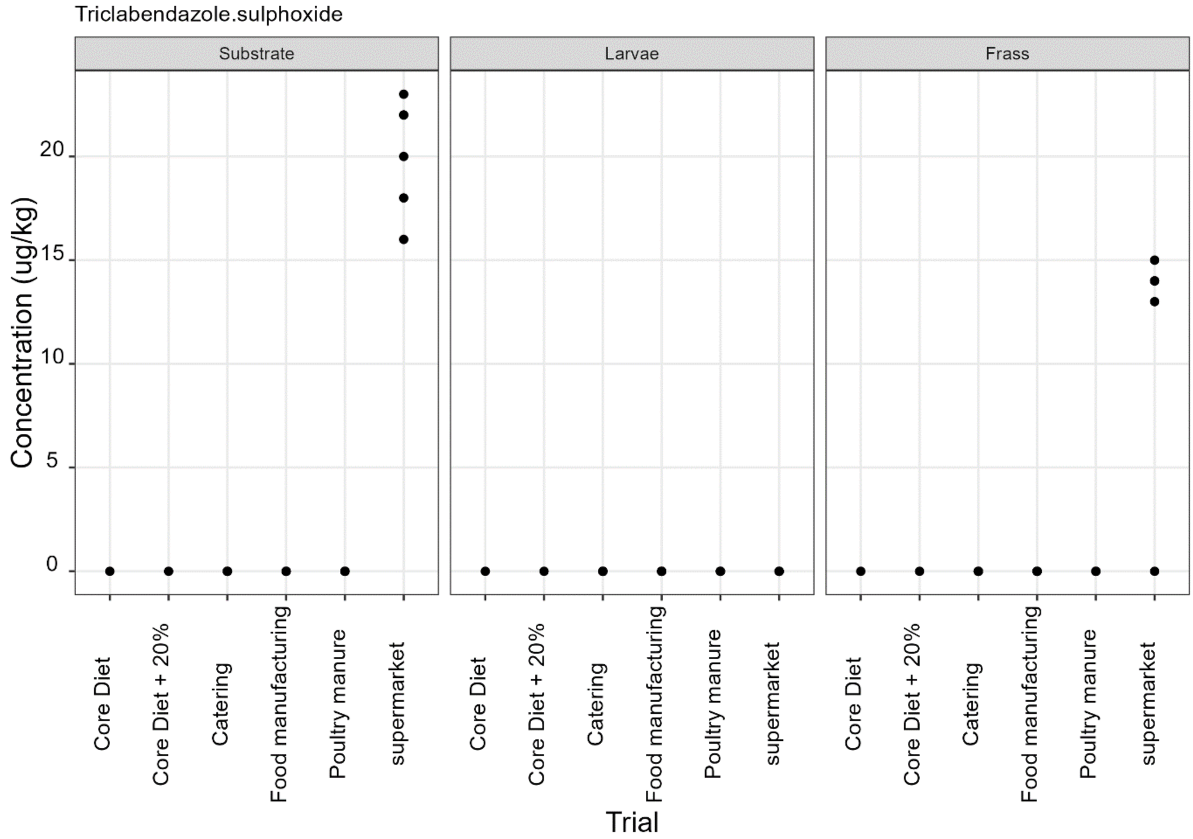
5.


Salinomycin
Substrate
Larvae
Frass
Concentration (ug/kg)
4000
3000
2000
1000
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

6.

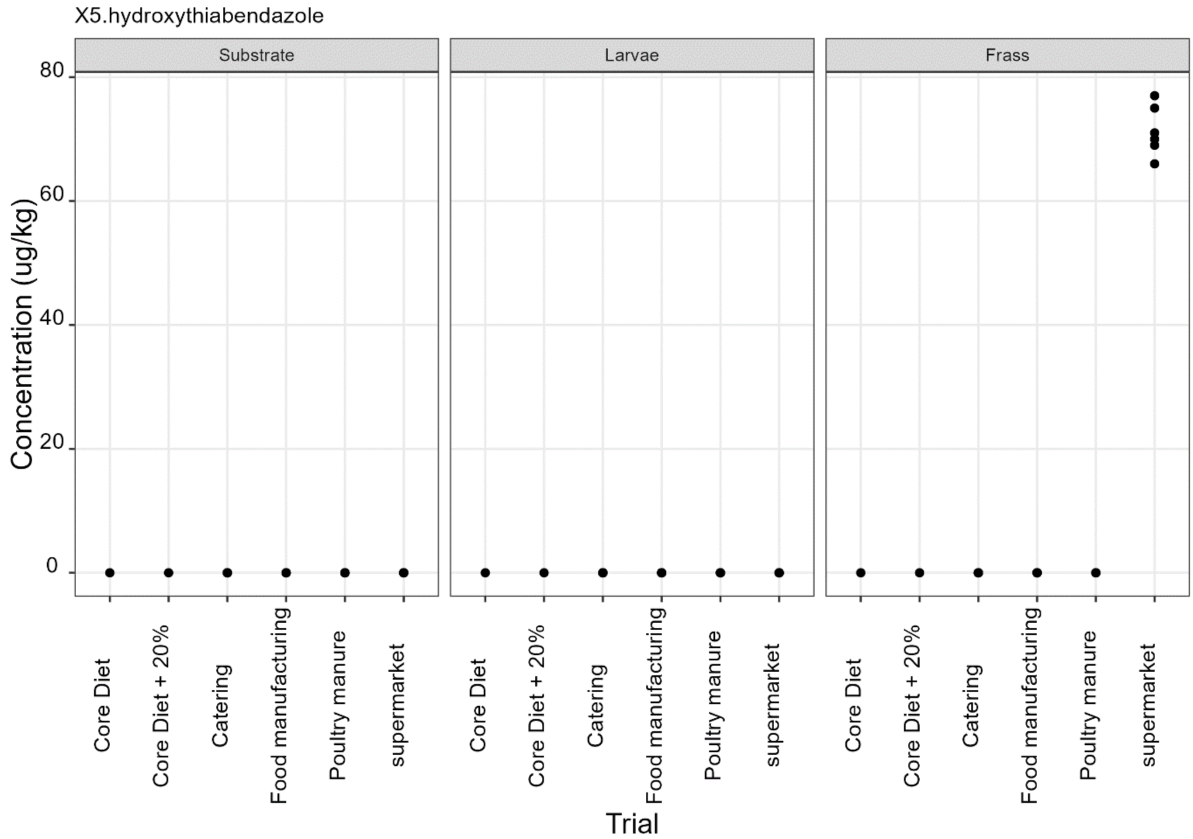

Thiabendazole
Substrate
Larvae
Frass
Concentration (ug/kg)
125
100
75
50
25
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

7.



Triclabendazole.sulphoxide
Substrate
Larvae
Frass
Concentration (ug/kg)
0
5
10
15
20
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

8.



X5.hydroxythiabendazole
Substrate
Larvae
Frass
Concentration (ug/kg)
0
20
40
60
80
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

Figure II (1 – 8). Concentration (μg/kg) of veterinary medicines analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table IV. Calculated quantities of veterinary medicines.

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet	Larvae	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet	Frass	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet	Substrate	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet	Larvae	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet	Frass	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet	Substrate	Flubendazole	1	0	0	0	NA	NA	NA
core diet	Larvae	Flubendazole	1	0	0	0	NA	NA	NA
core diet	Frass	Flubendazole	1	0	0	0	NA	NA	NA
core diet	Substrate	Lasalocid	1	0	0	0	NA	NA	NA
core diet	Larvae	Lasalocid	1	0	0	0	NA	NA	NA
core diet	Frass	Lasalocid	1	0	0	0	NA	NA	NA
core diet	Substrate	Narasin	1	0	0	0	NA	NA	NA
core diet	Larvae	Narasin	1	0	0	0	NA	NA	NA
core diet	Frass	Narasin	1	0	0	0	NA	NA	NA
core diet	Substrate	Salinomycin	1	0	0	0	NA	NA	NA
core diet	Larvae	Salinomycin	1	0	0	0	NA	NA	NA
core diet	Frass	Salinomycin	1	0	0	0	NA	NA	NA
core diet	Substrate	Thiabendazole	1	0	0	0	NA	NA	NA
core diet	Larvae	Thiabendazole	1	0	0	0	NA	NA	NA
core diet	Frass	Thiabendazole	1	0	0	0	NA	NA	NA
core diet	Substrate	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA
core diet	Larvae	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA
core diet	Frass	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	5.hydroxythiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Dinitrocarbanilide nicarbazin.	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Flubendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Flubendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Flubendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Lasalocid	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Lasalocid	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Lasalocid	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Narasin	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Narasin	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Narasin	1	0	0	0	NA	NA	NA

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Salinomycin	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Salinomycin	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Salinomycin	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	Triclabendazole.sulphoxide	1	0	0	0	NA	NA	NA
Catering	Substrate	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Catering	Larvae	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Catering	Frass	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Catering	Substrate	Dinitrocarbanilide nicarbazin.	6	4	12.25	27	NA	0.08163	-2
Catering	Larvae	Dinitrocarbanilide nicarbazin.	6	0	0	0	0	NA	NA
Catering	Frass	Dinitrocarbanilide nicarbazin.	6	6	36.25	54	2.959	-0.5517	-0.2897
Catering	Substrate	Flubendazole	6	0	0	0	NA	NA	NA
Catering	Larvae	Flubendazole	6	0	0	0	NA	NA	NA
Catering	Frass	Flubendazole	6	0	0	0	NA	NA	NA
Catering	Substrate	Lasalocid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Lasalocid	6	0	0	0	NA	NA	NA
Catering	Frass	Lasalocid	6	0	0	0	NA	NA	NA
Catering	Substrate	Narasin	6	6	3.413	5.9	NA	-0.1904	0.5201
Catering	Larvae	Narasin	6	0	0	0	0	NA	NA
Catering	Frass	Narasin	6	6	5.088	20	1.491	-2.211	-0.8599
Catering	Substrate	Salinomycin	6	0	0	0	NA	NA	NA
Catering	Larvae	Salinomycin	6	0	0	0	NA	NA	NA
Catering	Frass	Salinomycin	6	0	0	0	NA	NA	NA
Catering	Substrate	Thiabendazole	6	0	0	0	NA	NA	NA
Catering	Larvae	Thiabendazole	6	0	0	0	NA	NA	NA
Catering	Frass	Thiabendazole	6	0	0	0	NA	NA	NA
Catering	Substrate	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Catering	Larvae	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Catering	Frass	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Frass	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Flubendazole	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Flubendazole	6	3	20.5	23	Inf	0.09756	-0.1463
Manufacturing	Frass	Flubendazole	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Lasalocid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Lasalocid	6	6	86.88	117.5	Inf	-0.3165	-0.1007
Manufacturing	Frass	Lasalocid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Narasin	6	0	0	0	NA	NA	NA

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Narasin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Narasin	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Salinomycin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Salinomycin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Salinomycin	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Thiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Thiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Thiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Frass	5.hydroxythiabendazole	4	0	0	0	NA	NA	NA
Poultry manure	Substrate	Dinitrocarbanilide nicarbazin.	6	6	5775	6400	NA	0.03463	-0.02597
Poultry manure	Larvae	Dinitrocarbanilide nicarbazin.	6	6	363.8	380	0.063	0.04123	0.006872
Poultry manure	Frass	Dinitrocarbanilide nicarbazin.	4	4	2725	3100	0.4719	-0.09174	NA
Poultry manure	Substrate	Flubendazole	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Flubendazole	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Flubendazole	4	0	0	0	NA	NA	NA
Poultry manure	Substrate	Lasalocid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Lasalocid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Lasalocid	4	0	0	0	NA	NA	NA
Poultry manure	Substrate	Narasin	6	6	3562	4800	NA	-0.6036	-0.03509
Poultry manure	Larvae	Narasin	6	6	20.25	27	0.005685	0.3457	0.4198
Poultry manure	Frass	Narasin	4	4	385	700	0.1081	0.9091	NA
Poultry manure	Substrate	Salinomycin	6	6	3875	4600	NA	0	0.3484
Poultry manure	Larvae	Salinomycin	6	6	14.75	17	0.003806	0.2034	0.1695
Poultry manure	Frass	Salinomycin	4	4	195	210	0.05032	0	NA
Poultry manure	Substrate	Thiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Thiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Thiabendazole	4	0	0	0	NA	NA	NA
Poultry manure	Substrate	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Triclabendazole.sulphoxide	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Triclabendazole.sulphoxide	4	0	0	0	NA	NA	NA
supermarket	Substrate	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
supermarket	Larvae	5.hydroxythiabendazole	6	0	0	0	NA	NA	NA
supermarket	Frass	5.hydroxythiabendazole	6	6	70.88	77	Inf	0.02116	-0.0388

Trial	Sample Type	Veterinary medicine	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
supermarket	Substrate	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
supermarket	Larvae	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
supermarket	Frass	Dinitrocarbanilide nicarbazin.	6	0	0	0	NA	NA	NA
supermarket	Substrate	Flubendazole	6	0	0	0	NA	NA	NA
supermarket	Larvae	Flubendazole	6	0	0	0	NA	NA	NA
supermarket	Frass	Flubendazole	6	0	0	0	NA	NA	NA
supermarket	Substrate	Lasalocid	6	0	0	0	NA	NA	NA
supermarket	Larvae	Lasalocid	6	0	0	0	NA	NA	NA
supermarket	Frass	Lasalocid	6	6	3.89	5.2	Inf	-0.06684	0.4216
supermarket	Substrate	Narasin	6	0	0	0	NA	NA	NA
supermarket	Larvae	Narasin	6	0	0	0	NA	NA	NA
supermarket	Frass	Narasin	6	0	0	0	NA	NA	NA
supermarket	Substrate	Salinomycin	6	0	0	0	NA	NA	NA
supermarket	Larvae	Salinomycin	6	0	0	0	NA	NA	NA
supermarket	Frass	Salinomycin	6	0	0	0	NA	NA	NA
supermarket	Substrate	Thiabendazole	6	6	74.38	79	NA	-0.04706	-0.06386
supermarket	Larvae	Thiabendazole	6	0	0	0	0	NA	NA
supermarket	Frass	Thiabendazole	6	6	112.5	120	1.513	-0.08889	-0.04444
supermarket	Substrate	Triclabendazole.sulphoxide	6	6	20.12	23	NA	-0.07455	-0.01243
supermarket	Larvae	Triclabendazole.sulphoxide	6	0	0	0	0	NA	NA
supermarket	Frass	Triclabendazole.sulphoxide	6	5	12.38	15	0.6153	-0.525	0.3433

Inf = inferred, NA = not applicable

Appendix D - Pesticides

The aim of the analysis was to

- estimate the quantity of each pesticide in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of bioaccumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

Individual results are shown in the figures and the table shows the calculated quantities.

Pesticides which were not detected in any extracts from any samples are not reported here. They are: 2,4.DB, 6.benzyl.aminopurine, abamectin, acephate, acetamiprid, acetochlor, acibenzolar.S.methyl, aclonifen, acrinathrin, alachlor, aldicarb, aldicarb.sulfone, aldicarb.sulfoxide, aldrin, allethrin, ametoctradin, amidosulfuron, asulam, atrazine, azinphos.ethyl, azinphos.methyl, BAC10, benalaxyl, bendiocarb, benthiavalicarb.isopropyl, bifenox, bifenthrin, biphenyl, bispyribac.sodium, bitertanol, bixafen, bromophos.ethyl, bromopropylate, bromoxynil, bromuconazole, bupirimate, buprofezin, butachlor, butocarboxim, butocarboxim.sulfoxide, butoxycarboxim, cadusafos, carbaryl, carbendazim, carbetamide, carbofuran, carbofuran..3.hydroxy., carboxin, chlorantraniliprole, chlorbufam, chlordane..cis., chlordane..trans., chlorfenapyr, chlorfenvinphos, chlorfluazuron, chloridazon, chlorobenzilate, chlorothalonil, chlorpropham, chlorpyrifos, chlorpyrifos.methyl, chlorthal.dimethyl, chlortoluron, chlozolinate, chromafenozide, clethodim, clofentezine, clomazone, clothianidin, coumaphos, cyanazine, cyazofamid, cycloate, cycloxydim, cyflufenamid, cyfluthrin, cyhalofop.butyl, cyhalothrin.lambda, cymoxanil, cypermethrin, cyproconazole, cyromazine, DDD.pp, DDE.pp, DDT.op, DDT.pp, deltamethrin, demeton.S.methyl, demeton.S.methyl.sulfone, desmedipham, diafenthiuron, diazinon, dichlobenil, dichlofluanid, dichlorvos, diclobutrazol, dicloran, dicofol, dicrotophos, dieldrin, diethofencarb, difenoconazole, diflubenzuron, diflufenican, dimethenamid, dimethoate, dimethomorph, dimoxystrobin, diniconazole, dinotefuran, disulfoton, disulfoton.sulfone, disulfoton.sulfoxide, diuron, DMF, DMPF, DMSA, dodine, emamectin.benzoate, endosulfan..I., endosulfan..II., endosulfan.sulfate, endrin, EPN, epoxiconazole, EPTC, ethiofencarb, ethiofencarb.sulfone, ethiofencarb.sulfoxide, ethion, ethiprole, ethirimol, ethofumesate, ethoprophos, etofenprox, etoxazole, etridiazole, etrimfos, famoxadone, fenamidone, fenamiphos, fenamiphos.sulfone, fenamiphos.sulfoxide, fenarimol, fenazaquin, fenbuconazole, fenbutatin.oxide, fenitrothion, fenoprop, fenoxycarb, fenpropathrin, fenpropidin, fenpropimorph, fenpyrazamine, fenpyroximate, fensulfothion, fensulfothion.sulfone, fensulfothion.oxon, fensulfothion.oxon.sulfone, fenthion, fenthion.sulfone, fenthion.sulfoxide, fentin.acetate, fenvalerate, fipronil, fipronil.de.sulfinyl, fipronil.sulfone, flonicamid, fluazifop..free.acid., fluazifop.p.butyl, fluazinam, flubendiamide, flucythrinate, flufenacet, flufenoxuron, fluometuron, fluopicolide, fluopyram, fluoxastrobin, fluquinconazole, flurochloridone, fluroxypyr, flusilazole, flutolanil, flutriafol, fluvalinate, fluxapyroxad, folpet, fonofos, formetanate.HCl, fosthiazate, furalaxyl, furathiocarb, halauxifen.methyl, halofenozide, halosulfuron.methyl, HCH.alpha, HCH.beta, HCH.gamma, heptachlor, heptachlor.epoxide.cis, heptachlor.epoxide.trans, heptenophos, hexachlorobenzene, hexaconazole, hexazinone, hexythiazox, imazaquin, imidacloprid, indoxacarb, ioxynil, ipconazole, iprodione, iprovalicarb, isazofos, isocarbofos, isofenphos, isofenphos.methyl, isoflucypram, isoprocarb, isoprothiolane, isoproturon, isopyrazam, isoxaben, isoxaflutole, kresoxim.methyl, lenacil, linuron, lufenuron, malaoxon, malathion, MCPA, MCPB, mecarbam, mecoprop, mepanipyrim, mephosfolan, mepronil, mesosulfuron.methyl, metaflumizone, metalaxyl, metamitron, metazachlor, metconazole, methabenzthiazuron, methacrifos, methamidophos, methidathion, methiocarb, methiocarb.sulfone, methiocarb.sulfoxide, methomyl, methoxychlor, methoxyfenozide, metobromuron, metolachlor, metolcarb, metosulam, metoxuron, metribuzin, metsulfuron.methyl, mevinphos, molinate, monocrotophos, monolinuron, monuron, myclobutanil, napropamide, nitenpyram, nitrofen, nitrothal.isopropyl, novaluron, nuarimol, ofurace, omethoate, oxadiargyl, oxadiazon, oxadixyl, oxamyl, oxasulfuron, oxychlordane, oxydemeton.methyl, oxyfluorfen, paclobutrazol, paraoxon.methyl, parathion.ethyl, parathion.methyl, penconazole, pencycuron, pendimethalin, penflufen, pentachloroaniline, pentanochlor, penthiopyrad, permethrin, phenmedipham, phenthoate, phorate, phorate.sulfone, phorate.sulfoxide, phosalone, phosmet, phosphamidon, phoxim, phthalimide, picolinafen, picoxystrobin, piperonyl.butoxide, pirimicarb, pirimicarb.desmethyl, pirimiphos.ethyl, pirimiphos.methyl, prochloraz, procymidone, profenofos, promecarb, prometryn, propachlor, propamocarb..free.base., propaquizafop, propargite, propetamphos, propham, propoxur, propyzamide, proquinazid, prosulfocarb, prosulfuron, prothioconazole.desthio, prothiofos, pydiflumetofen, pymetrozine, pyrazophos, pyrethrins, pyridaben, pyridalyl, pyridaphenthion, pyrifenox, quassia, quinalphos, quinmerac, quinoclamine, quinoxyfen, quintozene, quizalofop.P, rimsulfuron, rotenone, simazine, spinetoram, spinosad, spirodiclofen, spiromesifen, spirotetramat, spirotetramat.enol, spiroxamine, sulcotrione, sulfoxaflor, tebuconazole, tebufenozide, tebufenpyrad, tebupirimphos, tebuthiuron, tecnazene, teflubenzuron, tefluthrin, tepraloxydim, terbufos, terbufos.sulfone, terbufos.sulfoxide, terbuthylazine, terbutryn, tetrachlorvinphos, tetraconazole, tetradifon, tetrahydrophthalimide, tetramethrin, TFNA, TFNG, thiacloprid, thiamethoxam, thifensulfuron.methyl, thiodicarb, thiophanate.methyl, tolclofos.methyl, tolfenpyrad, tolylfluanid, triadimefon, triadimenol, triallate, triasulfuron, triazophos, tribenuron.methyl, triclopyr, tricyclazole, trifloxystrobin, triflumizole, triflumuron, trifluralin, triflusulfuron.methyl, triforine, triticonazole, tritosulfuron, vamidothion, vinclozolin, and zoxamide.

Reporting limits are shown below:

Table V. Reporting limits for pesticides analysis.

Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
2,4-D	0.01	ethirimol	0.01	napropamide	0.01
2,4-DB	0.01	ethofumesate	0.01	nitenpyram	0.01
2-phenylphenol	0.01	ethoprophos	0.01	nitrofen	0.01
6-benzyl aminopurine	0.01	etofenprox	0.01	nitrothal-isopropyl	0.01
abamectin	0.01	etoxazole	0.01	novaluron	0.01
acephate	0.01	etridiazole	0.01	nuarimol	0.01

Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
acetamiprid	0.01	etrimfos	0.01	ofurace	0.01
acetochlor	0.01	famoxadone	0.01	omethoate	0.01
acibenzolar-S-methyl	0.01	fenamidone	0.01	oxadiargyl	0.01
aclonifen	0.01	fenamiphos	0.01	oxadiazon	0.01
acrinathrin	0.01	fenamiphos sulfone	0.01	oxadixyl	0.01
alachlor	0.01	fenamiphos sulfoxide	0.01	oxamyl	0.01
aldicarb	0.01	fenarimol	0.01	oxasulfuron	0.01
aldicarb sulfone	0.01	fenazaquin	0.01	oxychlordane	0.01
aldicarb sulfoxide	0.01	fenbuconazole	0.01	oxydemeton-methyl	0.01
aldrin	0.01	fenbutatin oxide	0.01	oxyfluorfen	0.01
allethrin	0.01	fenhexamid	0.01	paclobutrazol	0.01
ametoctradin	0.01	fenitrothion	0.01	paraoxon-methyl	0.01
amidosulfuron	0.01	fenoprop	0.01	parathion-ethyl	0.01
asulam	0.01	fenoxycarb	0.01	parathion-methyl	0.01
atrazine	0.01	fenpropathrin	0.01	penconazole	0.01
azinphos-ethyl	0.01	fenpropidin	0.01	pencycuron	0.01
azinphos-methyl	0.01	fenpropimorph	0.01	pendimethalin	0.01
azoxystrobin	0.01	fenpyrazamine	0.01	penflufen	0.01
BAC10	0.05	fenpyroximate	0.01	pentachloroaniline	0.01
BAC12	0.05	fensulfothion	0.01	pentanochlor	0.01
BAC14	0.05	fensulfothion sulfone	0.01	penthiopyrad	0.01
BAC16	0.05	fensulfothion-oxon	0.01	permethrin	0.01
benalaxyl	0.01	fensulfothion-oxon-sulfone	0.01	phenmedipham	0.01
bendiocarb	0.01	fenthion	0.01	phenthoate	0.01
benthiavalicarb-isopropyl	0.01	fenthion sulfone	0.01	phorate	0.01
bifenox	0.01	fenthion sulfoxide	0.01	phorate sulfone	0.01
bifenthrin	0.01	fentin acetate	0.01	phorate sulfoxide	0.01
biphenyl	0.02	fenvalerate	0.01	phosalone	0.01
bispyribac-sodium	0.01	fipronil	0.002	phosmet	0.01
bitertanol	0.01	fipronil de-sulfinyl	0.002	phosphamidon	0.01
bixafen	0.01	fipronil sulfone	0.002	phoxim	0.01

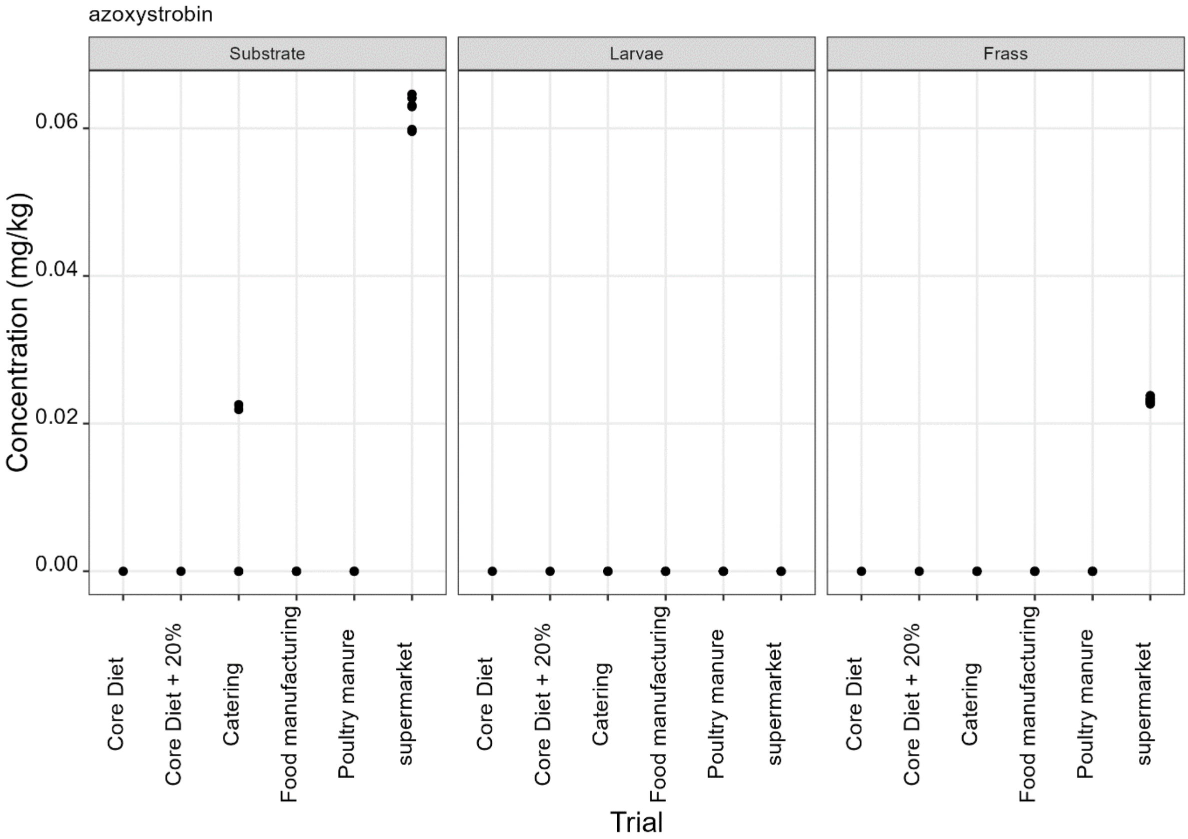
Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
boscalid	0.01	flonicamid	0.01	phthalimide#	0.01
bromophos-ethyl	0.01	fluazifop (free acid)	0.01	picolinafen	0.01
bromopropylate	0.01	fluazifop-p-butyl	0.01	picoxystrobin	0.01
bromoxynil	0.01	fluazinam	0.01	piperonyl butoxide	0.01
bromuconazole	0.01	flubendiamide	0.01	pirimicarb	0.01
bupirimate	0.01	flucythrinate	0.01	pirimicarb-desmethyl	0.01
buprofezin	0.01	fludioxonil	0.010	pirimiphos-ethyl	0.01
butachlor	0.01	flufenacet	0.01	pirimiphos-methyl	0.01
butocarboxim	0.01	flufenoxuron	0.01	prochloraz	0.01
butocarboxim sulfoxide	0.01	fluometuron	0.01	procymidone	0.01
butoxycarboxim	0.01	fluopicolide	0.01	profenofos	0.01
cadusafos	0.01	fluopyram	0.01	promecarb	0.01
carbaryl	0.01	fluoxastrobin	0.01	prometryn	0.01
carbendazim	0.01	fluquinconazole	0.01	propachlor	0.01
carbetamide	0.01	flurochloridone	0.01	propamocarb (free base)	0.01
carbofuran	0.001	fluroxypyr	0.01	propaquizafop	0.01
carbofuran (3-hydroxy)	0.001	flusilazole	0.01	propargite	0.01
carboxin	0.01	flutolanil	0.01	propetamphos	0.01
chlorantraniliprole	0.01	flutriafol	0.01	propham	0.01
chlorbufam	0.01	fluvalinate	0.01	propiconazole	0.01
chlordane (cis)	0.01	fluxapyroxad	0.01	propoxur	0.01
chlordane (trans)	0.01	folpet#	0.01	propyzamide	0.01
chlorfenapyr	0.01	fonofos	0.01	proquinazid	0.01
chlorfenvinphos	0.01	formetanate-HCl	0.01	prosulfocarb	0.01
chlorfluazuron	0.01	fosthiazate	0.01	prosulfuron	0.01
chloridazon	0.01	furalaxyl	0.01	prothioconazole-desthio	0.01
chlorobenzilate	0.01	furathiocarb	0.001	prothiofos	0.01
chlorothalonil	0.01	halauxifen-methyl	0.01	pydiflumetofen	0.01
chlorpropham	0.01	halofenozide	0.01	pymetrozine	0.01
chlorpyrifos	0.01	halosulfuron-methyl	0.01	pyraclostrobin	0.01

Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
chlorpyrifos-methyl	0.01	haloxyfop (free acid)	0.01	pyrazophos	0.01
chlorthal-dimethyl	0.01	HCH-alpha	0.01	pyrethrins	0.01
chlortoluron	0.01	HCH-beta	0.01	pyridaben	0.01
chlozolinate	0.01	HCH-gamma	0.01	pyridalyl	0.01
chromafenozide	0.01	heptachlor	0.01	pyridaphenthion	0.01
clethodim	0.01	heptachlor epoxide-cis	0.01	pyrifenox	0.01
clofentezine	0.01	heptachlor epoxide-trans	0.01	pyrimethanil	0.01
clomazone	0.01	heptenophos	0.01	pyriproxyfen	0.01
clothianidin	0.01	hexachlorobenzene	0.01	quassia	0.01
coumaphos	0.01	hexaconazole	0.01	quinalphos	0.01
cyanazine	0.01	hexazinone	0.01	quinmerac	0.01
cyazofamid	0.01	hexythiazox	0.01	quinoclamine	0.01
cycloate	0.01	imazalil	0.01	quinoxyfen	0.01
cycloxydim	0.01	imazaquin	0.01	quintozene	0.01
cyflufenamid	0.01	imidacloprid	0.01	quizalofop P	0.01
cyfluthrin	0.01	indoxacarb	0.01	rimsulfuron	0.01
cyhalofop butyl	0.01	ioxynil	0.01	rotenone	0.01
cyhalothrin-lambda	0.01	ipconazole	0.01	simazine	0.01
cymoxanil	0.01	iprodione	0.01	spinetoram	0.01
cypermethrin	0.01	iprovalicarb	0.01	spinosad	0.01
cyproconazole	0.01	isazofos	0.01	spirodiclofen	0.01
cyprodinil	0.01	isocarbofos	0.01	spiromesifen	0.01
cyromazine	0.01	isofenphos	0.01	spirotetramat	0.01
DDAC	0.05	isofenphos-methyl	0.01	spirotetramat enol	0.01
DDD-pp	0.01	isoflucypram	0.01	spiroxamine	0.01
DDE-pp	0.01	isoprocarb	0.01	sulcotrione	0.01
DDT-op	0.01	isoprothiolane	0.01	sulfoxaflor	0.01
DDT-pp	0.01	isoproturon	0.01	tebuconazole	0.01
deltamethrin	0.01	isopyrazam	0.01	tebufenozide	0.01
demeton-S-methyl	0.01	isoxaben	0.01	tebufenpyrad	0.01
demeton-S-methyl sulfone	0.01	isoxaflutole	0.01	tebupirimphos	0.01
desmedipham	0.01	kresoxim-methyl	0.01	tebuthiuron	0.01
diafenthiuron	0.01	lenacil	0.01	tecnazene	0.01

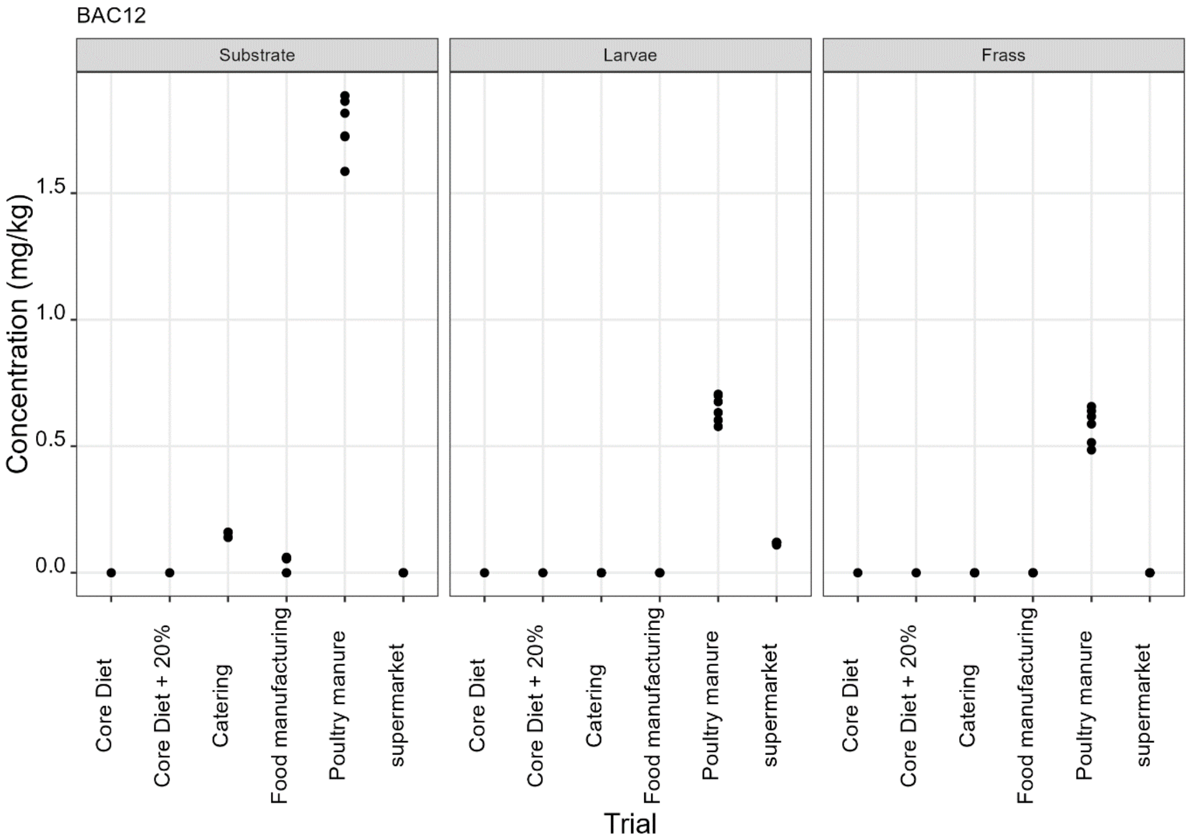
Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
diazinon	0.01	linuron	0.01	teflubenzuron	0.01
dichlobenil	0.01	lufenuron	0.01	tefluthrin	0.01
dichlofluanid	0.01	malaoxon	0.01	tepraloxydim	0.01
dichlorprop	0.01	malathion	0.01	terbufos	0.01
dichlorvos	0.01	mandipropamid	0.01	terbufos sulfone	0.01
diclobutrazol	0.01	MCPA	0.01	terbufos sulfoxide	0.01
dicloran	0.01	MCPB	0.01	terbuthylazine	0.01
dicofol	0.01	mecarbam	0.01	terbutryn	0.01
dicrotophos	0.01	mecoprop	0.01	tetrachlorvinphos	0.01
dieldrin	0.01	mepanipyrim	0.01	tetraconazole	0.01
diethofencarb	0.01	mephosfolan	0.01	tetradifon	0.01
difenoconazole	0.01	mepronil	0.01	tetrahydrophthalimide	0.05
diflubenzuron	0.01	mesosulfuron-methyl	0.01	tetramethrin	0.01
diflufenican	0.01	metaflumizone	0.01	TFNA	0.01
dimethenamid	0.01	metalaxyl	0.01	TFNG	0.01
dimethoate	0.01	metamitron	0.01	thiabendazole	0.01
dimethomorph	0.01	metazachlor	0.01	thiacloprid	0.01
dimoxystrobin	0.01	metconazole	0.01	thiamethoxam	0.01
diniconazole	0.01	methabenzthiazuron	0.01	thifensulfuron-methyl	0.01
dinotefuran	0.01	methacrifos	0.01	thiodicarb	0.01
diphenylamine	0.01	methamidophos	0.01	thiophanate-methyl	0.01
disulfoton	0.01	methidathion	0.01	tolclofos-methyl	0.01
disulfoton sulfone	0.01	methiocarb	0.01	tolfenpyrad	0.01
disulfoton sulfoxide	0.01	methiocarb sulfone	0.01	tolylfluanid	0.01
diuron	0.01	methiocarb sulfoxide	0.01	triadimefon	0.01
DMF	0.01	methomyl	0.01	triadimenol	0.01
DMPF	0.01	methoxychlor	0.01	triallate	0.01
DMSA	0.01	methoxyfenozide	0.01	triasulfuron	0.01
dodine	0.01	metobromuron	0.01	triazophos	0.01
emamectin benzoate	0.01	metolachlor	0.01	tribenuron-methyl	0.01
endosulfan (I)	0.01	metolcarb	0.01	triclopyr	0.01
endosulfan (II)	0.01	metosulam	0.01	tricyclazole	0.01

Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg	Pesticide	Reporting limit mg/kg
endosulfan sulfate	0.01	metoxuron	0.01	trifloxystrobin	0.01
endrin	0.01	metrafenone	0.01	triflumizole	0.01
EPN	0.01	metribuzin	0.01	triflumuron	0.01
epoxiconazole	0.01	metsulfuron-methyl	0.01	trifluralin	0.01
EPTC	0.01	mevinphos	0.01	triflusulfuron-methyl	0.01
ethiofencarb	0.01	molinate	0.01	triforine	0.01
ethiofencarb sulfone	0.01	monocrotophos	0.01	triticonazole	0.01
ethiofencarb sulfoxide	0.01	monolinuron	0.01	tritosulfuron	0.01
ethion	0.01	monuron	0.01	vamidothion	0.01
ethiprole	0.01	myclobutanil	0.01	vinclozolin	0.01
zoxamide	0.01				

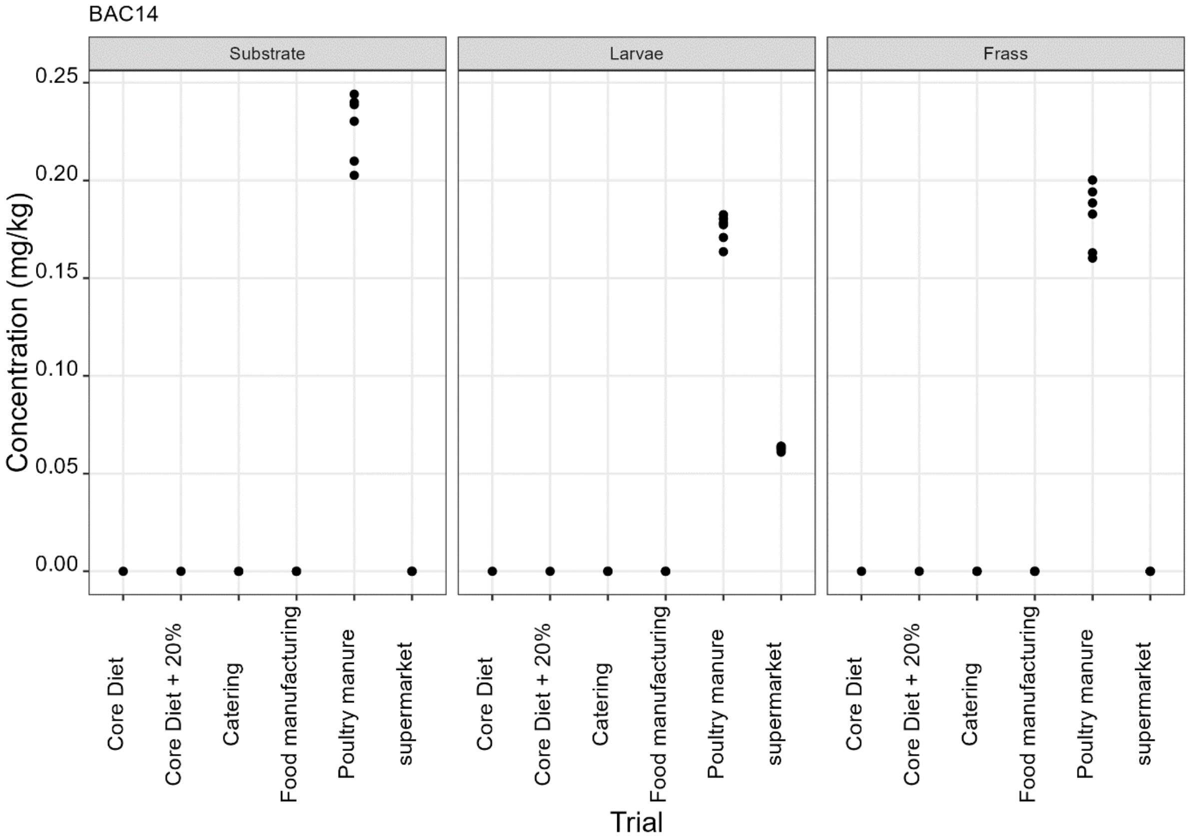
1.


azoxystrobin
Substrate
Larvae
Frass
Concentration (mg/kg)
0.06
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

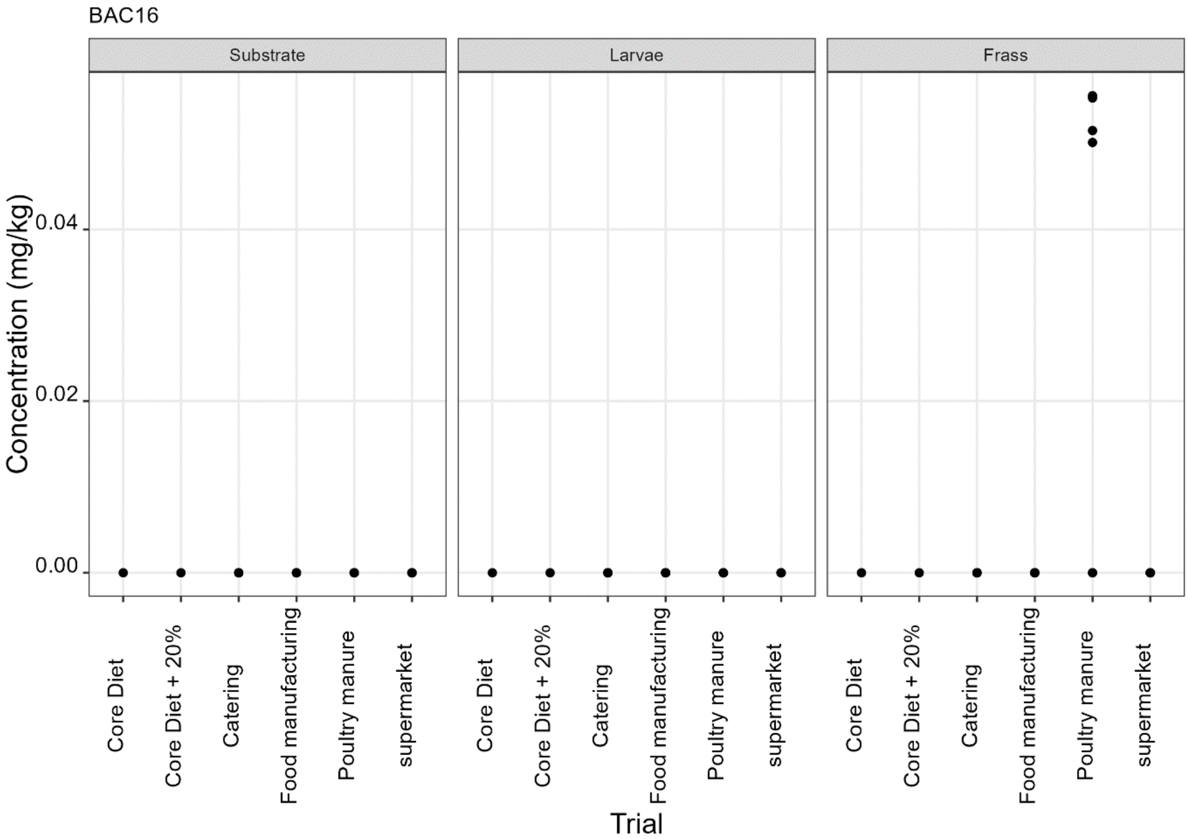
2.


BAC12
Substrate
Larvae
Frass
Concentration (mg/kg)
1.5
1.0
0.5
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

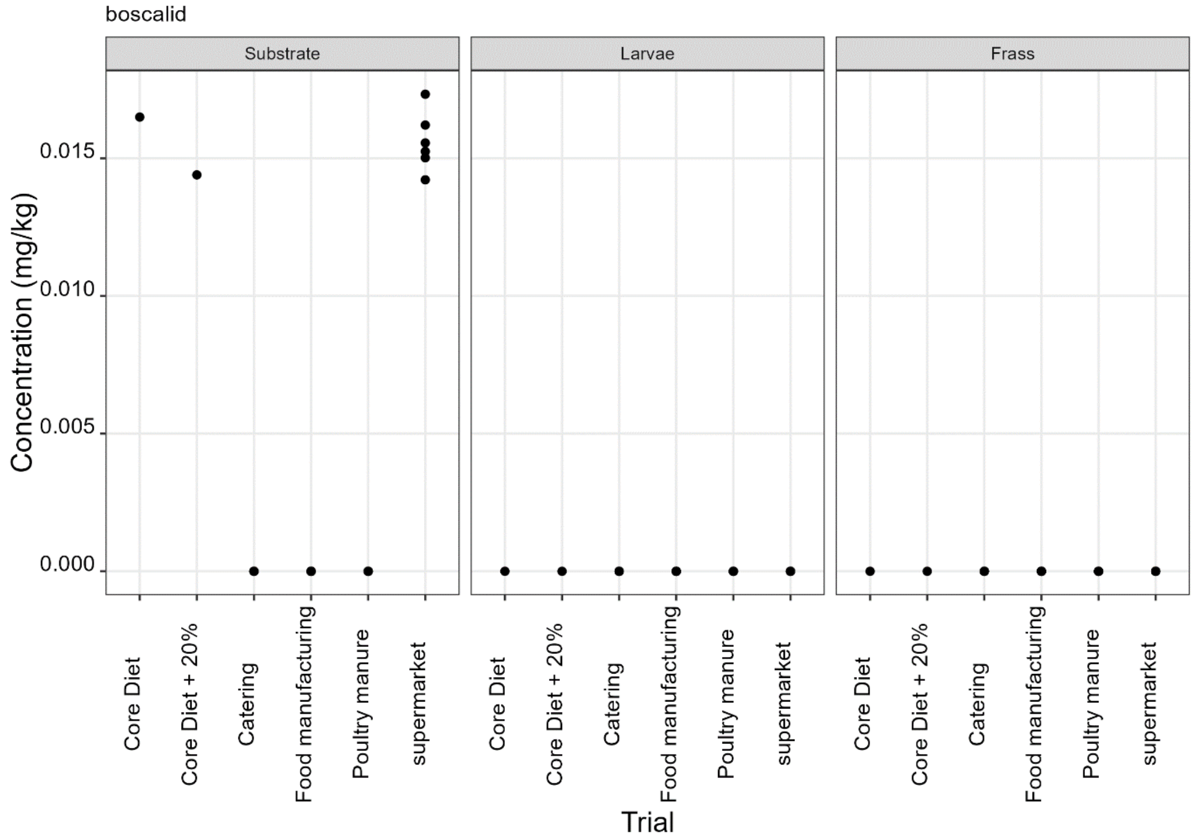
3.


BAC14
Substrate
Larvae
Frass
Concentration (mg/kg)
0.25
0.20
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

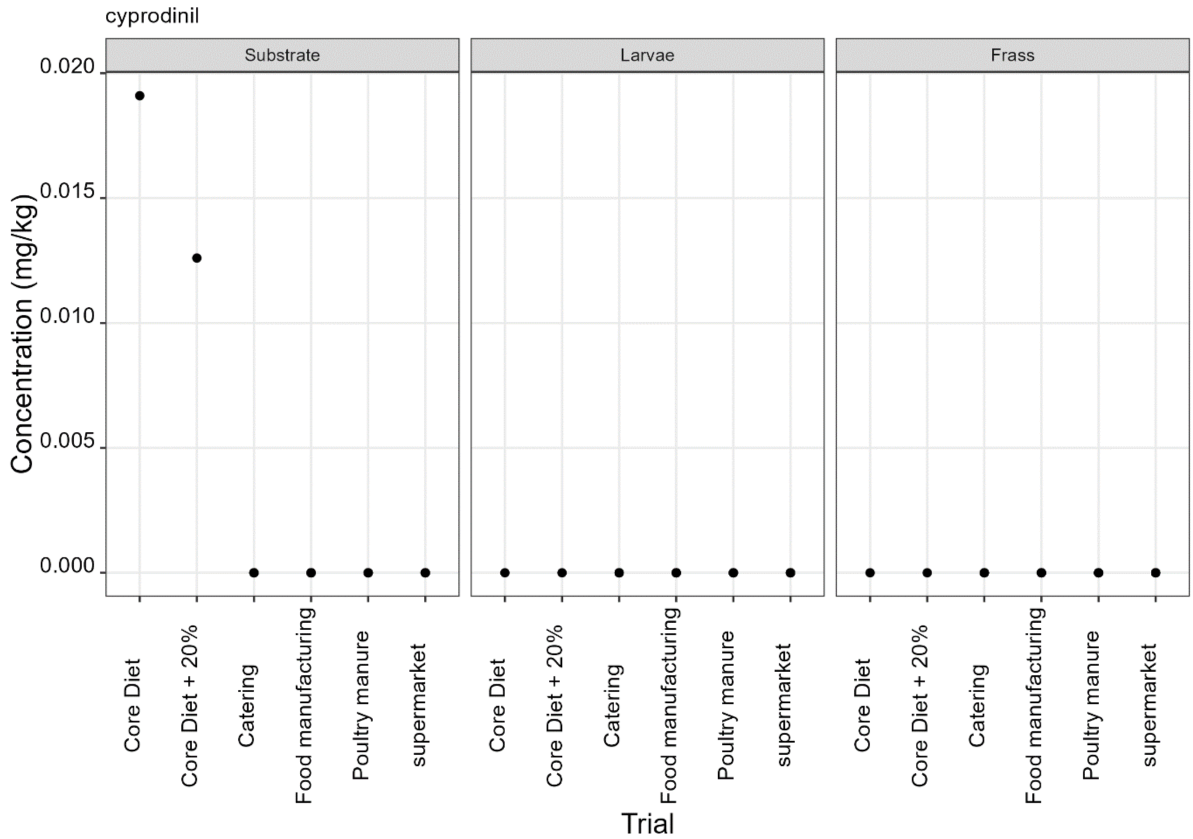
4.


BAC16
Substrate
Larvae
Frass
Concentration (mg/kg)
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

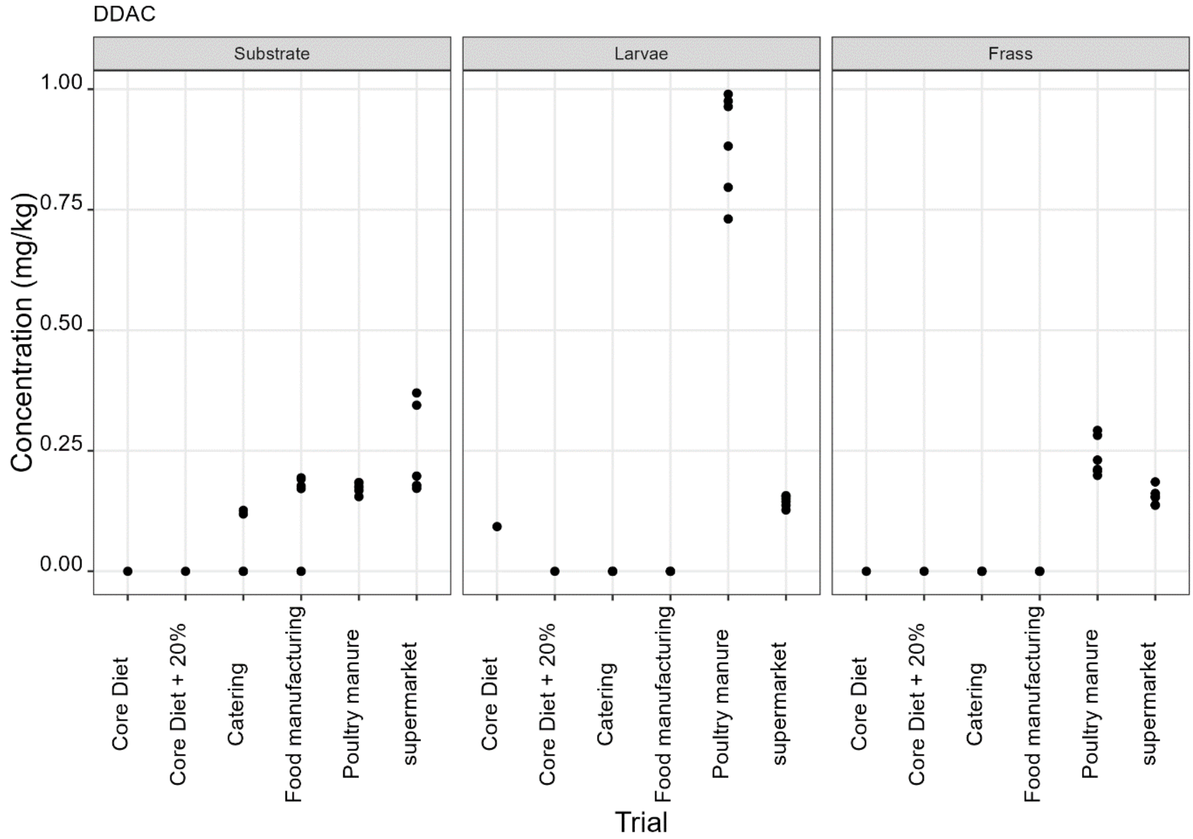
5.


boscalid
Substrate
Larvae
Frass
Concentration (mg/kg)
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

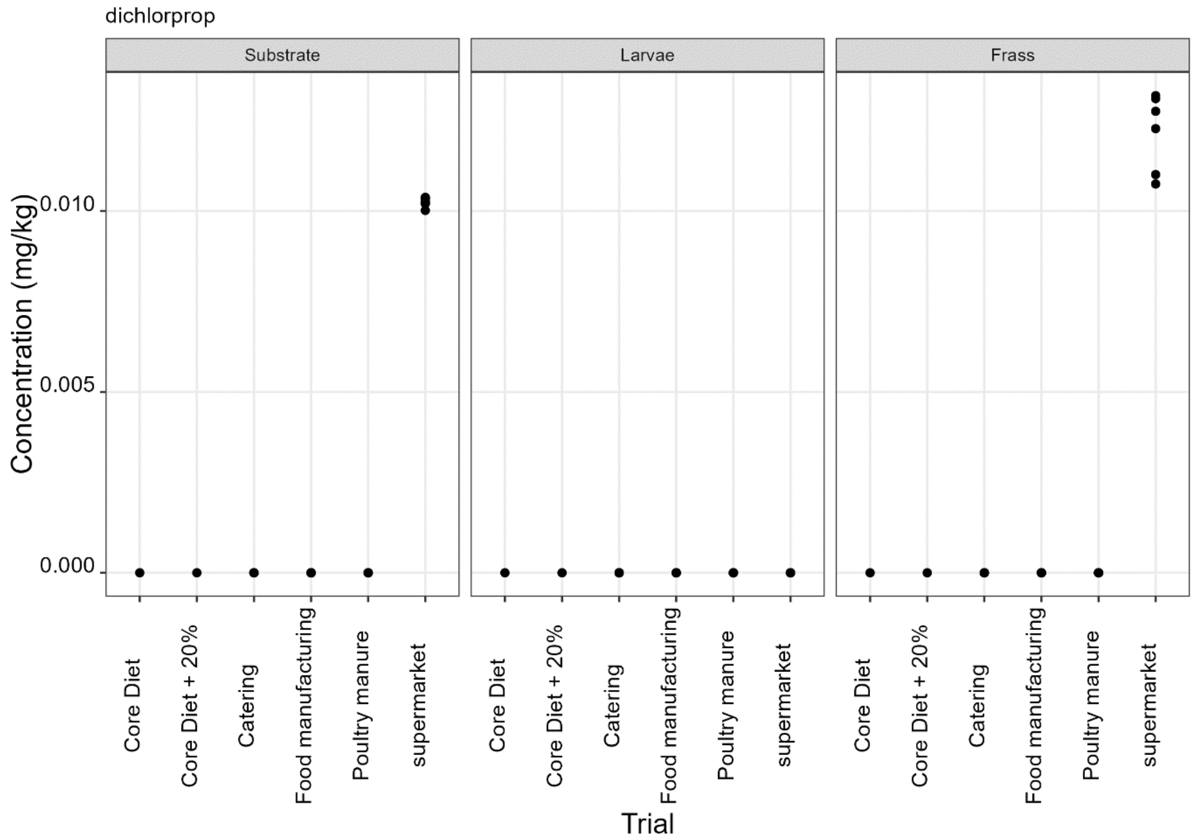
6.


cyprodinil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.020
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

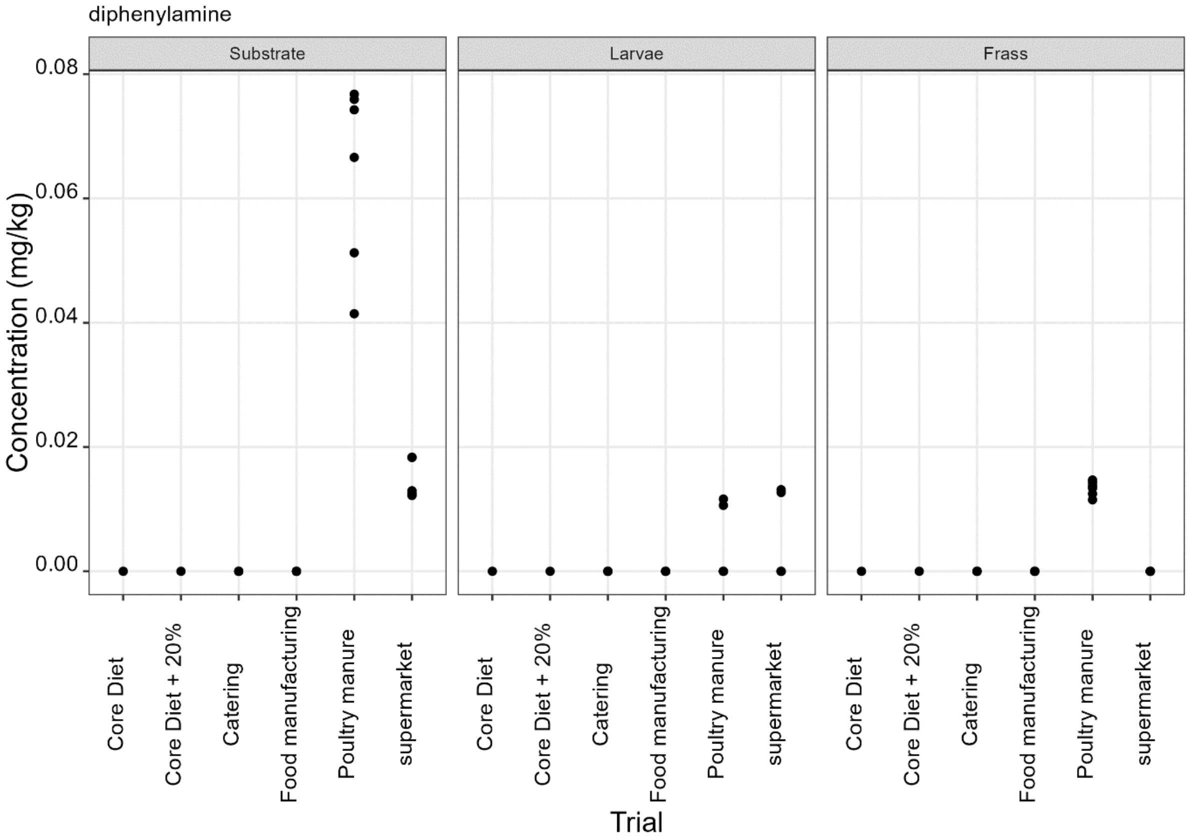
7.


DDAC
Substrate
Larvae
Frass
Concentration (mg/kg)
1.00
0.75
0.50
0.25
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

8.

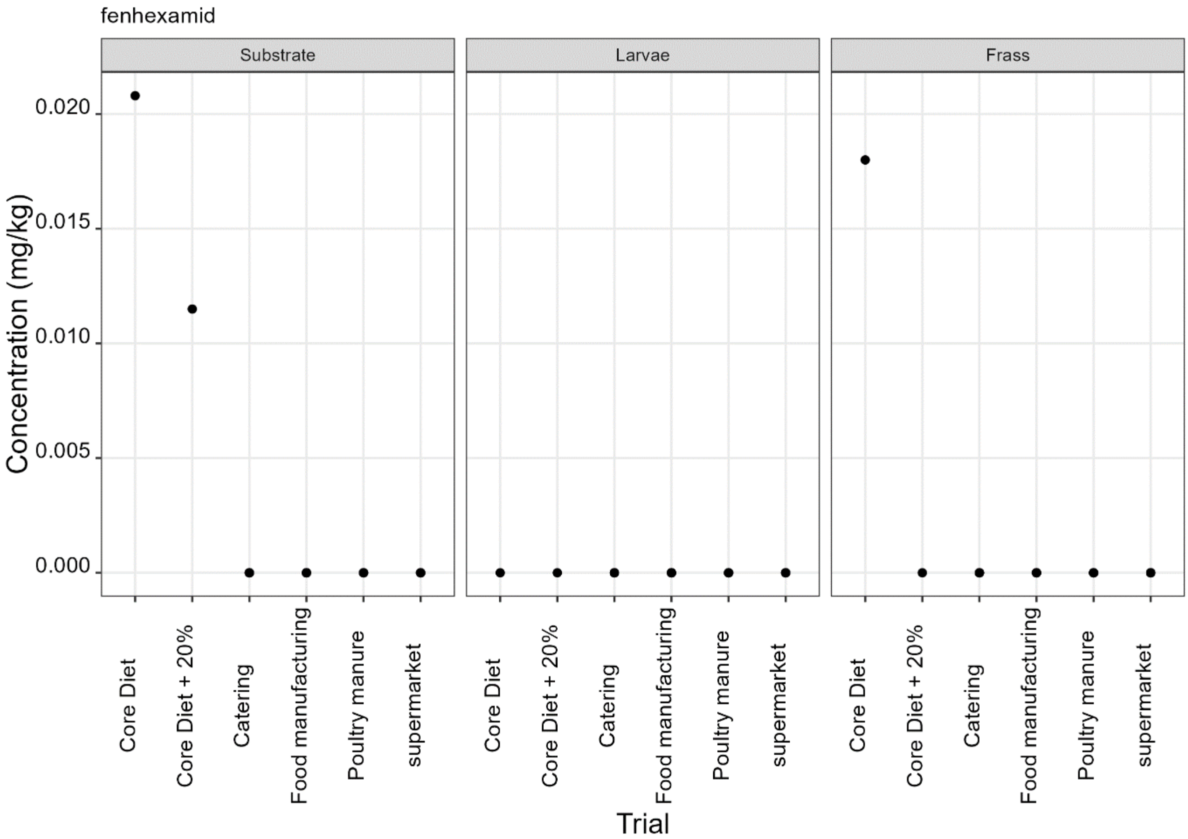

dichlorprop
Substrate
Larvae
Frass
Concentration (mg/kg)
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

9.



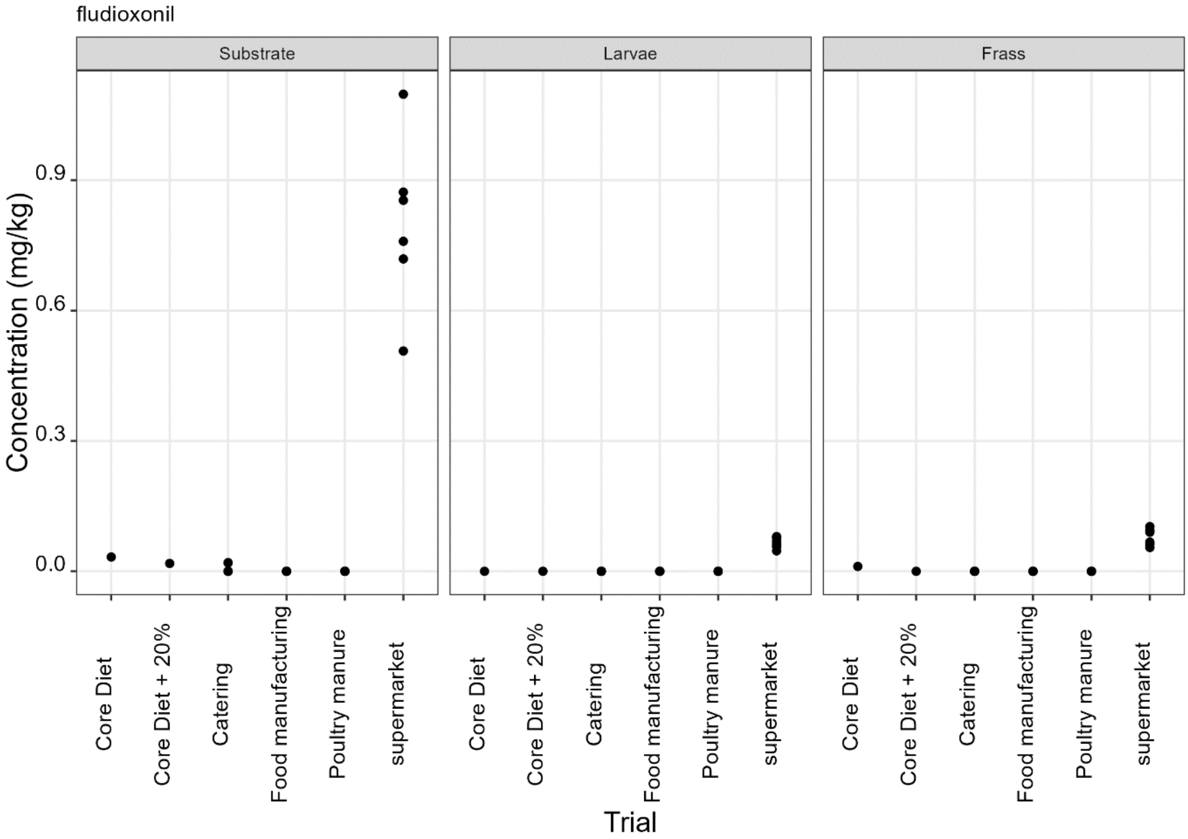
diphenylamine
Substrate
Larvae
Frass
Concentration (mg/kg)
0.08
0.06
0.04
0.02
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

10.

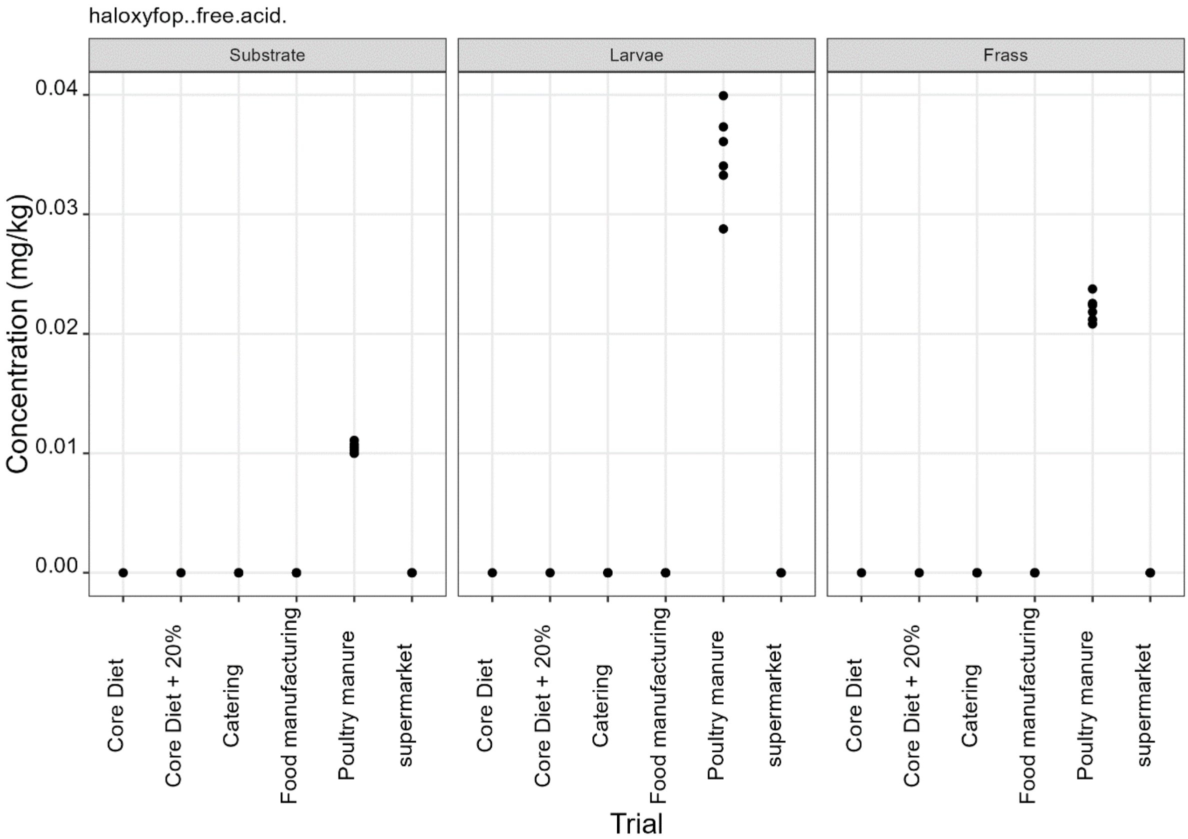


fenhexamid
Substrate
Larvae
Frass
Concentration (mg/kg)
0.020
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

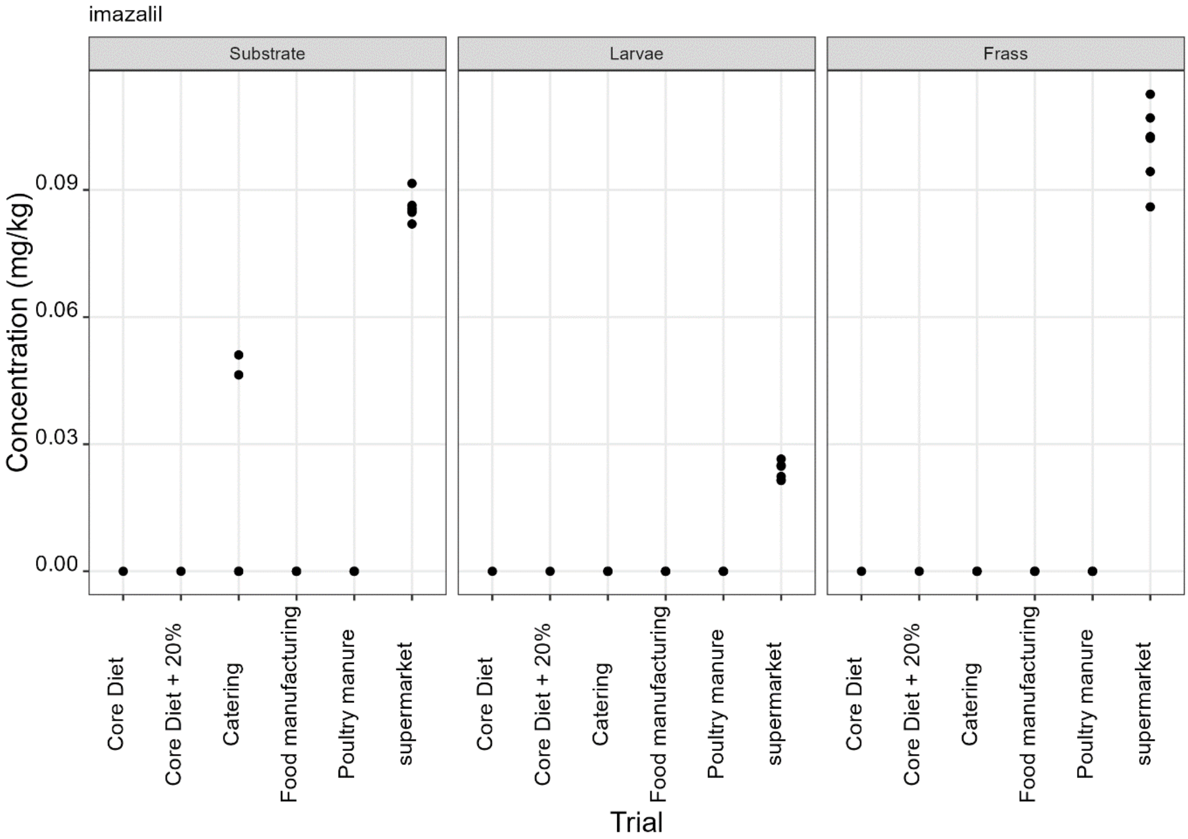
11.


fludioxonil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.9
0.6
0.3
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

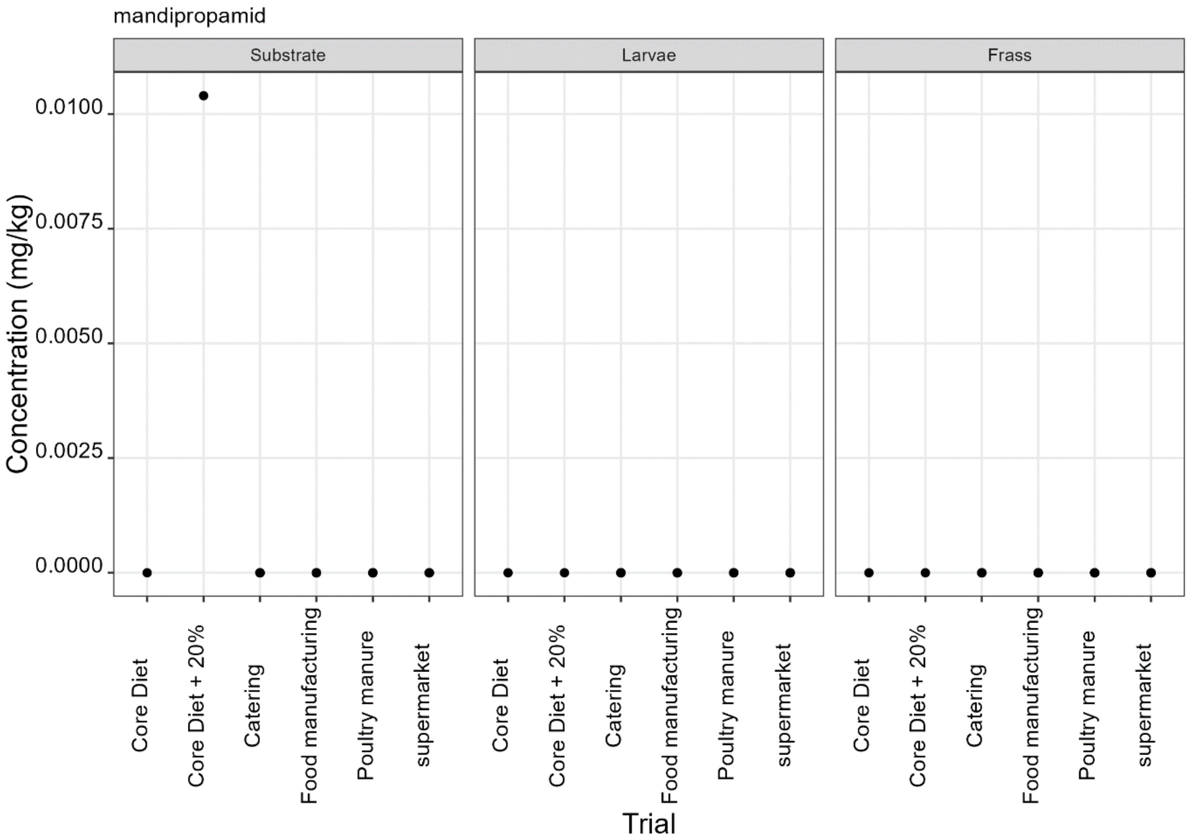
12.


haloxyfop..free.acid.
Substrate
Larvae
Frass
Concentration (mg/kg)
0.04
0.03
0.02
0.01
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

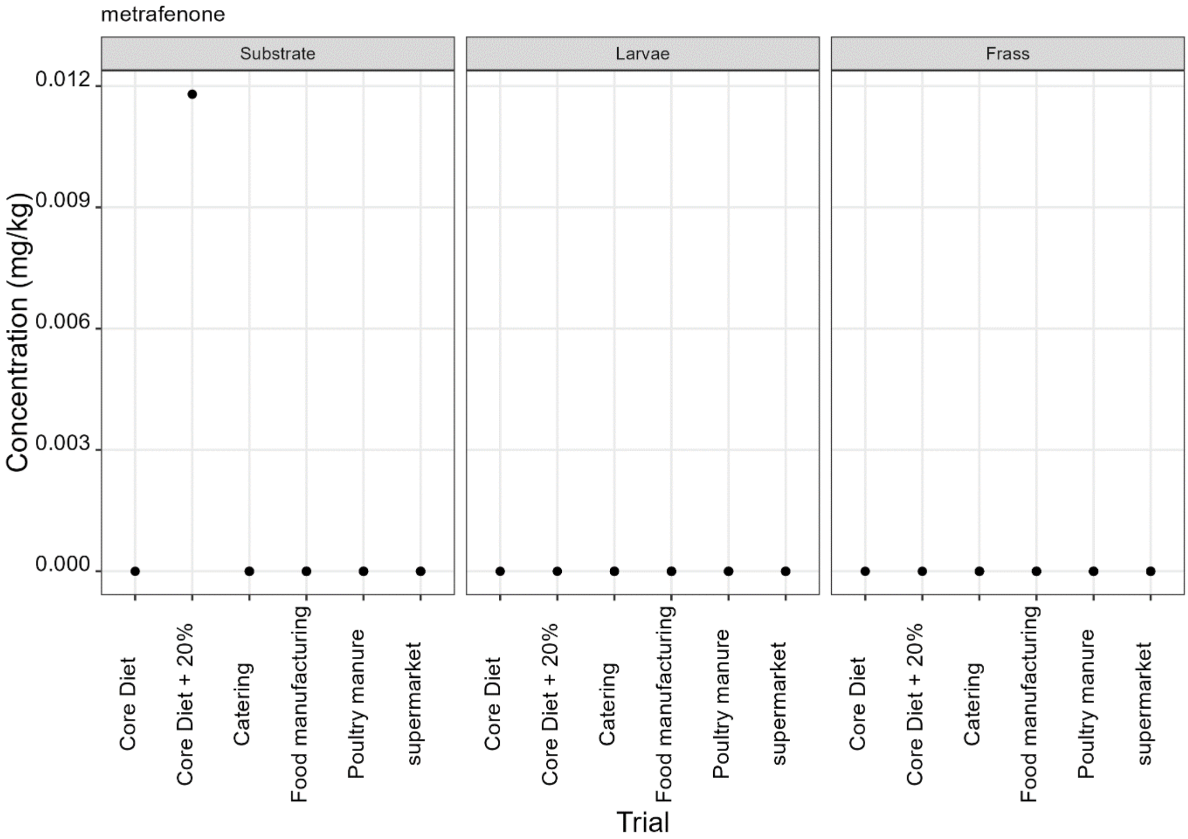
13.


imazalil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.09
0.06
0.03
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

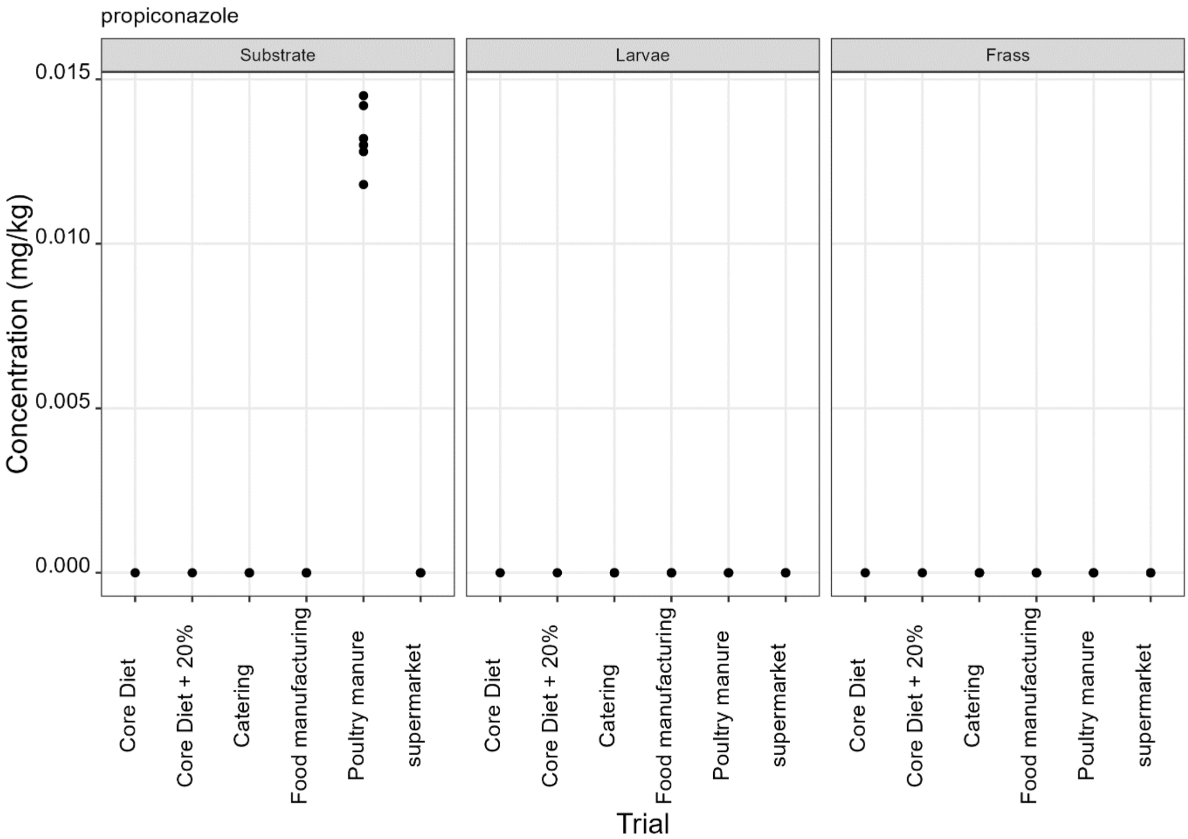
14.


mandipropamid
Substrate
Larvae
Frass
Concentration (mg/kg)
0.0100
0.0075
0.0050
0.0025
0.0000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

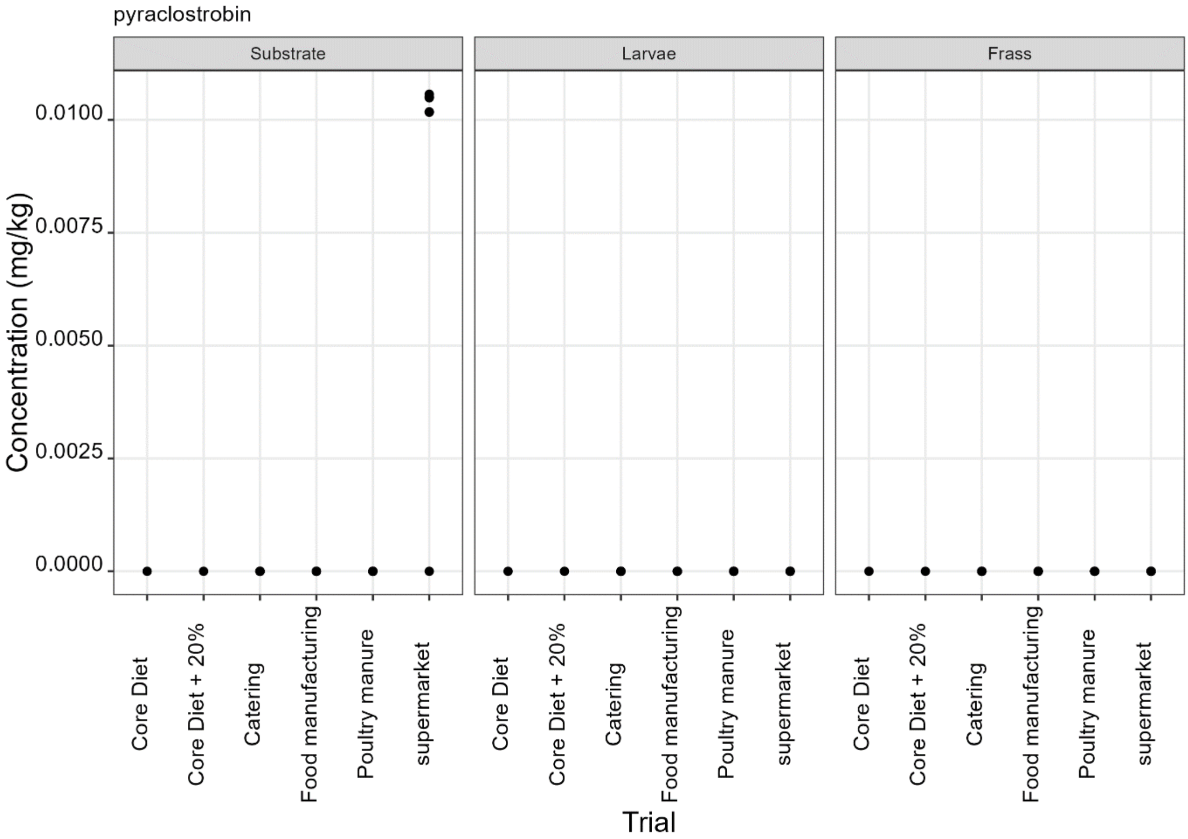
15.


metrafenone
Substrate
Larvae
Frass
Concentration (mg/kg)
0.012
0.009
0.006
0.003
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

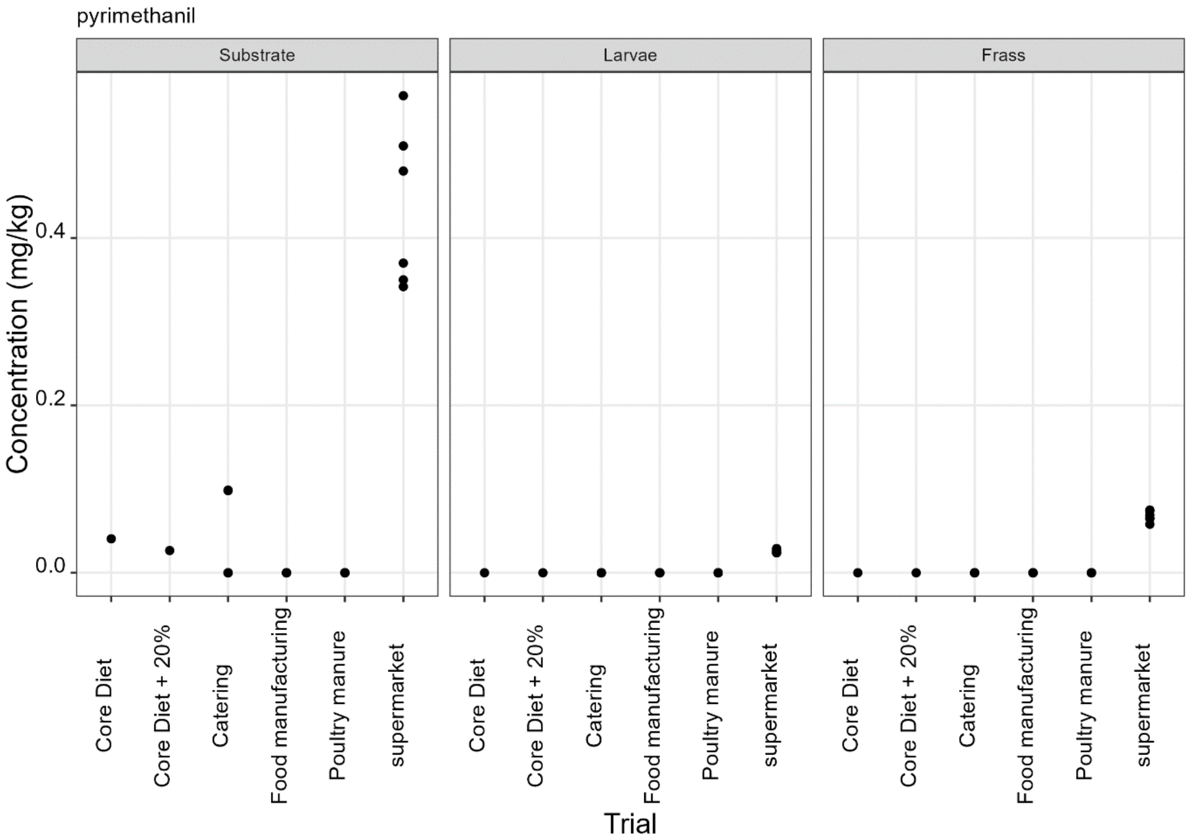
16.


propiconazole
Substrate
Larvae
Frass
Concentration (mg/kg)
0.015
0.010
0.005
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

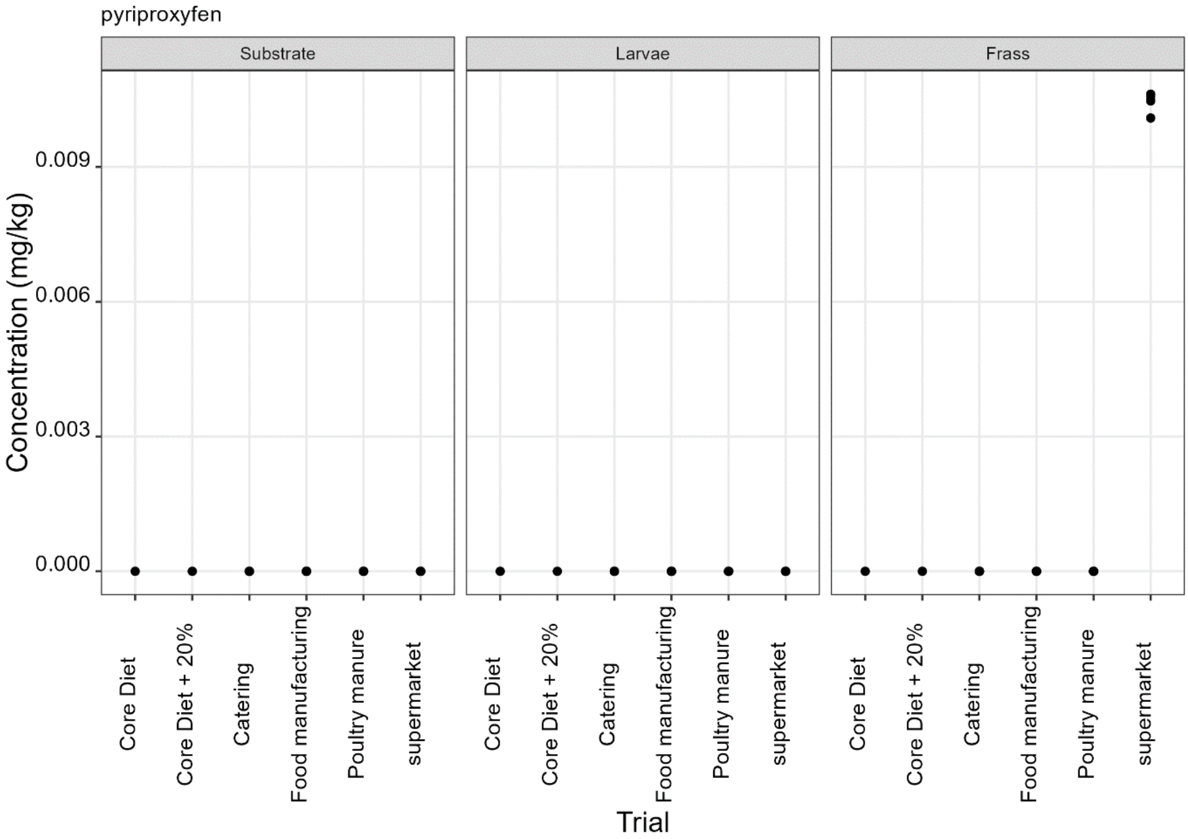
17.


pyraclostrobin
Substrate
Larvae
Frass
Concentration (mg/kg)
0.0100
0.0075
0.0050
0.0025
0.0000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

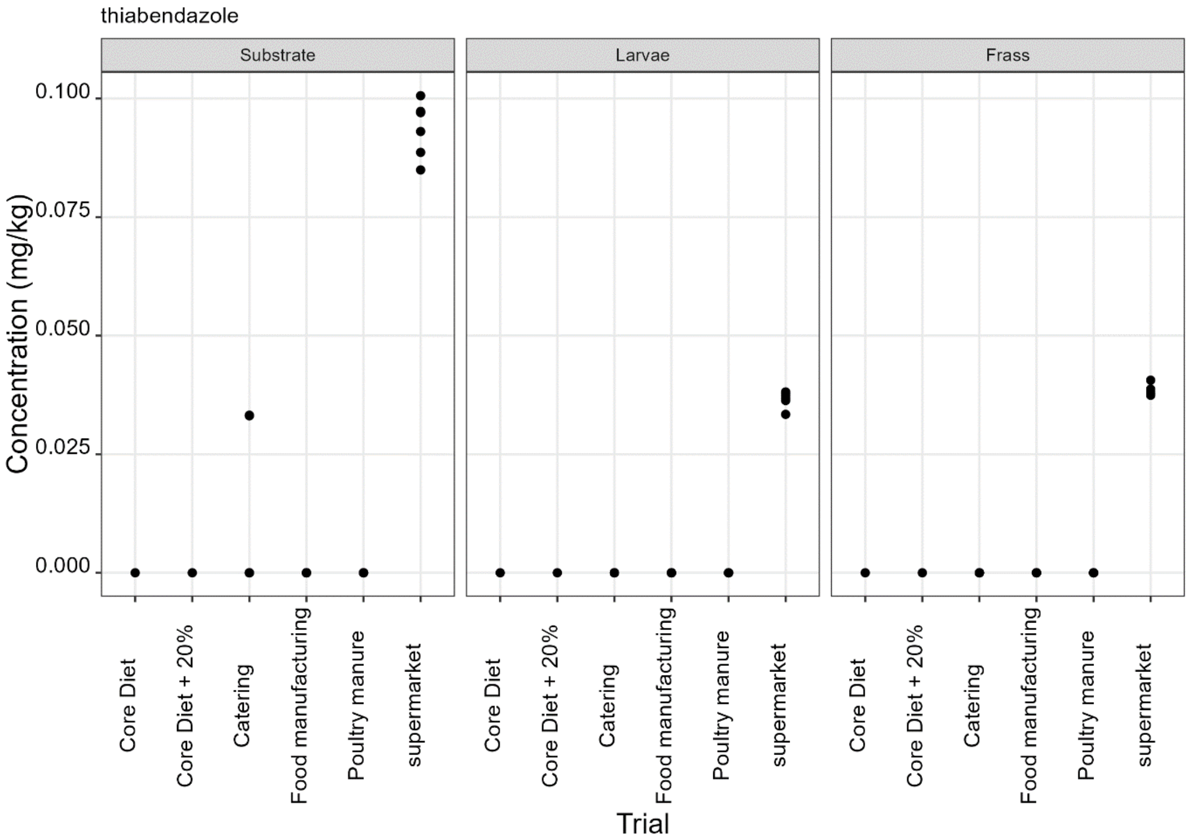
18.


pyrimethanil
Substrate
Larvae
Frass
Concentration (mg/kg)
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

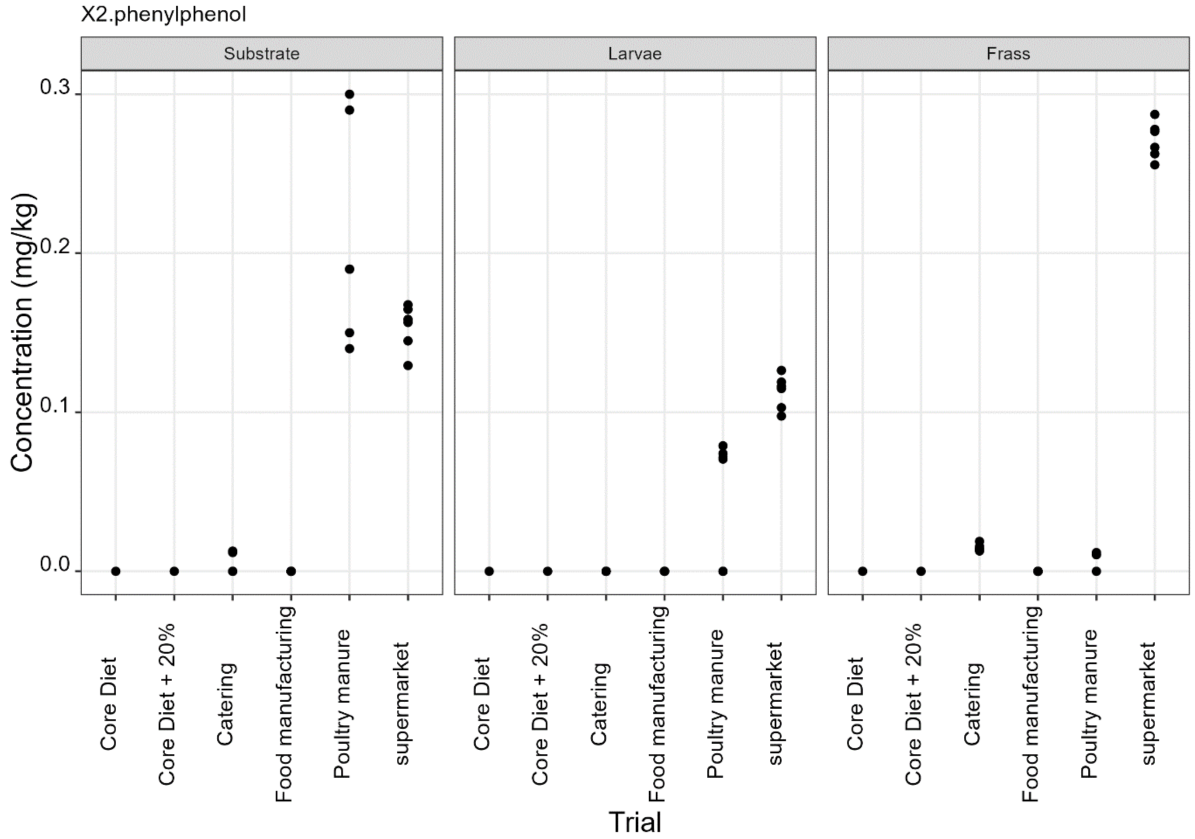
19.


pyriproxyfen
Substrate
Larvae
Frass
0.009
0.006
0.003
0.000
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

20.


thiabendazole
Substrate
Larvae
Frass
0.100
0.075
0.050
0.025
0.000
Concentration (mg/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

21.


X2.phenylphenol
Substrate
Larvae
Frass
Concentration (mg/kg)
0.3
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

22.

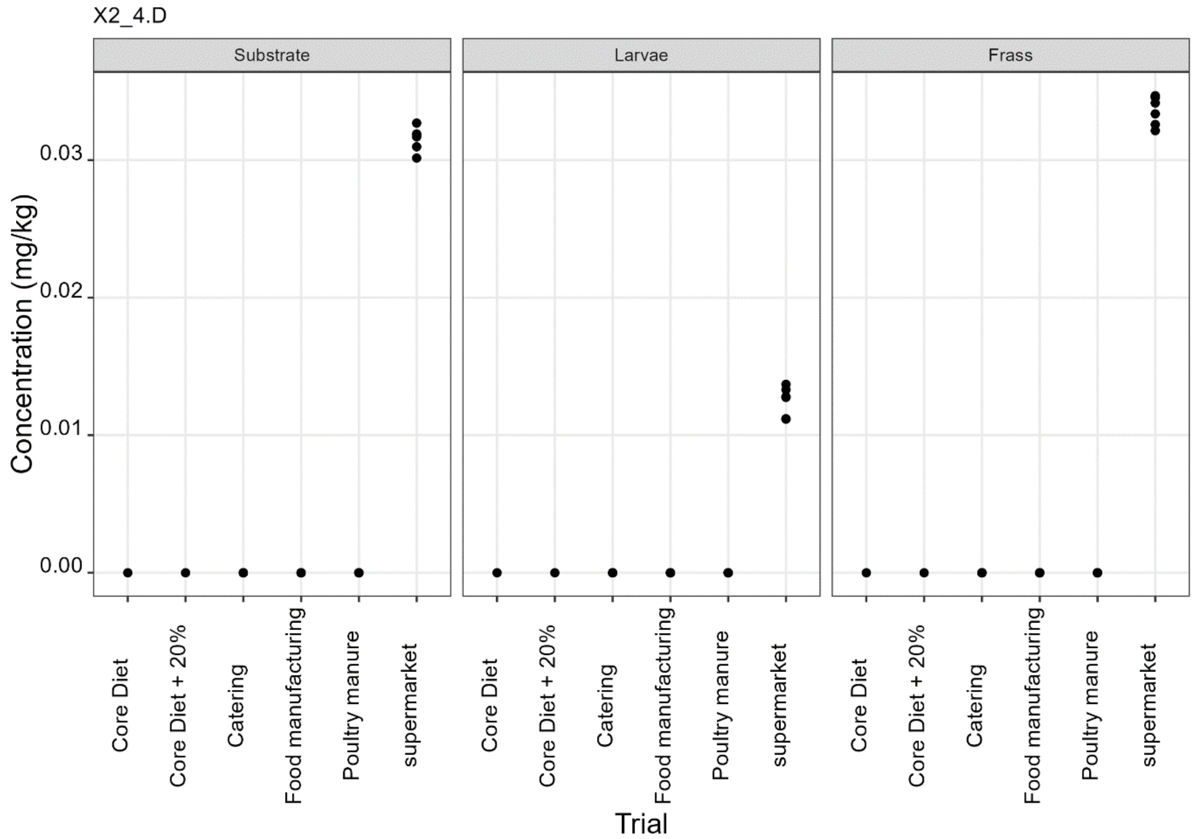

X2_4.D
Substrate
Larvae
Frass
Concentration (mg/kg)
0.03
0.02
0.01
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

Figure III (1 – 22). Concentration (mg/kg) of pesticides analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table VI. Calculated quantities of pesticides.

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	2,4.D	1	0	0	0	NA	NA	NA
core diet	Larvae	2,4.D	1	0	0	0	NA	NA	NA
core diet	Frass	2,4.D	1	0	0	0	NA	NA	NA
core diet	Substrate	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet	Larvae	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet	Frass	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet	Substrate	azoxystrobin	1	0	0	0	NA	NA	NA
core diet	Larvae	azoxystrobin	1	0	0	0	NA	NA	NA
core diet	Frass	azoxystrobin	1	0	0	0	NA	NA	NA
core diet	Substrate	BAC12	1	0	0	0	NA	NA	NA
core diet	Larvae	BAC12	1	0	0	0	NA	NA	NA
core diet	Frass	BAC12	1	0	0	0	NA	NA	NA
core diet	Substrate	BAC14	1	0	0	0	NA	NA	NA
core diet	Larvae	BAC14	1	0	0	0	NA	NA	NA
core diet	Frass	BAC14	1	0	0	0	NA	NA	NA
core diet	Substrate	BAC16	1	0	0	0	NA	NA	NA
core diet	Larvae	BAC16	1	0	0	0	NA	NA	NA
core diet	Frass	BAC16	1	0	0	0	NA	NA	NA
core diet	Substrate	boscalid	1	1	0.0165	0.0165	NA	NA	NA
core diet	Larvae	boscalid	1	0	0	0	0	NA	NA
core diet	Frass	boscalid	1	0	0	0	0	NA	NA
core diet	Substrate	cyprodinil	1	1	0.0191	0.0191	NA	NA	NA
core diet	Larvae	cyprodinil	1	0	0	0	0	NA	NA
core diet	Frass	cyprodinil	1	0	0	0	0	NA	NA
core diet	Substrate	DDAC	1	0	0	0	NA	NA	NA
core diet	Larvae	DDAC	1	1	0.0927	0.0927	Inf	NA	NA
core diet	Frass	DDAC	1	0	0	0	NA	NA	NA
core diet	Substrate	dichlorprop	1	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Larvae	dichlorprop	1	0	0	0	NA	NA	NA
core diet	Frass	dichlorprop	1	0	0	0	NA	NA	NA
core diet	Substrate	diphenylamine	1	0	0	0	NA	NA	NA
core diet	Larvae	diphenylamine	1	0	0	0	NA	NA	NA
core diet	Frass	diphenylamine	1	0	0	0	NA	NA	NA
core diet	Substrate	fenhexamid	1	1	0.0208	0.0208	NA	NA	NA
core diet	Larvae	fenhexamid	1	0	0	0	0	NA	NA
core diet	Frass	fenhexamid	1	1	0.018	0.018	0.8654	NA	NA
core diet	Substrate	fludioxonil	1	1	0.0329	0.0329	NA	NA	NA
core diet	Larvae	fludioxonil	1	0	0	0	0	NA	NA
core diet	Frass	fludioxonil	1	1	0.0112	0.0112	0.3404	NA	NA
core diet	Substrate	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA
core diet	Larvae	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA
core diet	Frass	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA
core diet	Substrate	imazalil	1	0	0	0	NA	NA	NA
core diet	Larvae	imazalil	1	0	0	0	NA	NA	NA
core diet	Frass	imazalil	1	0	0	0	NA	NA	NA
core diet	Substrate	mandipropamid	1	0	0	0	NA	NA	NA
core diet	Larvae	mandipropamid	1	0	0	0	NA	NA	NA
core diet	Frass	mandipropamid	1	0	0	0	NA	NA	NA
core diet	Substrate	metrafenone	1	0	0	0	NA	NA	NA
core diet	Larvae	metrafenone	1	0	0	0	NA	NA	NA
core diet	Frass	metrafenone	1	0	0	0	NA	NA	NA
core diet	Substrate	propiconazole	1	0	0	0	NA	NA	NA
core diet	Larvae	propiconazole	1	0	0	0	NA	NA	NA
core diet	Frass	propiconazole	1	0	0	0	NA	NA	NA
core diet	Substrate	pyraclostrobin	1	0	0	0	NA	NA	NA
core diet	Larvae	pyraclostrobin	1	0	0	0	NA	NA	NA
core diet	Frass	pyraclostrobin	1	0	0	0	NA	NA	NA
core diet	Substrate	pyrimethanil	1	1	0.0406	0.0406	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Larvae	pyrimethanil	1	0	0	0	0	NA	NA
core diet	Frass	pyrimethanil	1	0	0	0	0	NA	NA
core diet	Substrate	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet	Larvae	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet	Frass	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet	Substrate	thiabendazole	1	0	0	0	NA	NA	NA
core diet	Larvae	thiabendazole	1	0	0	0	NA	NA	NA
core diet	Frass	thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	2,4.D	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	2,4.D	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	2,4.D	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	2.phenylphenol	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	azoxystrobin	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	azoxystrobin	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	azoxystrobin	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	BAC12	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	BAC12	1	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	BAC12	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	BAC14	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	BAC14	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	BAC14	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	BAC16	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	BAC16	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	BAC16	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	boscalid	1	1	0.0144	0.0144	NA	NA	NA
core diet + 20%	Larvae	boscalid	1	0	0	0	0	NA	NA
core diet + 20%	Frass	boscalid	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	cyprodinil	1	1	0.0126	0.0126	NA	NA	NA
core diet + 20%	Larvae	cyprodinil	1	0	0	0	0	NA	NA
core diet + 20%	Frass	cyprodinil	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	DDAC	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	DDAC	1	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	DDAC	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	dichlorprop	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	dichlorprop	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	dichlorprop	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	diphenylamine	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	diphenylamine	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	diphenylamine	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	fenhexamid	1	1	0.0115	0.0115	NA	NA	NA
core diet + 20%	Larvae	fenhexamid	1	0	0	0	0	NA	NA
core diet + 20%	Frass	fenhexamid	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	fludioxonil	1	1	0.0181	0.0181	NA	NA	NA
core diet + 20%	Larvae	fludioxonil	1	0	0	0	0	NA	NA
core diet + 20%	Frass	fludioxonil	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	haloxyfop..free.acid.	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	imazalil	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	imazalil	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	imazalil	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	mandipropamid	1	1	0.0104	0.0104	NA	NA	NA
core diet + 20%	Larvae	mandipropamid	1	0	0	0	0	NA	NA
core diet + 20%	Frass	mandipropamid	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	metrafenone	1	1	0.0118	0.0118	NA	NA	NA
core diet + 20%	Larvae	metrafenone	1	0	0	0	0	NA	NA
core diet + 20%	Frass	metrafenone	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	propiconazole	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	propiconazole	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	propiconazole	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	pyraclostrobin	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	pyraclostrobin	1	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Frass	pyraclostrobin	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	pyrimethanil	1	1	0.0266	0.0266	NA	NA	NA
core diet + 20%	Larvae	pyrimethanil	1	0	0	0	0	NA	NA
core diet + 20%	Frass	pyrimethanil	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	pyriproxyfen	1	0	0	0	NA	NA	NA
core diet + 20%	Substrate	thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Larvae	thiabendazole	1	0	0	0	NA	NA	NA
core diet + 20%	Frass	thiabendazole	1	0	0	0	NA	NA	NA
Catering	Substrate	2,4.D	6	0	0	0	NA	NA	NA
Catering	Larvae	2,4.D	6	0	0	0	NA	NA	NA
Catering	Frass	2,4.D	6	0	0	0	NA	NA	NA
Catering	Substrate	2.phenylphenol	6	4	0.006112	0.0127	NA	-0.05726	-2
Catering	Larvae	2.phenylphenol	6	0	0	0	0	NA	NA
Catering	Frass	2.phenylphenol	6	6	0.01444	0.0188	2.363	0.2389	-0.1437
Catering	Substrate	azoxystrobin	6	2	0.01112	0.02257	NA	0	2
Catering	Larvae	azoxystrobin	6	0	0	0	0	NA	NA
Catering	Frass	azoxystrobin	6	0	0	0	0	NA	NA
Catering	Substrate	BAC12	6	6	0.15	0.16	NA	0	-0.1333
Catering	Larvae	BAC12	6	0	0	0	0	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	BAC12	6	0	0	0	0	NA	NA
Catering	Substrate	BAC14	6	0	0	0	NA	NA	NA
Catering	Larvae	BAC14	6	0	0	0	NA	NA	NA
Catering	Frass	BAC14	6	0	0	0	NA	NA	NA
Catering	Substrate	BAC16	6	0	0	0	NA	NA	NA
Catering	Larvae	BAC16	6	0	0	0	NA	NA	NA
Catering	Frass	BAC16	6	0	0	0	NA	NA	NA
Catering	Substrate	boscalid	6	0	0	0	NA	NA	NA
Catering	Larvae	boscalid	6	0	0	0	NA	NA	NA
Catering	Frass	boscalid	6	0	0	0	NA	NA	NA
Catering	Substrate	cyprodinil	6	0	0	0	NA	NA	NA
Catering	Larvae	cyprodinil	6	0	0	0	NA	NA	NA
Catering	Frass	cyprodinil	6	0	0	0	NA	NA	NA
Catering	Substrate	DDAC	6	2	0.06132	0.12635	NA	0	2
Catering	Larvae	DDAC	6	0	0	0	0	NA	NA
Catering	Frass	DDAC	6	0	0	0	0	NA	NA
Catering	Substrate	dichlorprop	6	0	0	0	NA	NA	NA
Catering	Larvae	dichlorprop	6	0	0	0	NA	NA	NA
Catering	Frass	dichlorprop	6	0	0	0	NA	NA	NA
Catering	Substrate	diphenylamine	6	0	0	0	NA	NA	NA
Catering	Larvae	diphenylamine	6	0	0	0	NA	NA	NA
Catering	Frass	diphenylamine	6	0	0	0	NA	NA	NA
Catering	Substrate	fenhexamid	6	0	0	0	NA	NA	NA
Catering	Larvae	fenhexamid	6	0	0	0	NA	NA	NA
Catering	Frass	fenhexamid	6	0	0	0	NA	NA	NA
Catering	Substrate	fludioxonil	6	2	0.009833	0.0199	NA	0	2
Catering	Larvae	fludioxonil	6	0	0	0	0	NA	NA
Catering	Frass	fludioxonil	6	0	0	0	0	NA	NA
Catering	Substrate	Haloxyfop free acid	6	0	0	0	NA	NA	NA
Catering	Larvae	Haloxyfop free acid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Haloxyfop free acid	6	0	0	0	NA	NA	NA
Catering	Substrate	imazalil	6	2	0.02436	0.05106	NA	0	2
Catering	Larvae	imazalil	6	0	0	0	0	NA	NA
Catering	Frass	imazalil	6	0	0	0	0	NA	NA
Catering	Substrate	mandipropamid	6	0	0	0	NA	NA	NA
Catering	Larvae	mandipropamid	6	0	0	0	NA	NA	NA
Catering	Frass	mandipropamid	6	0	0	0	NA	NA	NA
Catering	Substrate	metrafenone	6	0	0	0	NA	NA	NA
Catering	Larvae	metrafenone	6	0	0	0	NA	NA	NA
Catering	Frass	metrafenone	6	0	0	0	NA	NA	NA
Catering	Substrate	propiconazole	6	0	0	0	NA	NA	NA
Catering	Larvae	propiconazole	6	0	0	0	NA	NA	NA
Catering	Frass	propiconazole	6	0	0	0	NA	NA	NA
Catering	Substrate	pyraclostrobin	6	0	0	0	NA	NA	NA
Catering	Larvae	pyraclostrobin	6	0	0	0	NA	NA	NA
Catering	Frass	pyraclostrobin	6	0	0	0	NA	NA	NA
Catering	Substrate	pyrimethanil	6	2	0.04922	0.09875	NA	0	2
Catering	Larvae	pyrimethanil	6	0	0	0	0	NA	NA
Catering	Frass	pyrimethanil	6	0	0	0	0	NA	NA
Catering	Substrate	pyriproxyfen	6	0	0	0	NA	NA	NA
Catering	Larvae	pyriproxyfen	6	0	0	0	NA	NA	NA
Catering	Frass	pyriproxyfen	6	0	0	0	NA	NA	NA
Catering	Substrate	thiabendazole	6	2	0.01659	0.03323	NA	0	1.999
Catering	Larvae	thiabendazole	6	0	0	0	0	NA	NA
Catering	Frass	thiabendazole	6	0	0	0	0	NA	NA
Manufacturing	Substrate	2,4.D	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	2,4.D	6	0	0	0	NA	NA	NA
Manufacturing	Frass	2,4.D	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	2.phenylphenol	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	2.phenylphenol	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	2.phenylphenol	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	azoxystrobin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	azoxystrobin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	azoxystrobin	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	BAC12	6	4	0.02899	0.0615	NA	-0.1845	-2
Manufacturing	Larvae	BAC12	6	0	0	0	0	NA	NA
Manufacturing	Frass	BAC12	6	0	0	0	0	NA	NA
Manufacturing	Substrate	BAC14	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	BAC14	6	0	0	0	NA	NA	NA
Manufacturing	Frass	BAC14	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	BAC16	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	BAC16	6	0	0	0	NA	NA	NA
Manufacturing	Frass	BAC16	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	boscalid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	boscalid	6	0	0	0	NA	NA	NA
Manufacturing	Frass	boscalid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	cyprodinil	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	cyprodinil	6	0	0	0	NA	NA	NA
Manufacturing	Frass	cyprodinil	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	DDAC	6	4	0.09175	0.19395	NA	-0.2027	-2
Manufacturing	Larvae	DDAC	6	0	0	0	0	NA	NA
Manufacturing	Frass	DDAC	6	0	0	0	0	NA	NA
Manufacturing	Substrate	dichlorprop	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	dichlorprop	6	0	0	0	NA	NA	NA
Manufacturing	Frass	dichlorprop	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	diphenylamine	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	diphenylamine	6	0	0	0	NA	NA	NA
Manufacturing	Frass	diphenylamine	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	fenhexamid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	fenhexamid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	fenhexamid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	fludioxonil	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	fludioxonil	6	0	0	0	NA	NA	NA
Manufacturing	Frass	fludioxonil	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	imazalil	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	imazalil	6	0	0	0	NA	NA	NA
Manufacturing	Frass	imazalil	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	mandipropamid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	mandipropamid	6	0	0	0	NA	NA	NA
Manufacturing	Frass	mandipropamid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	metrafenone	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	metrafenone	6	0	0	0	NA	NA	NA
Manufacturing	Frass	metrafenone	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	propiconazole	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	propiconazole	6	0	0	0	NA	NA	NA
Manufacturing	Frass	propiconazole	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	pyraclostrobin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	pyraclostrobin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	pyraclostrobin	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	pyrimethanil	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	pyrimethanil	6	0	0	0	NA	NA	NA
Manufacturing	Frass	pyrimethanil	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	pyriproxyfen	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	pyriproxyfen	6	0	0	0	NA	NA	NA
Manufacturing	Frass	pyriproxyfen	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	thiabendazole	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	thiabendazole	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	thiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	2,4.D	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	2,4.D	6	0	0	0	NA	NA	NA
Poultry manure	Frass	2,4.D	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	2.phenylphenol	6	6	0.2312	0.3	NA	-0.1946	0.5515
Poultry manure	Larvae	2.phenylphenol	6	4	0.03687	0.0789	0.1595	-0.1469	-2
Poultry manure	Frass	2.phenylphenol	6	5	0.009641	0.01178	0.0417	0.543	0.3483
Poultry manure	Substrate	azoxystrobin	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	azoxystrobin	6	0	0	0	NA	NA	NA
Poultry manure	Frass	azoxystrobin	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	BAC12	6	6	1.767	1.885	NA	-0.1234	0.002547
Poultry manure	Larvae	BAC12	6	6	0.6598	0.7055	0.3734	-0.1159	0.09397
Poultry manure	Frass	BAC12	6	6	0.5628	0.657	0.3185	-0.01066	-0.2229
Poultry manure	Substrate	BAC14	6	6	0.2294	0.24415	NA	-0.1562	0.04517
Poultry manure	Larvae	BAC14	6	6	0.1767	0.1825	0.7703	0.01217	0.04188
Poultry manure	Frass	BAC14	6	6	0.1765	0.2002	0.7694	0.0653	-0.1686

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	BAC16	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	BAC16	6	0	0	0	NA	NA	NA
Poultry manure	Frass	BAC16	6	4	0.02658	0.0556	Inf	0.02107	-2
Poultry manure	Substrate	boscalid	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	boscalid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	boscalid	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	cyprodinil	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	cyprodinil	6	0	0	0	NA	NA	NA
Poultry manure	Frass	cyprodinil	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	DDAC	6	6	0.175	0.1841	NA	-0.1049	0.05186
Poultry manure	Larvae	DDAC	6	6	0.913	0.9897	5.217	0.08078	0.1525
Poultry manure	Frass	DDAC	6	6	0.2304	0.29225	1.317	0.3136	-0.179
Poultry manure	Substrate	dichlorprop	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	dichlorprop	6	0	0	0	NA	NA	NA
Poultry manure	Frass	dichlorprop	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	diphenylamine	6	6	0.0659	0.07678	NA	-0.4554	0.1378
Poultry manure	Larvae	diphenylamine	6	2	0.005558	0.01162	0.08434	0	2
Poultry manure	Frass	diphenylamine	6	6	0.01301	0.01469	0.1974	0.02921	-0.1564
Poultry manure	Substrate	fenhexamid	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	fenhexamid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	fenhexamid	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	fludioxonil	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	fludioxonil	6	0	0	0	NA	NA	NA
Poultry manure	Frass	fludioxonil	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Haloxyfop free.acid	6	6	0.01036	0.011086	NA	0.06443	-0.04351
Poultry manure	Larvae	Haloxyfop free.acid	6	6	0.03533	0.03993	3.41	-0.09196	0.07188
Poultry manure	Frass	Haloxyfop free.acid	6	6	0.02214	0.023754	2.137	0.04356	0.01358
Poultry manure	Substrate	imazalil	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	imazalil	6	0	0	0	NA	NA	NA
Poultry manure	Frass	imazalil	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	mandipropamid	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	mandipropamid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	mandipropamid	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	metrafenone	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	metrafenone	6	0	0	0	NA	NA	NA
Poultry manure	Frass	metrafenone	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	propiconazole	6	6	0.01335	0.0145	NA	-0.09738	0.04494
Poultry manure	Larvae	propiconazole	6	0	0	0	0	NA	NA
Poultry manure	Frass	propiconazole	6	0	0	0	0	NA	NA
Poultry manure	Substrate	pyraclostrobin	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	pyraclostrobin	6	0	0	0	NA	NA	NA
Poultry manure	Frass	pyraclostrobin	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	pyrimethanil	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	pyrimethanil	6	0	0	0	NA	NA	NA
Poultry manure	Frass	pyrimethanil	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	pyriproxyfen	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	pyriproxyfen	6	0	0	0	NA	NA	NA
Poultry manure	Frass	pyriproxyfen	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	thiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	thiabendazole	6	0	0	0	NA	NA	NA
Poultry manure	Frass	thiabendazole	6	0	0	0	NA	NA	NA
supermarket	Substrate	2,4.D	6	6	0.03162	0.03269	NA	-0.05155	0.01581
supermarket	Larvae	2,4.D	6	6	0.01274	0.0137	0.4029	0.1248	0.1197
supermarket	Frass	2,4.D	6	6	0.03327	0.03468	1.052	0.009769	-0.05463
supermarket	Substrate	2.phenylphenol	6	6	0.1495	0.1676	NA	-0.00334	-0.1649
supermarket	Larvae	2.phenylphenol	6	6	0.1097	0.1263	0.7338	-0.02689	-0.172
supermarket	Frass	2.phenylphenol	6	6	0.2681	0.2873	1.793	0.04103	-0.06733
supermarket	Substrate	azoxystrobin	6	6	0.0617	0.06462	NA	-0.00276	-0.06442
supermarket	Larvae	azoxystrobin	6	0	0	0	0	NA	NA
supermarket	Frass	azoxystrobin	6	6	0.02328	0.0238	0.3773	-0.0116	0.02728
supermarket	Substrate	BAC12	6	0	0	0	NA	NA	NA
supermarket	Larvae	BAC12	6	6	0.1175	0.12	Inf	0	-0.04255
supermarket	Frass	BAC12	6	0	0	0	NA	NA	NA
supermarket	Substrate	BAC14	6	0	0	0	NA	NA	NA
supermarket	Larvae	BAC14	6	6	0.06262	0.064	Inf	0.02395	-0.01996
supermarket	Frass	BAC14	6	0	0	0	NA	NA	NA
supermarket	Substrate	BAC16	6	0	0	0	NA	NA	NA
supermarket	Larvae	BAC16	6	0	0	0	NA	NA	NA
supermarket	Frass	BAC16	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
supermarket	Substrate	boscalid	6	6	0.01548	0.01733	NA	0.1214	-0.0449
supermarket	Larvae	boscalid	6	0	0	0	0	NA	NA
supermarket	Frass	boscalid	6	0	0	0	0	NA	NA
supermarket	Substrate	cyprodinil	6	0	0	0	NA	NA	NA
supermarket	Larvae	cyprodinil	6	0	0	0	NA	NA	NA
supermarket	Frass	cyprodinil	6	0	0	0	NA	NA	NA
supermarket	Substrate	DDAC	6	6	0.2693	0.37	NA	0.02488	0.653
supermarket	Larvae	DDAC	6	6	0.148	0.1569	0.5496	-0.05203	0.1115
supermarket	Frass	DDAC	6	6	0.1528	0.1854	0.5674	0.1859	-0.08508
supermarket	Substrate	dichlorprop	6	6	0.01025	0.01038	NA	0.000976	0.009268
supermarket	Larvae	dichlorprop	6	0	0	0	0	NA	NA
supermarket	Frass	dichlorprop	6	6	0.01227	0.01319	1.197	-0.185	0.04116
supermarket	Substrate	diphenylamine	6	6	0.01541	0.01836	NA	0.03082	0.3791
supermarket	Larvae	diphenylamine	6	2	0.006452	0.01313	0.4187	0	2
supermarket	Frass	diphenylamine	6	0	0	0	0	NA	NA
supermarket	Substrate	fenhexamid	6	0	0	0	NA	NA	NA
supermarket	Larvae	fenhexamid	6	0	0	0	NA	NA	NA
supermarket	Frass	fenhexamid	6	0	0	0	NA	NA	NA
supermarket	Substrate	fludioxonil	6	6	0.8018	1.098	NA	0.1548	0.001621
supermarket	Larvae	fludioxonil	6	6	0.06393	0.07985	0.07973	-0.3157	-0.1362
supermarket	Frass	fludioxonil	6	6	0.08024	0.1029	0.1001	-0.4229	0.1205
supermarket	Substrate	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
supermarket	Larvae	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
supermarket	Frass	Haloxyfop free.acid	6	0	0	0	NA	NA	NA
supermarket	Substrate	imazalil	6	6	0.08545	0.09154	NA	-0.04295	-0.03013
supermarket	Larvae	imazalil	6	6	0.02414	0.02649	0.2825	0.08761	0.1329
supermarket	Frass	imazalil	6	6	0.09814	0.1126	1.149	-0.0754	-0.1623
supermarket	Substrate	mandipropamid	6	0	0	0	NA	NA	NA
supermarket	Larvae	mandipropamid	6	0	0	0	NA	NA	NA
supermarket	Frass	mandipropamid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Pesticide	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
supermarket	Substrate	metrafenone	6	0	0	0	NA	NA	NA
supermarket	Larvae	metrafenone	6	0	0	0	NA	NA	NA
supermarket	Frass	metrafenone	6	0	0	0	NA	NA	NA
supermarket	Substrate	propiconazole	6	0	0	0	NA	NA	NA
supermarket	Larvae	propiconazole	6	0	0	0	NA	NA	NA
supermarket	Frass	propiconazole	6	0	0	0	NA	NA	NA
supermarket	Substrate	pyraclostrobin	6	5	0.007761	0.01057	NA	0.02641	-0.6897
supermarket	Larvae	pyraclostrobin	6	0	0	0	0	NA	NA
supermarket	Frass	pyraclostrobin	6	0	0	0	0	NA	NA
supermarket	Substrate	pyrimethanil	6	6	0.4167	0.57	NA	0.08399	-0.2917
supermarket	Larvae	pyrimethanil	6	6	0.02588	0.029	0.06211	0.05796	0.08694
supermarket	Frass	pyrimethanil	6	6	0.06937	0.075	0.1665	-0.07928	0.1478
supermarket	Substrate	pyriproxyfen	6	0	0	0	NA	NA	NA
supermarket	Larvae	pyriproxyfen	6	0	0	0	NA	NA	NA
supermarket	Frass	pyriproxyfen	6	6	0.01043	0.01062	Inf	0.004314	-0.02852
supermarket	Substrate	thiabendazole	6	6	0.09396	0.1006	NA	-0.1293	0.02307
supermarket	Larvae	thiabendazole	6	6	0.03677	0.03814	0.3913	0.07207	0.01795
supermarket	Frass	thiabendazole	6	6	0.03835	0.04061	0.4082	-0.02829	-0.01428

Inf = inferred, NA = not applicable

Appendix E - Mycotoxins

The aim of the analysis was to

- estimate the quantity of each mycotoxin in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of bioaccumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

Individual results are shown in the figures and the table shows the calculated quantities.

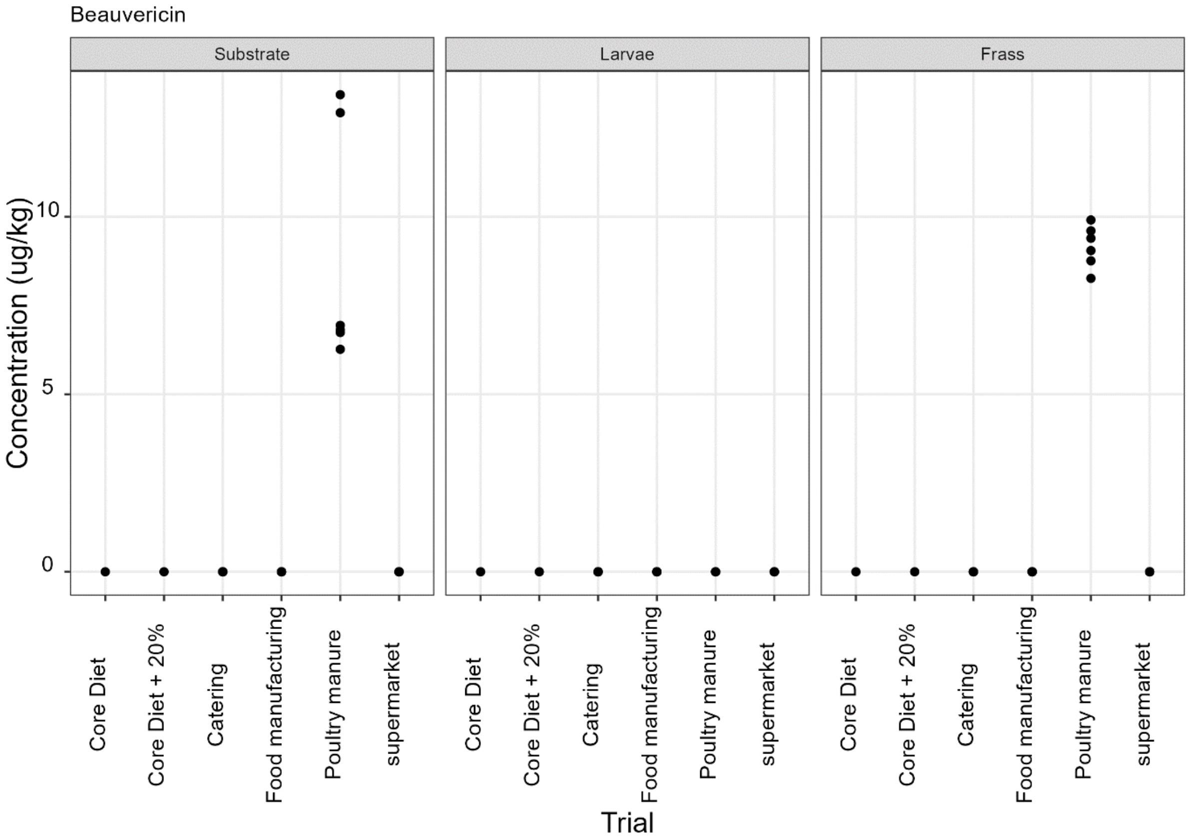
Mycotoxins which were not detected in any extracts from any samples are not reported here. They are: aflatoxin.B1, aflatoxin.B2, aflatoxin.G1, aflatoxin.G2, aflatoxin.M1, aflatoxin.M2, altenuene, citrinin, cytochalasin A, cytochalasin B, cytochalasin C, cytochalasin D, cytochalasin E, cytochalasin H, deepoxy.deoxynivalenol, deoxynivalenol, diacetoxyscirpenol, enniatin A, ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine, fusarenon.X, gliotoxin, HT2 toxin, meleagrin, mycophenolic acid, neosolaniol, nivalenol, ochratoxin.A, penitrem A, phomopsin A, sterigmatocystin, T2 toxin, tentoxin, verruculogen, alternariol, alternariol monomethyl ether, deoxynivalenol 3 glucoside, Emodin, 3-nitropropionic acid, patulin, a.zearalanol, b.zearalanol, zearalanone, a.zearalenol, b.zearalenol, zearalenone, zearalenone-4-sulfate, fumonisin B1, fumonisin B2, fumonisin B3, fusaric acid and tenuazonic acid.

Reporting limits are shown below:

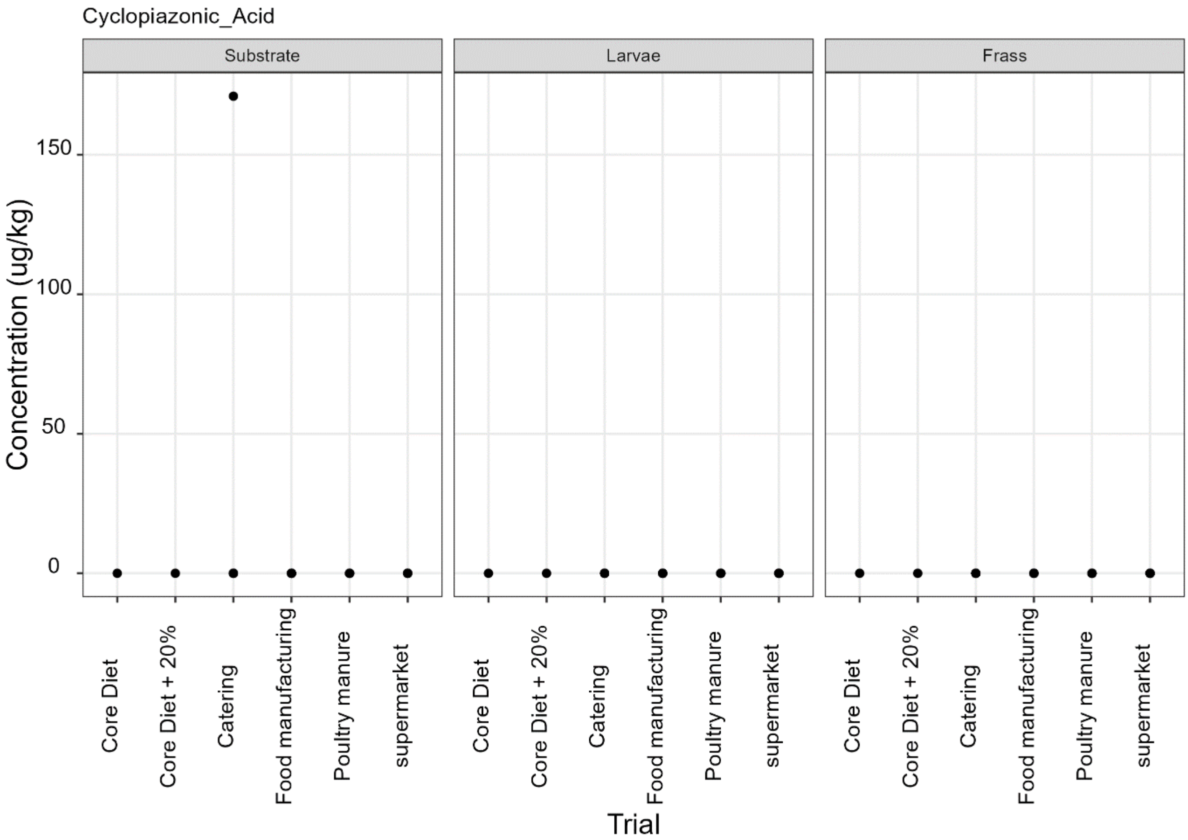
Table VII. Reporting limits for mycotoxins analysis.

Mycotoxin	Reporting limit µg/kg	Mycotoxin	Reporting limit µg/kg
Acetyl-Deoxynivalenol (3- and 15-)	50	Gliotoxin	250
Aflatoxin B1	2.5	HT2 Toxin	50
Aflatoxin B2	2.5	Meleagrin	50
Aflatoxin G1	2.5	Mycophenolic Acid	50
Aflatoxin G2	2.5	Neosolaniol	50
Aflatoxin M1	2.5	Nivalenol	100
Altenuene	50	Ochratoxin A	25
Beauvericin	5	Penicillic Acid	50
Citrinin	50	Penitrem A	50
Cytochalasin A	50	Phomopsin A	50
Cytochalasin B	50	Roquefortine C	5
Cytochalasin C	50	Sterigmatocystin	2.5
Cytochalasin D	50	T2 Toxin	50
Cytochalasin E	50	Tentoxin	50
Cytochalasin H	50	Verruculogen	50
Deepoxy-Deoxynivalenol	50	Alternariol	50
Deoxynivalenol	50	Alternariol_Monomethyl_Ether	50
Diacetoxyscirpenol	50	Deoxynivalenol-3-Glucoside	50
Enniatin A	5	Emodin	50
Enniatin A1	5	Moniliformin	50
Enniatin B	5	3-Nitropropionic_Acid	500
Enniatin B1	5	Patulin	250
Ergocornine	25	a-Zearalanol	25
Ergocorninine	5	b-Zearalanol	25
Ergocristine	25	Zearalanone	25
Ergocristinine	5	a-Zearalenol	25
Ergocryptine	25	b-Zearalenol	25
Ergocryptinine	5	Zearalenone	25
Ergometrine	25	Cyclopiazonic_Acid	50
Ergometrinine	5	Fumonisin_B1	50
Ergosine	25	Fumonisin_B2	50
Ergosinine	5	Fumonisin_B3	50
Ergotamine	25	Fusaric_Acid	50
Ergotaminine	5	Tenuazonic_Acid	50
Fusarenon X	50		

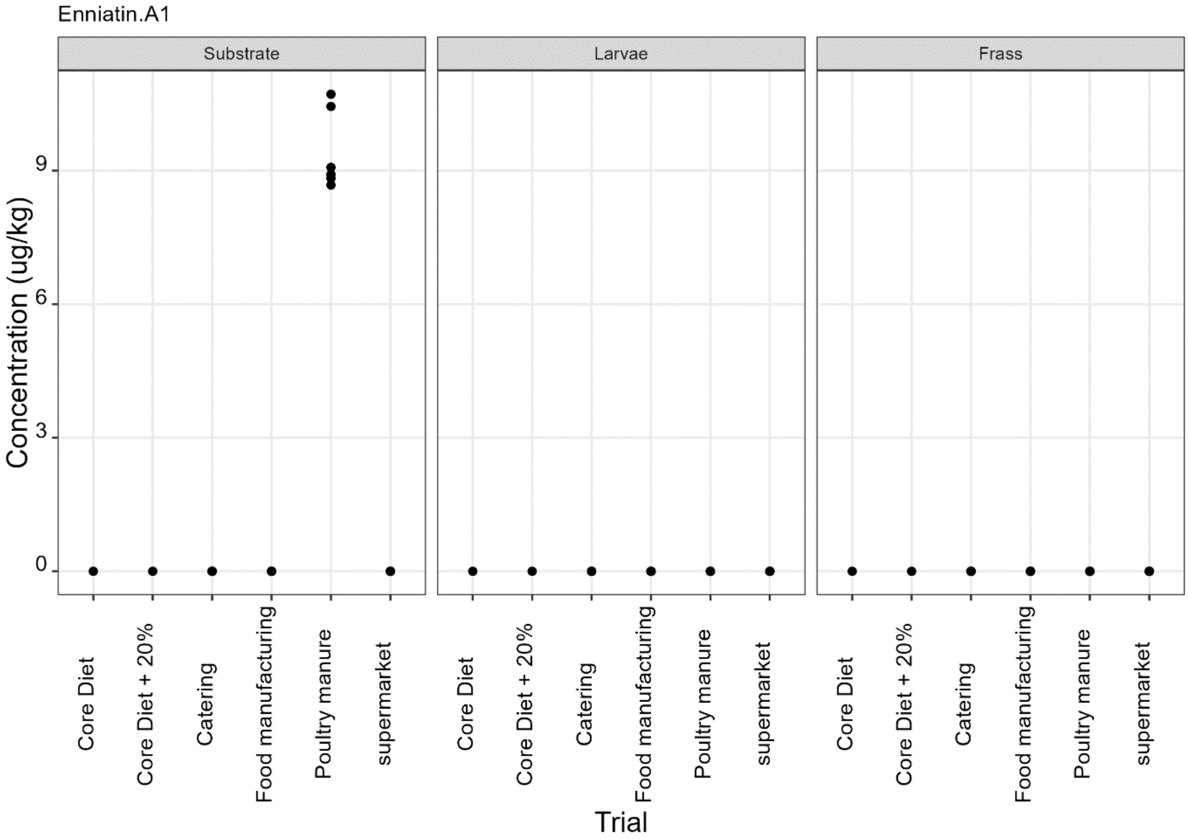
1.


Beauvericin
Substrate
Larvae
Frass
Concentration (ug/kg)
0
5
10
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

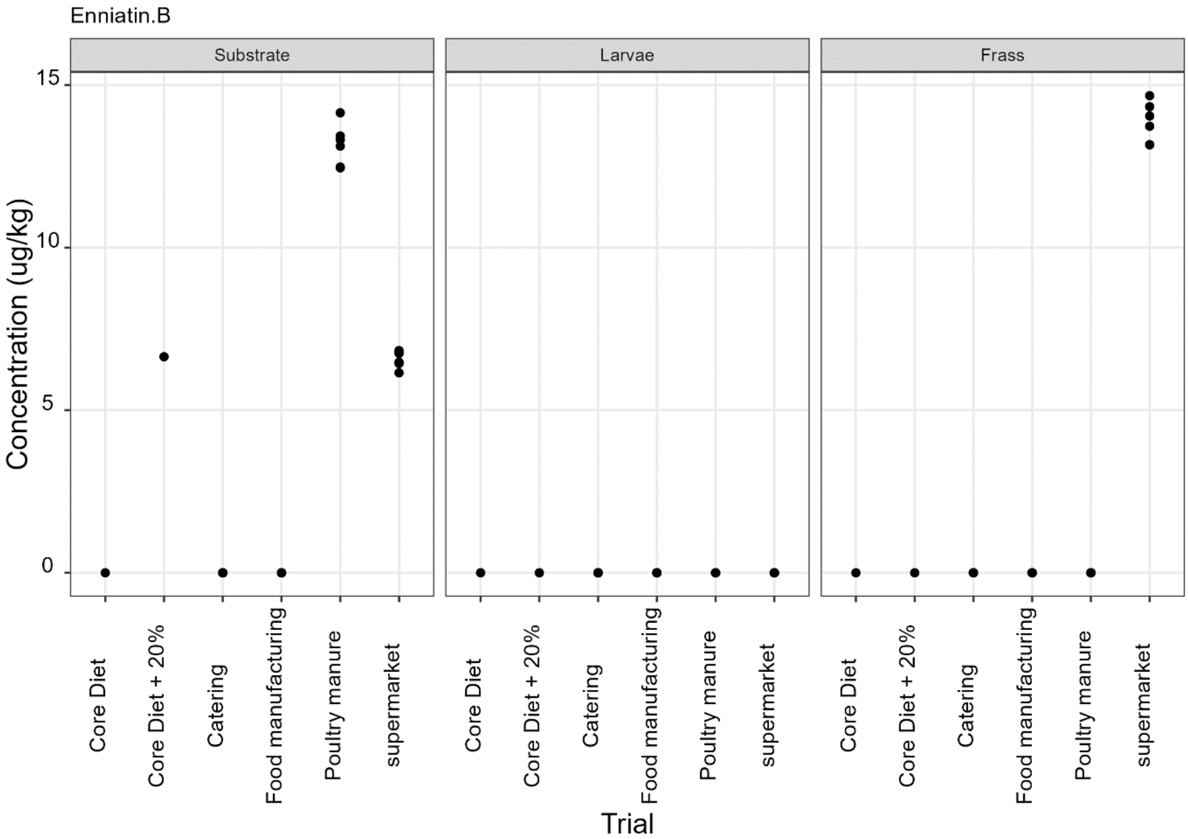
2.


Cyclopiazonic_Acid
Substrate
Larvae
Frass
Concentration (ug/kg)
0
50
100
150
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

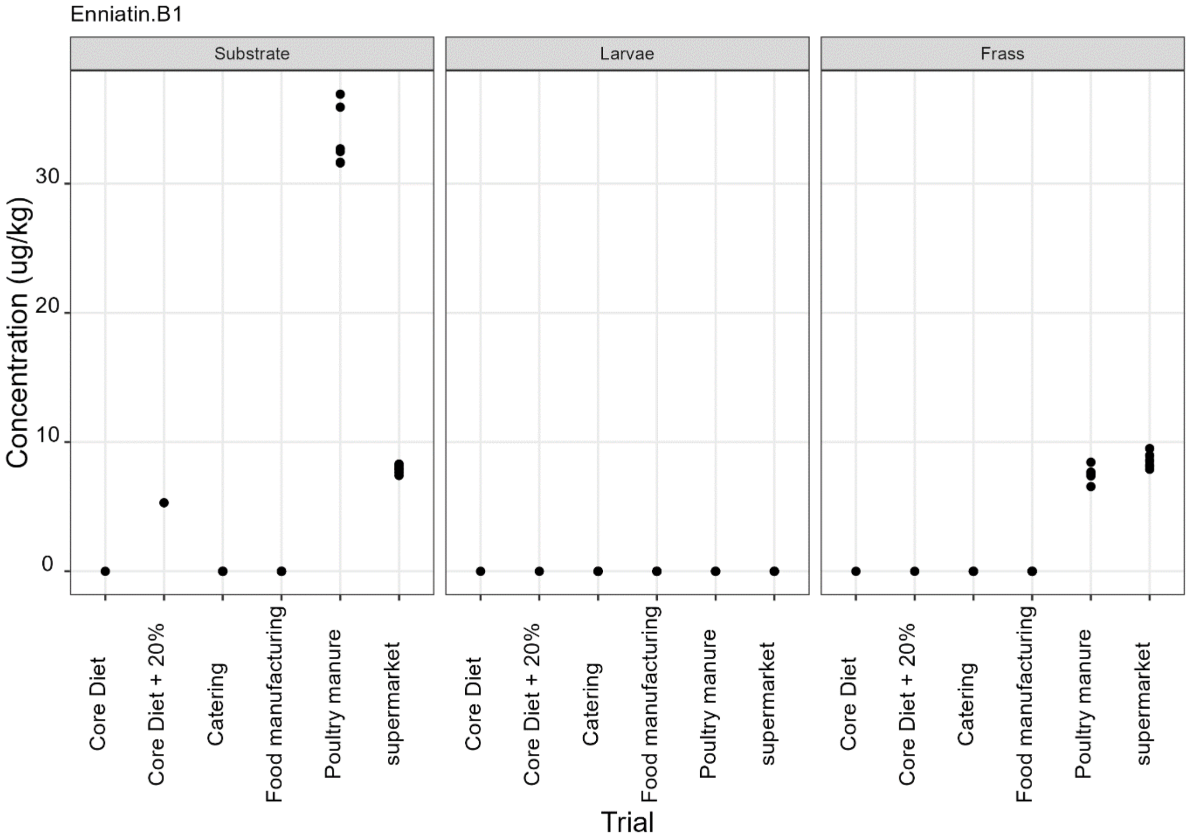
3.


Enniatin.A1
Substrate
Larvae
Frass
Concentration (ug/kg)
0
3
6
9
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

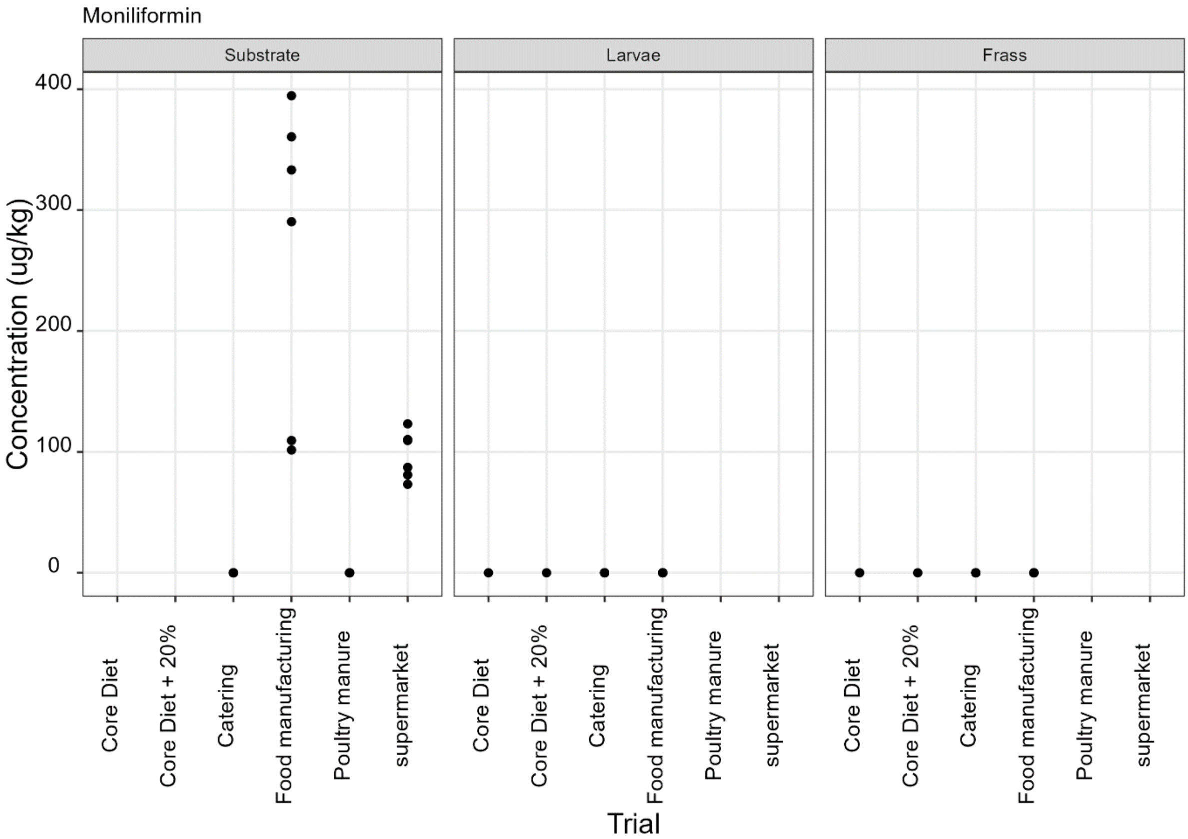
4.


Enniatin.B
Substrate
Larvae
Frass
Concentration (ug/kg)
0
5
10
15
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

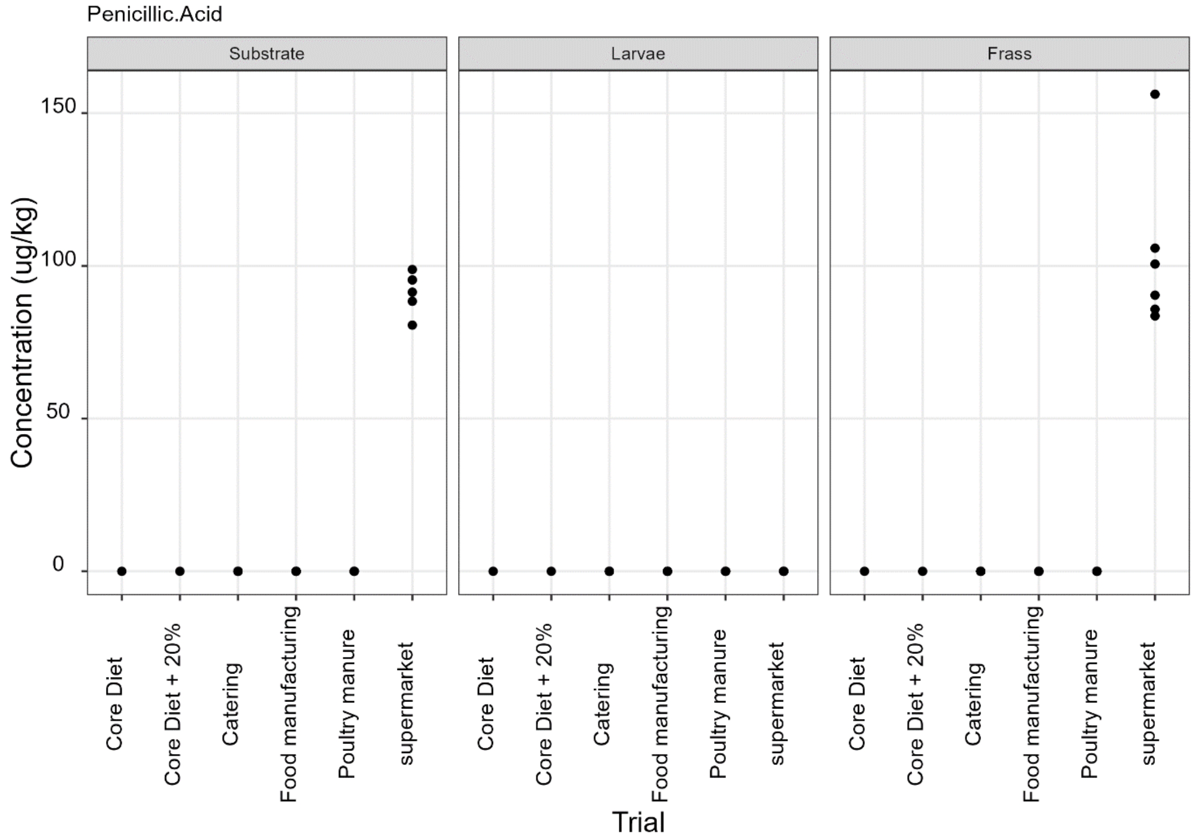
5.


Enniatin.B1
Substrate
Larvae
Frass
Concentration (ug/kg)
0
10
20
30
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

6.

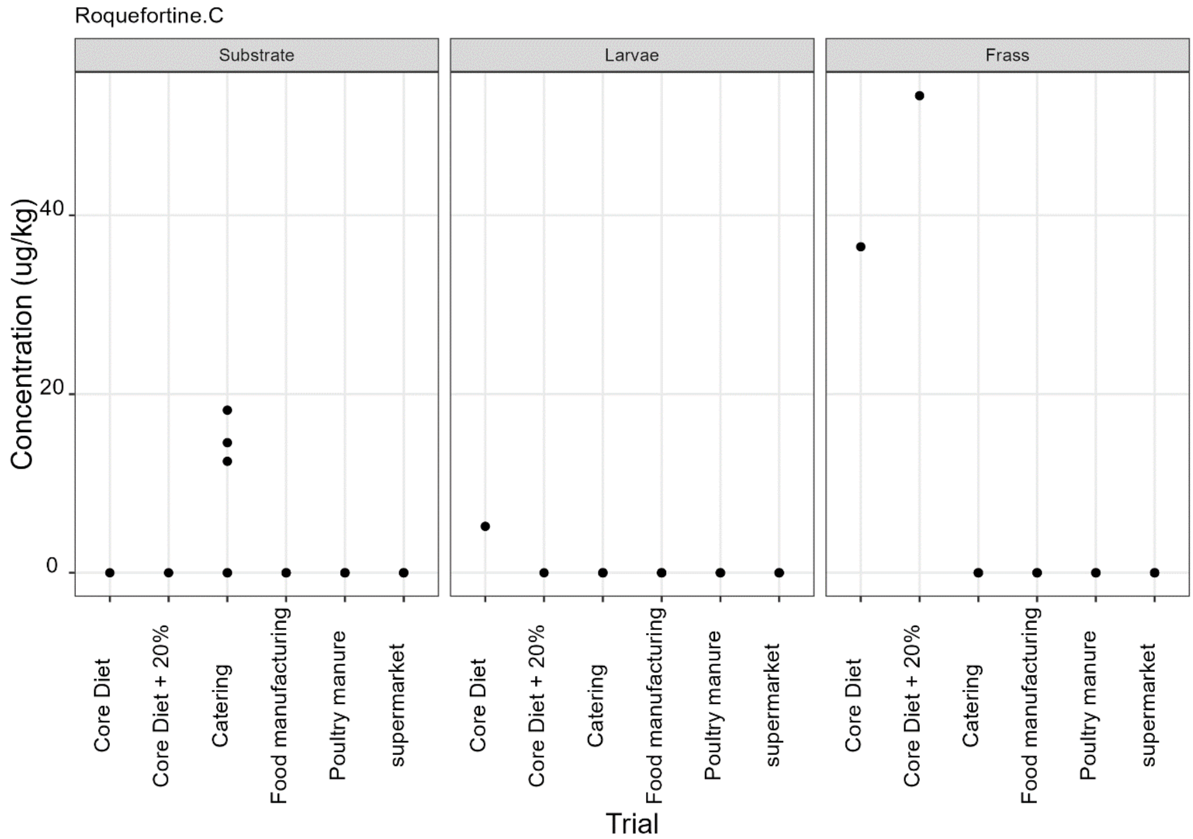

Moniliformin
Substrate
Larvae
Frass
Concentration (ug/kg)
0
100
200
300
400
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

7.



Penicillic.Acid
Substrate
Larvae
Frass
Concentration (ug/kg)
150
100
50
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

8.



Roquefortine.C
Substrate
Larvae
Frass
Concentration (ug/kg)
40
20
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

9.

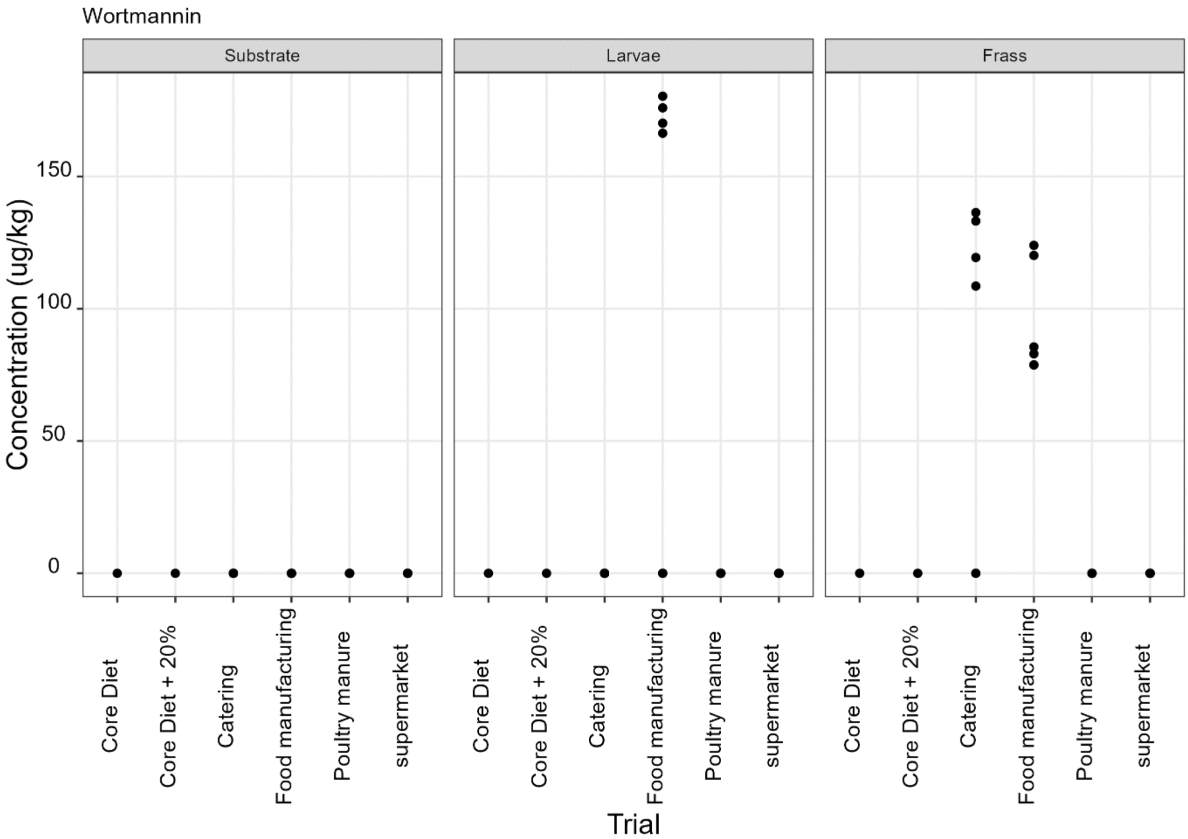


Figure IV (1 – 9). Concentration (μg/kg) of mycotoxins analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table VIII. Calculated quantities of mycotoxins.

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Beauvericin	2	0	0	0	NA	NA	NA
core diet	Larvae	Beauvericin	2	0	0	0	NA	NA	NA
core diet	Frass	Beauvericin	2	0	0	0	NA	NA	NA
core diet	Substrate	Enniatin.A1	2	0	0	0	NA	NA	NA
core diet	Larvae	Enniatin.A1	2	0	0	0	NA	NA	NA
core diet	Frass	Enniatin.A1	2	0	0	0	NA	NA	NA
core diet	Substrate	Enniatin.B	2	0	0	0	NA	NA	NA
core diet	Larvae	Enniatin.B	2	0	0	0	NA	NA	NA
core diet	Frass	Enniatin.B	2	0	0	0	NA	NA	NA
core diet	Substrate	Enniatin.B1	2	0	0	0	NA	NA	NA
core diet	Larvae	Enniatin.B1	2	0	0	0	NA	NA	NA
core diet	Frass	Enniatin.B1	2	0	0	0	NA	NA	NA
core diet	Substrate	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet	Larvae	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet	Frass	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet	Substrate	Roquefortine.C	2	0	0	0	NA	NA	NA
core diet	Larvae	Roquefortine.C	2	1	5.2	5.2	Inf	NA	NA
core diet	Frass	Roquefortine.C	2	1	36.49	36.49	Inf	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Moniliformin	1	0	NA	NA	NA	NA	NA
core diet	Larvae	Moniliformin	2	0	0	0	NA	NA	NA
core diet	Frass	Moniliformin	2	0	0	0	NA	NA	NA
core diet	Substrate	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet	Larvae	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet	Frass	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet	Substrate	Wortmannin	2	0	0	0	NA	NA	NA
core diet	Larvae	Wortmannin	2	0	0	0	NA	NA	NA
core diet	Frass	Wortmannin	2	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Beauvericin	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Beauvericin	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Beauvericin	2	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Enniatin.A1	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Enniatin.A1	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Enniatin.A1	2	0	0	0	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Substrate	Enniatin.B	2	1	6.644	6.6437	NA	NA	NA
core diet + 20%	Larvae	Enniatin.B	2	0	0	0	0	NA	NA
core diet + 20%	Frass	Enniatin.B	2	0	0	0	0	NA	NA
core diet + 20%	Substrate	Enniatin.B1	2	1	5.304	5.3038	NA	NA	NA
core diet + 20%	Larvae	Enniatin.B1	2	0	0	0	0	NA	NA
core diet + 20%	Frass	Enniatin.B1	2	0	0	0	0	NA	NA
core diet + 20%	Substrate	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Penicillic.Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Roquefortine.C	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Roquefortine.C	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Roquefortine.C	2	1	53.4	53.4	Inf	NA	NA
core diet + 20%	Substrate	Moniliformin	1	0	NA	NA	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Moniliformin	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Moniliformin	2	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Cyclopiazonic Acid	2	0	0	0	NA	NA	NA
core diet + 20%	Substrate	Wortmannin	2	0	0	0	NA	NA	NA
core diet + 20%	Larvae	Wortmannin	2	0	0	0	NA	NA	NA
core diet + 20%	Frass	Wortmannin	2	0	0	0	NA	NA	NA
Catering	Substrate	Beauvericin	6	0	0	0	NA	NA	NA
Catering	Larvae	Beauvericin	6	0	0	0	NA	NA	NA
Catering	Frass	Beauvericin	6	0	0	0	NA	NA	NA
Catering	Substrate	Enniatin.A1	6	0	0	0	NA	NA	NA
Catering	Larvae	Enniatin.A1	6	0	0	0	NA	NA	NA
Catering	Frass	Enniatin.A1	6	0	0	0	NA	NA	NA
Catering	Substrate	Enniatin.B	6	0	0	0	NA	NA	NA
Catering	Larvae	Enniatin.B	6	0	0	0	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Enniatin.B	6	0	0	0	NA	NA	NA
Catering	Substrate	Enniatin.B1	6	0	0	0	NA	NA	NA
Catering	Larvae	Enniatin.B1	6	0	0	0	NA	NA	NA
Catering	Frass	Enniatin.B1	6	0	0	0	NA	NA	NA
Catering	Substrate	Penicillic.Acid	6	0	0	0	NA	NA	NA
Catering	Larvae	Penicillic.Acid	6	0	0	0	NA	NA	NA
Catering	Frass	Penicillic.Acid	6	0	0	0	NA	NA	NA
Catering	Substrate	Roquefortine.C	6	3	9.75	18.2	NA	-0.6399	1.36
Catering	Larvae	Roquefortine.C	6	0	0	0	0	NA	NA
Catering	Frass	Roquefortine.C	6	0	0	0	0	NA	NA
Catering	Substrate	Moniliformin	6	0	0	0	NA	NA	NA
Catering	Larvae	Moniliformin	6	0	0	0	NA	NA	NA
Catering	Frass	Moniliformin	6	0	0	0	NA	NA	NA
Catering	Substrate	Cyclopiazonic Acid	6	1	21.38	171.006	NA	-3.999	-2
Catering	Larvae	Cyclopiazonic Acid	6	0	0	0	0	NA	NA
Catering	Frass	Cyclopiazonic Acid	6	0	0	0	0	NA	NA
Catering	Substrate	Wortmannin	6	0	0	0	NA	NA	NA
Catering	Larvae	Wortmannin	6	0	0	0	NA	NA	NA
Catering	Frass	Wortmannin	6	4	90.7	136.4	Inf	-1.486	0.5138

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Beauvericin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Beauvericin	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Beauvericin	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Enniatin.A1	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Enniatin.A1	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Enniatin.A1	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Enniatin.B	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Enniatin.B	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Enniatin.B	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Enniatin.B1	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Enniatin.B1	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Enniatin.B1	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Penicillic.Acid	6	0	0	0	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Larvae	Penicillic.Acid	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Penicillic.Acid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Roquefortine.C	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Roquefortine.C	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Roquefortine.C	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Moniliformin	6	6	225.1	394.6	NA	0.2923	-1.063
Manufacturing	Larvae	Moniliformin	6	0	0	0	0	NA	NA
Manufacturing	Frass	Moniliformin	6	0	0	0	0	NA	NA
Manufacturing	Substrate	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Manufacturing	Frass	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Manufacturing	Substrate	Wortmannin	6	0	0	0	NA	NA	NA
Manufacturing	Larvae	Wortmannin	6	4	86.62	180.4	Inf	-0.1143	-2

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Manufacturing	Frass	Wortmannin	6	6	101.8	124	Inf	-0.05403	0.3983
Poultry manure	Substrate	Beauvericin	6	6	8.36	13.439	NA	-0.7992	-0.3551
Poultry manure	Larvae	Beauvericin	6	0	0	0	0	NA	NA
Poultry manure	Frass	Beauvericin	6	6	9.037	9.9094	1.081	0.05195	-0.08413
Poultry manure	Substrate	Enniatin.A1	6	6	9.329	10.715	NA	-0.1957	-0.07195
Poultry manure	Larvae	Enniatin.A1	6	0	0	0	0	NA	NA
Poultry manure	Frass	Enniatin.A1	6	0	0	0	0	NA	NA
Poultry manure	Substrate	Enniatin.B	6	6	12.98	14.1456	NA	-0.035	-0.07995
Poultry manure	Larvae	Enniatin.B	6	0	0	0	0	NA	NA
Poultry manure	Frass	Enniatin.B	6	0	0	0	0	NA	NA
Poultry manure	Substrate	Enniatin.B1	6	6	33.17	36.92	NA	-0.128	-0.06791
Poultry manure	Larvae	Enniatin.B1	6	0	0	0	0	NA	NA
Poultry manure	Frass	Enniatin.B1	6	6	7.397	8.438	0.223	-0.05737	-0.1156

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Substrate	Penicillic.Acid	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Penicillic.Acid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Penicillic.Acid	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Roquefortine.C	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Roquefortine.C	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Roquefortine.C	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Moniliformin	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Moniliformin	0	0	NA	NA	NA	NA	NA
Poultry manure	Frass	Moniliformin	0	0	NA	NA	NA	NA	NA
Poultry manure	Substrate	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Poultry manure	Larvae	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
Poultry manure	Substrate	Wortmannin	6	0	0	0	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Wortmannin	6	0	0	0	NA	NA	NA
Poultry manure	Frass	Wortmannin	6	0	0	0	NA	NA	NA
supermarket	Substrate	Beauvericin	6	0	0	0	NA	NA	NA
supermarket	Larvae	Beauvericin	6	0	0	0	NA	NA	NA
supermarket	Frass	Beauvericin	6	0	0	0	NA	NA	NA
supermarket	Substrate	Enniatin.A1	6	0	0	0	NA	NA	NA
supermarket	Larvae	Enniatin.A1	6	0	0	0	NA	NA	NA
supermarket	Frass	Enniatin.A1	6	0	0	0	NA	NA	NA
supermarket	Substrate	Enniatin.B	6	6	6.499	6.834	NA	0.01574	-0.06346
supermarket	Larvae	Enniatin.B	6	0	0	0	0	NA	NA
supermarket	Frass	Enniatin.B	6	6	13.9	14.6712	2.139	-0.02223	0.01956
supermarket	Substrate	Enniatin.B1	6	6	7.803	8.2974	NA	0.01619	-0.06895
supermarket	Larvae	Enniatin.B1	6	0	0	0	0	NA	NA
supermarket	Frass	Enniatin.B1	6	6	8.495	9.4992	1.089	-0.1398	-0.03356
supermarket	Substrate	Penicillic.Acid	6	6	92.15	98.8	NA	0.102	0.03147
supermarket	Larvae	Penicillic.Acid	6	0	0	0	0	NA	NA
supermarket	Frass	Penicillic.Acid	6	6	98.98	156.2	1.074	-0.3061	-0.2884
supermarket	Substrate	Roquefortine.C	6	0	0	0	NA	NA	NA
supermarket	Larvae	Roquefortine.C	6	0	0	0	NA	NA	NA

Trial	Sample Type	Mycotoxin	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
supermarket	Frass	Roquefortine.C	6	0	0	0	NA	NA	NA
supermarket	Substrate	Moniliformin	6	6	96.92	123.2	NA	0.1434	-0.02734
supermarket	Larvae	Moniliformin	0	0	NA	NA	NA	NA	NA
supermarket	Frass	Moniliformin	0	0	NA	NA	NA	NA	NA
supermarket	Substrate	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
supermarket	Larvae	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
supermarket	Frass	Cyclopiazonic Acid	6	0	0	0	NA	NA	NA
supermarket	Substrate	Wortmannin	6	0	0	0	NA	NA	NA
supermarket	Larvae	Wortmannin	6	0	0	0	NA	NA	NA
supermarket	Frass	Wortmannin	6	0	0	0	NA	NA	NA

Inf = inferred, NA = not applicable

Appendix F - PAHs

The aim of the analysis was to

- estimate the quantity of each substance in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of bioaccumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (\,A + ((B1 + B2)/2))/2$$

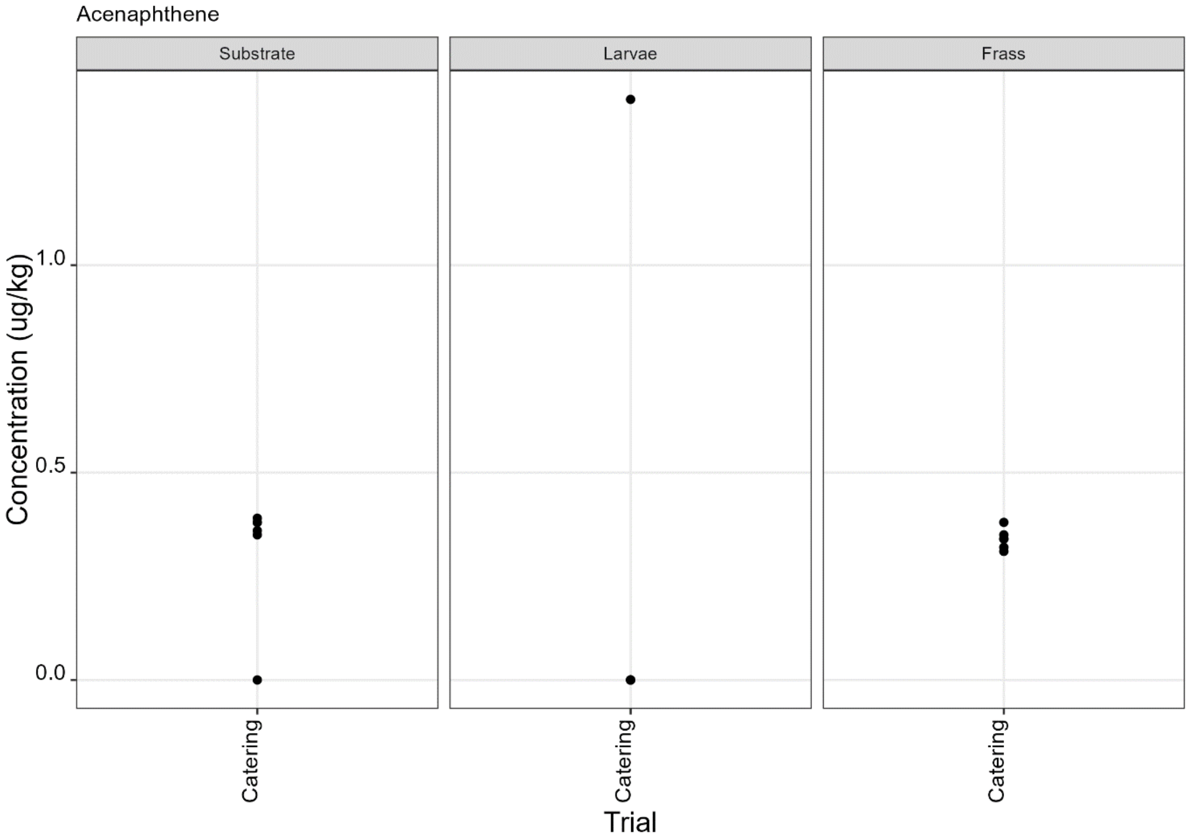
An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

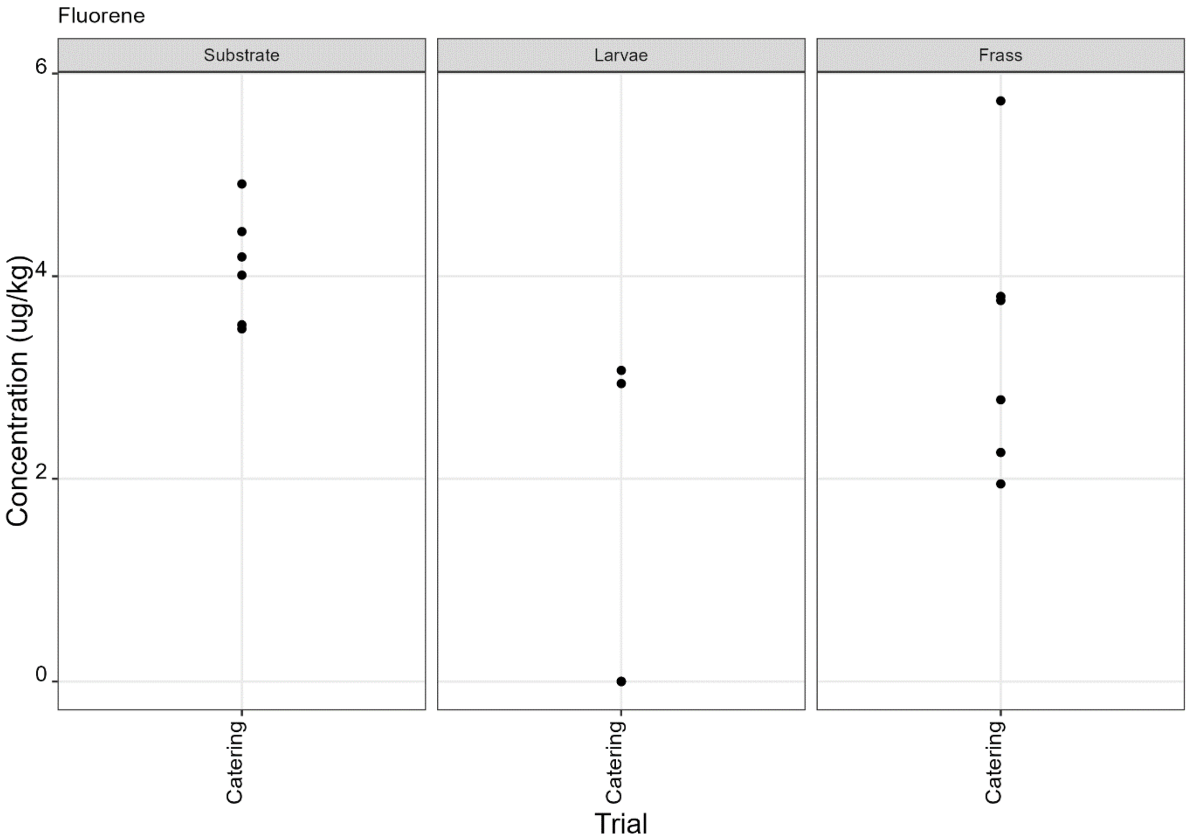
Individual results are shown in the figures and the table shows the calculated quantities.

Eleven substances were not detected in any samples. Results from these samples were excluded from further analysis: anthracene, benzo.ghi.fluoranthene, benzo.b.naphtho.2.1.d.thiophene, indeno.1.2.3.c.d.pyrene, dibenzo.a.h.anthracene, anthanthrene, dibenzo.a.l.pyrene, dibenzo.a.e.pyrene, dibenzo.a.i.pyrene, dibenzo.a.h.pyrene, and coronene.

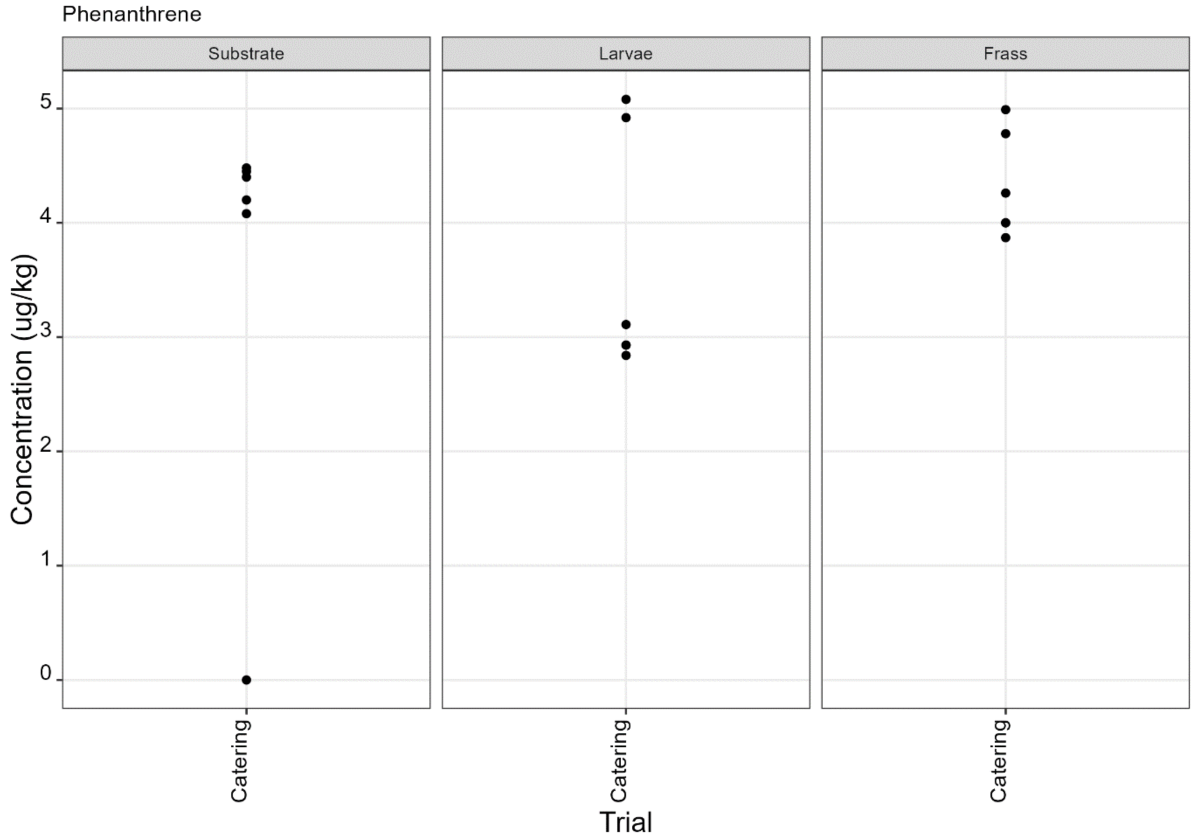
1.


Acenaphthene
Substrate
Larvae
Frass
Concentration (ug/kg)
1.0
0.5
0.0
Catering
Catering
Catering
Trial

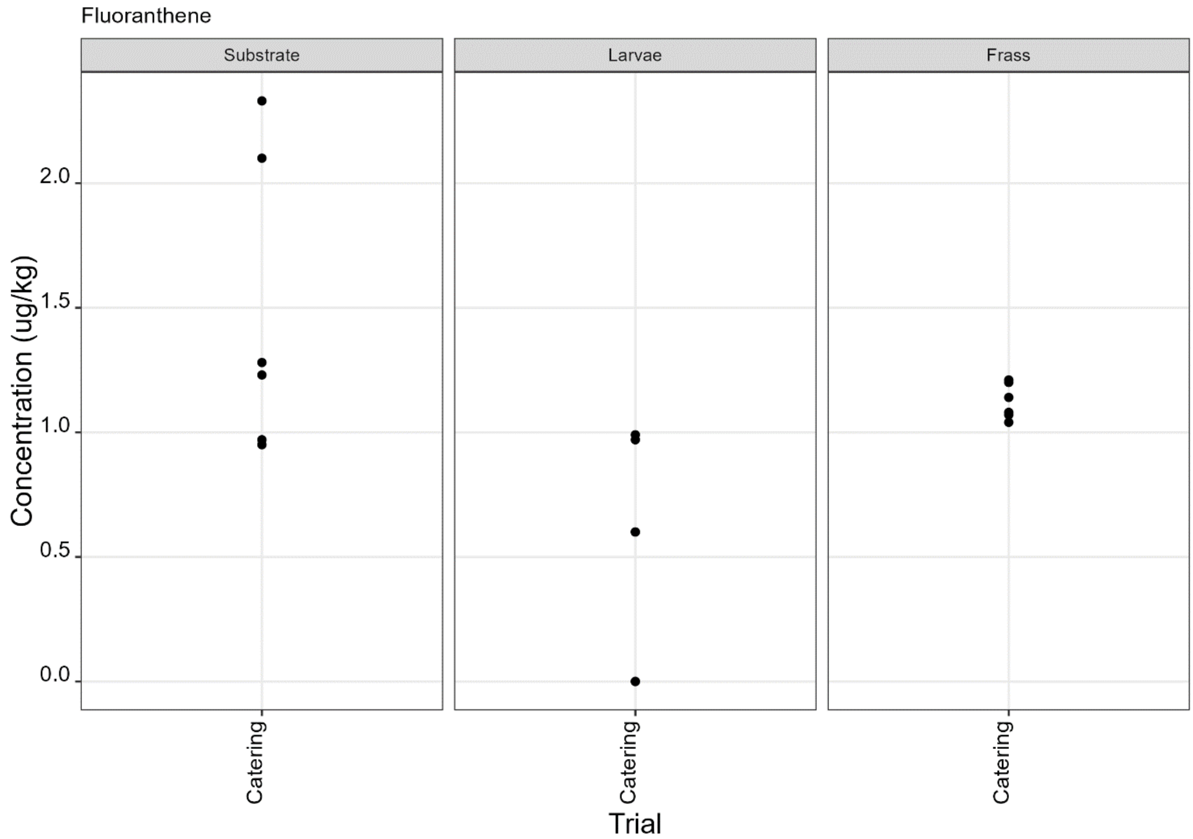
2.


Fluorene
Substrate
Larvae
Frass
Concentration (ug/kg)
6
4
2
0
Catering
Catering
Catering
Trial

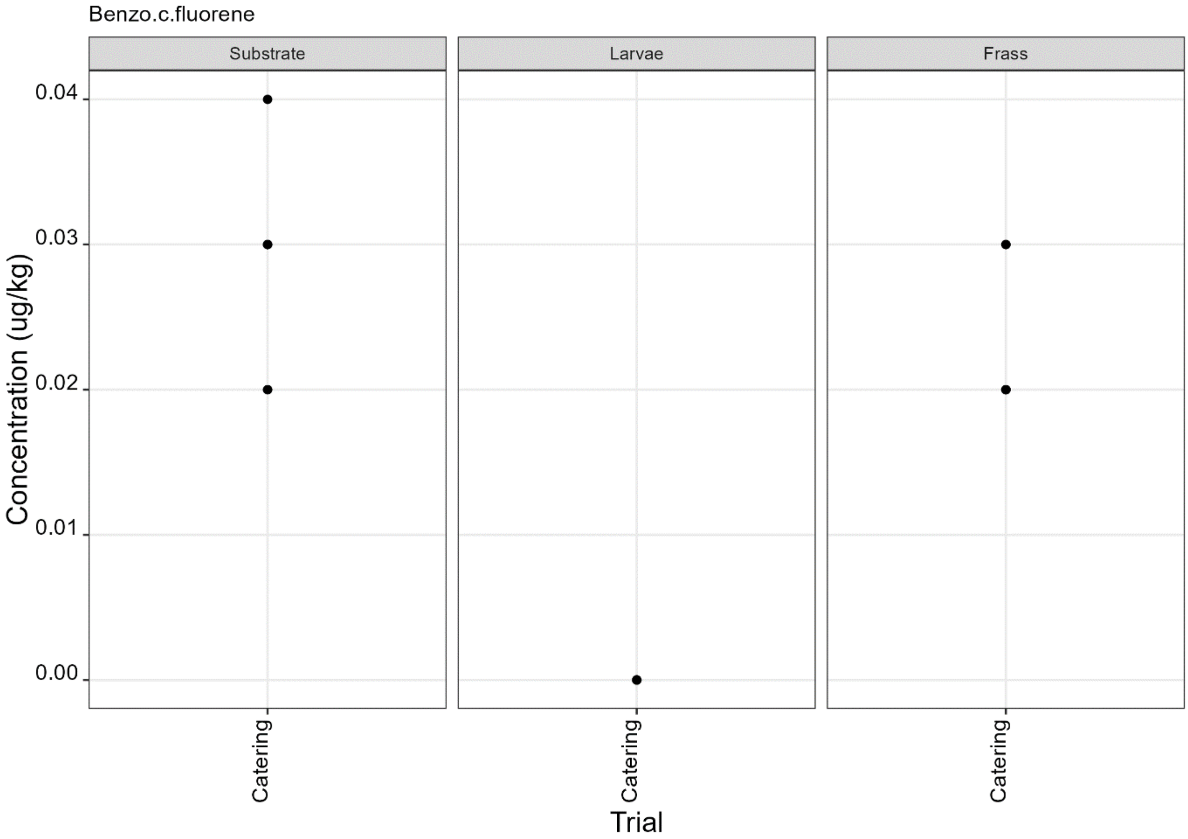
3.


Phenanthrene
Substrate
Larvae
Frass
Concentration (ug/kg)
0
1
2
3
4
5
Catering
Catering
Catering
Trial

4.

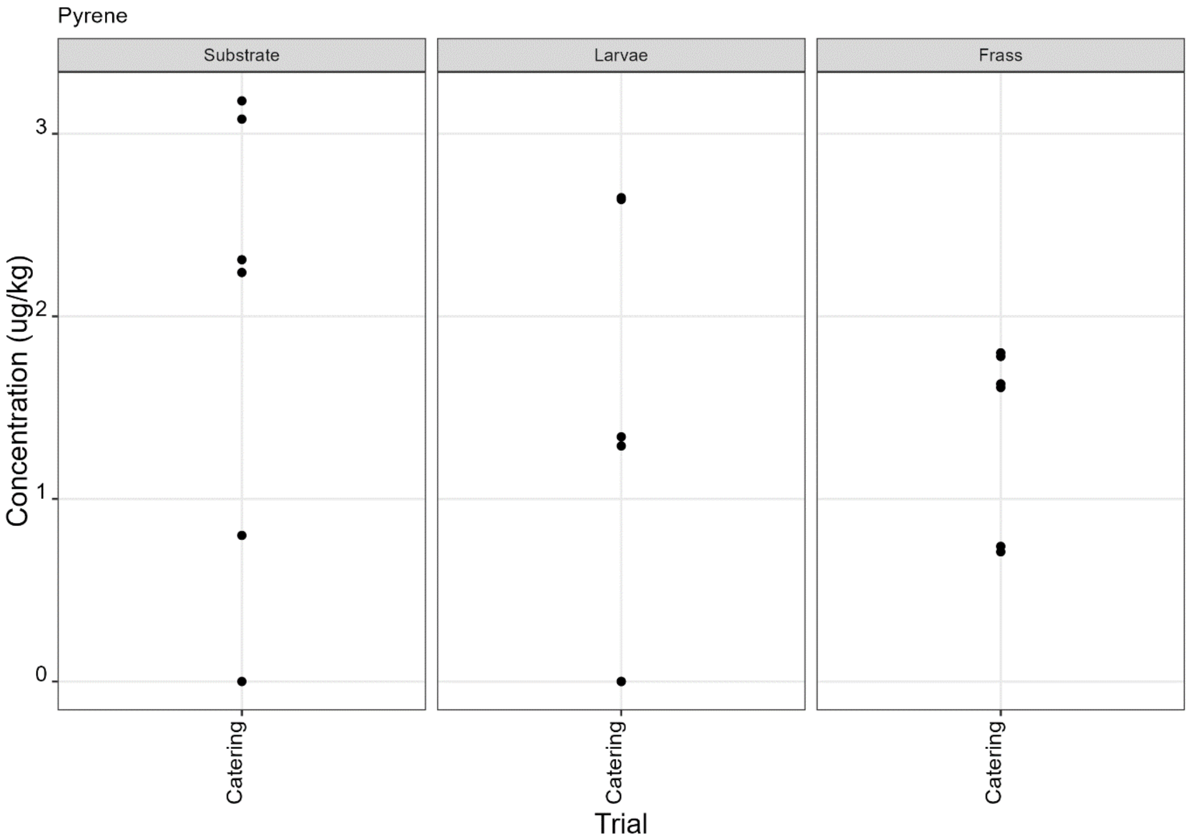

Fluoranthene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.0
0.5
1.0
1.5
2.0
Catering
Catering
Catering
Trial

5.



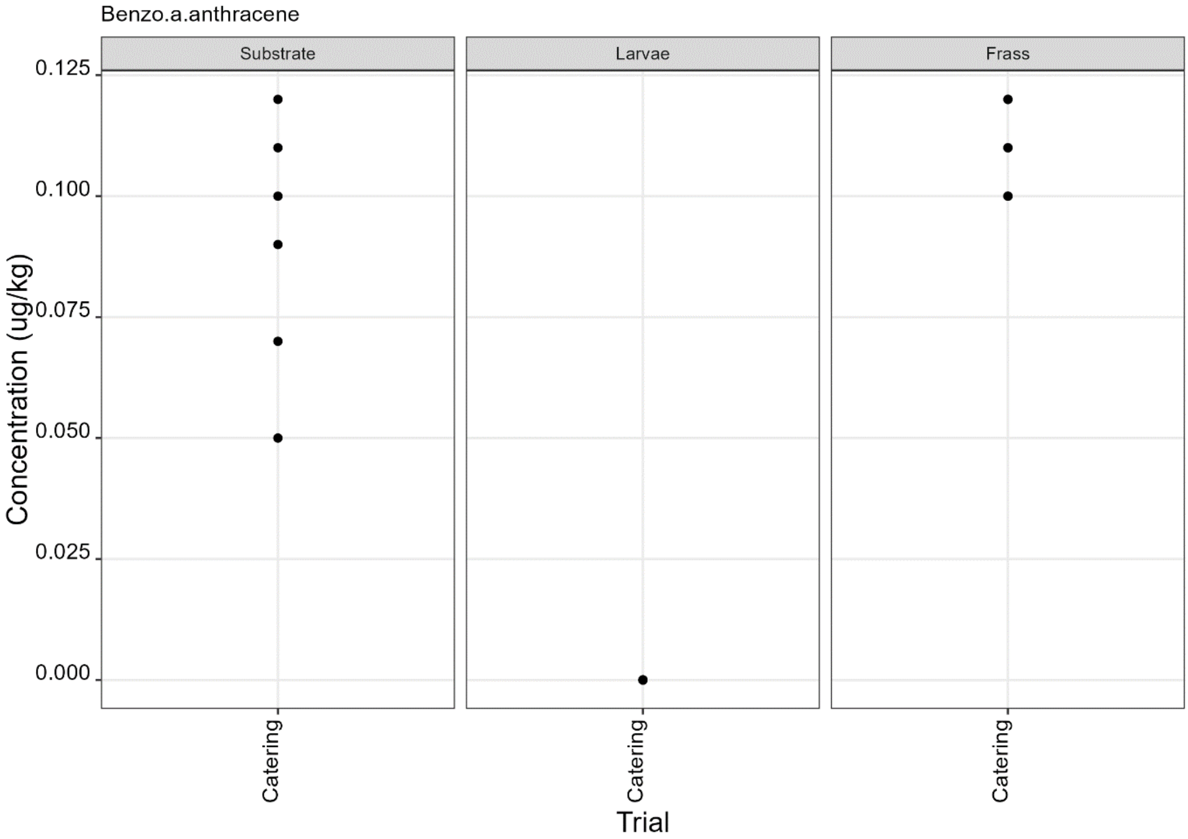
Benzo.c.fluorene
Substrate
Larvae
Frass
0.04
0.03
0.02
0.01
0.00
Concentration (ug/kg)
Catering
Catering
Catering
Trial

6.

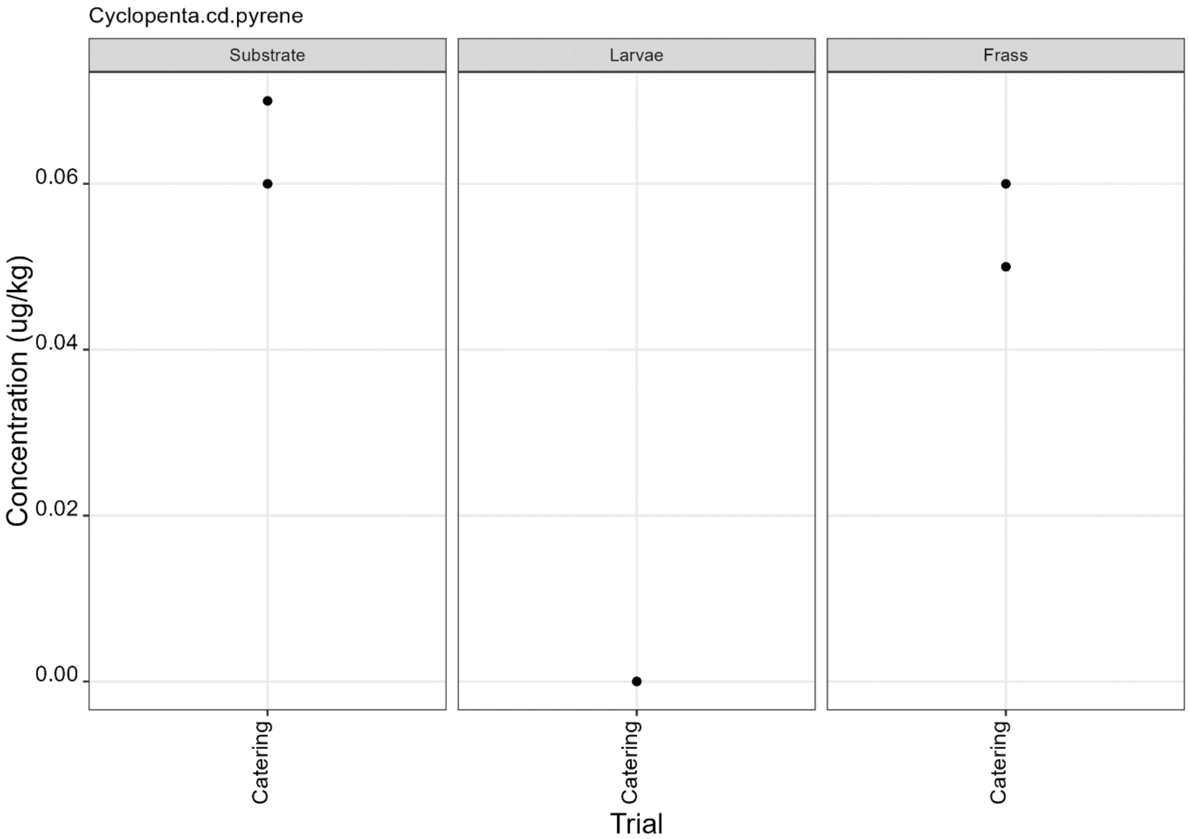


Pyrene
Substrate
Larvae
Frass
3
2
1
0
Concentration (ug/kg)
Catering
Catering
Catering
Trial

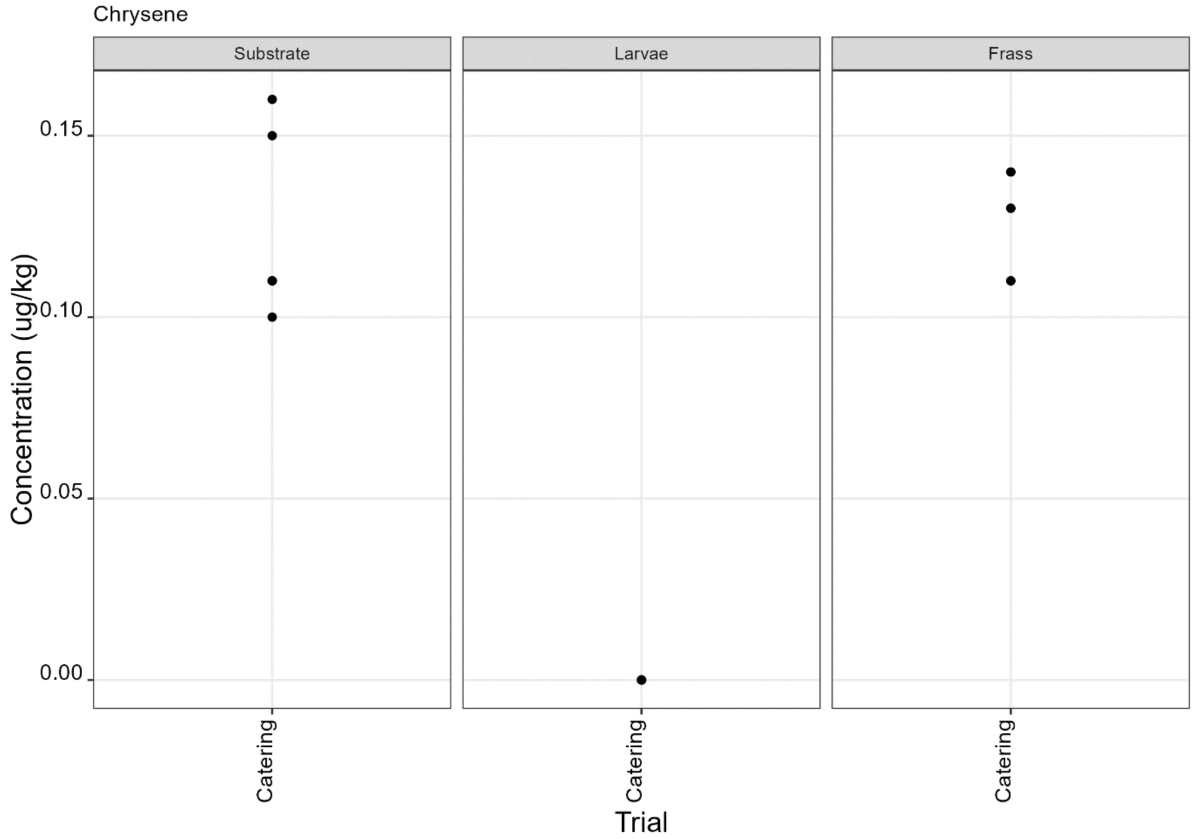
7.


Benzo.a.anthracene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.125
0.100
0.075
0.050
0.025
0.000
Catering
Catering
Catering
Trial

8.

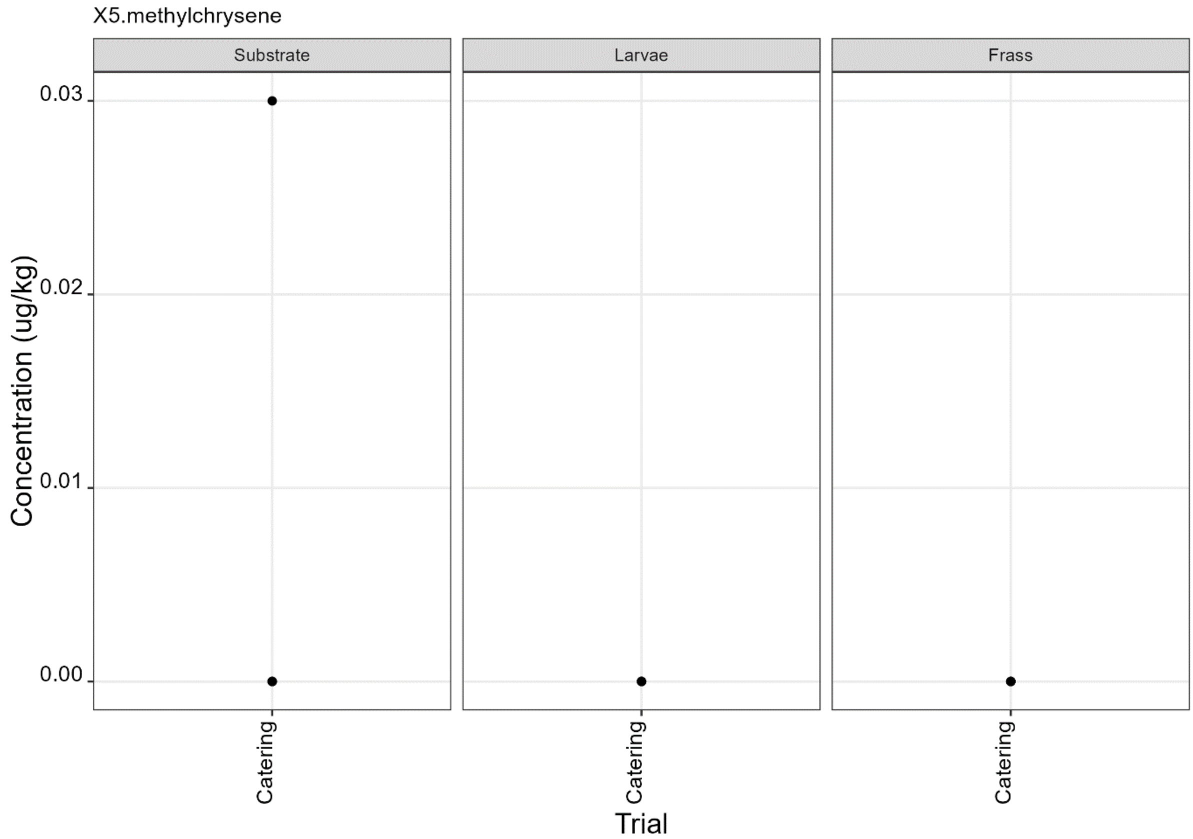

Cyclopenta.cd.pyrene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.06
0.04
0.02
0.00
Catering
Catering
Catering
Trial

9.



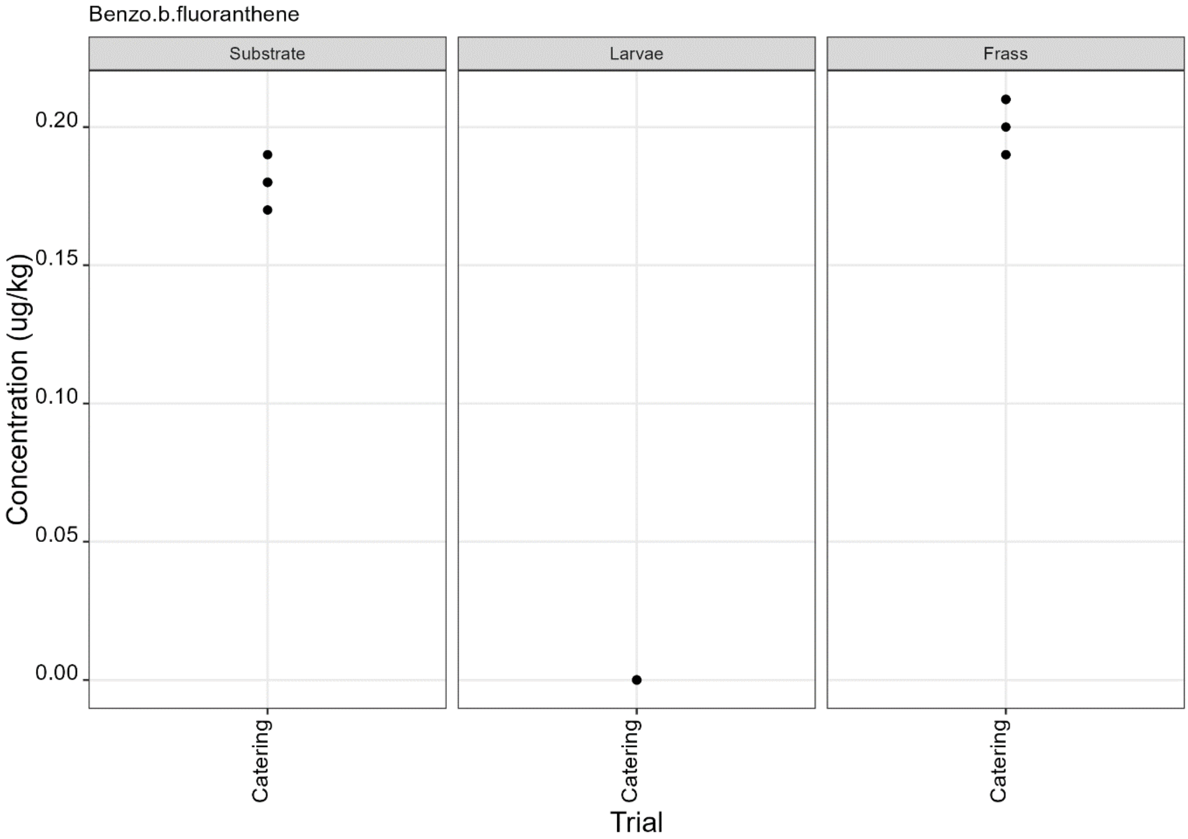
Chrysene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.15
0.10
0.05
0.00
Catering
Catering
Catering
Trial

10.

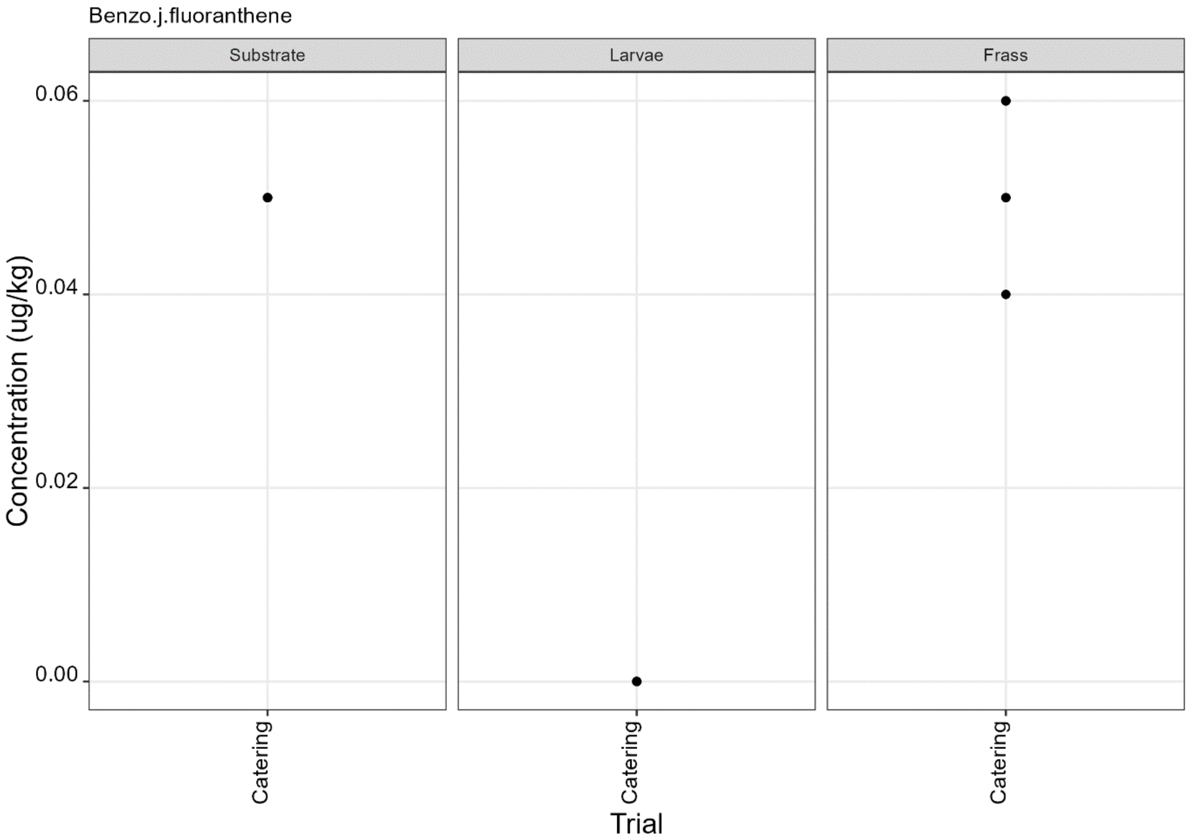


X5.methylchrysene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.03
0.02
0.01
0.00
Catering
Catering
Catering
Trial

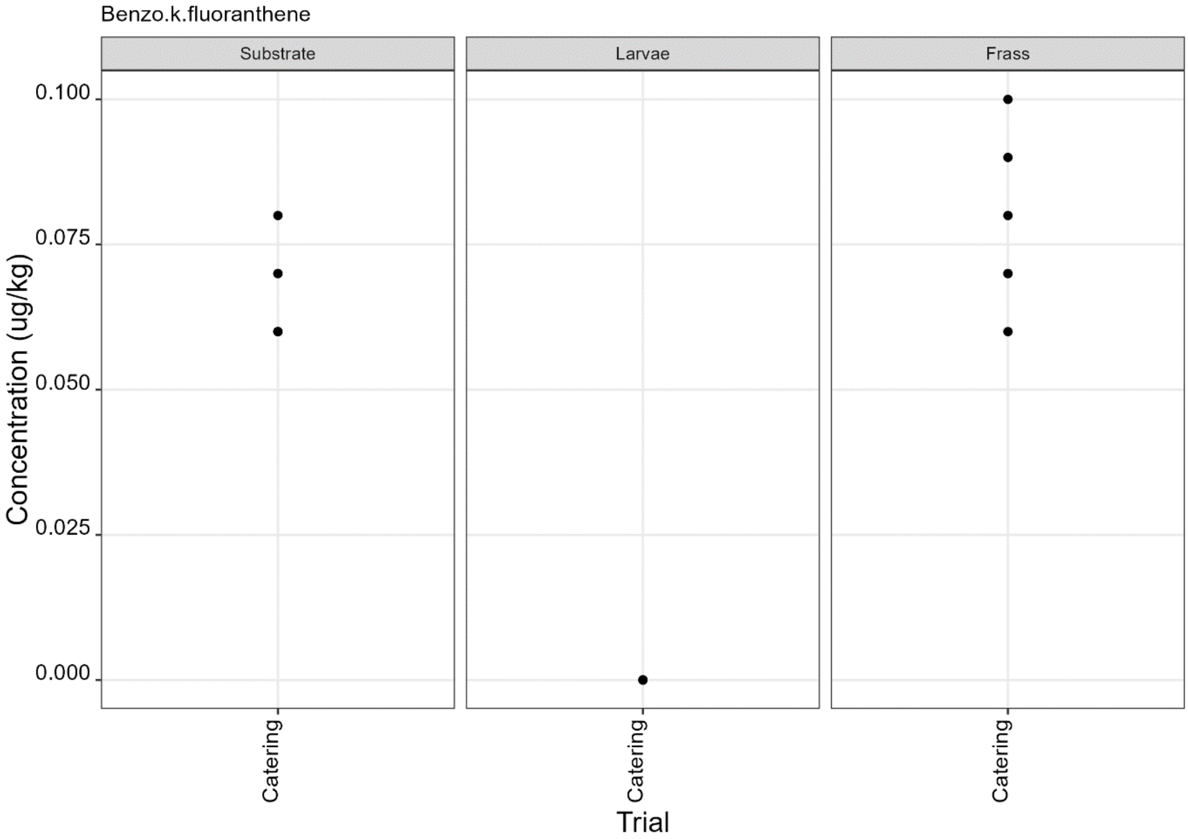
11.


Benzo.b.fluoranthene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.20
0.15
0.10
0.05
0.00
Catering
Catering
Catering
Trial

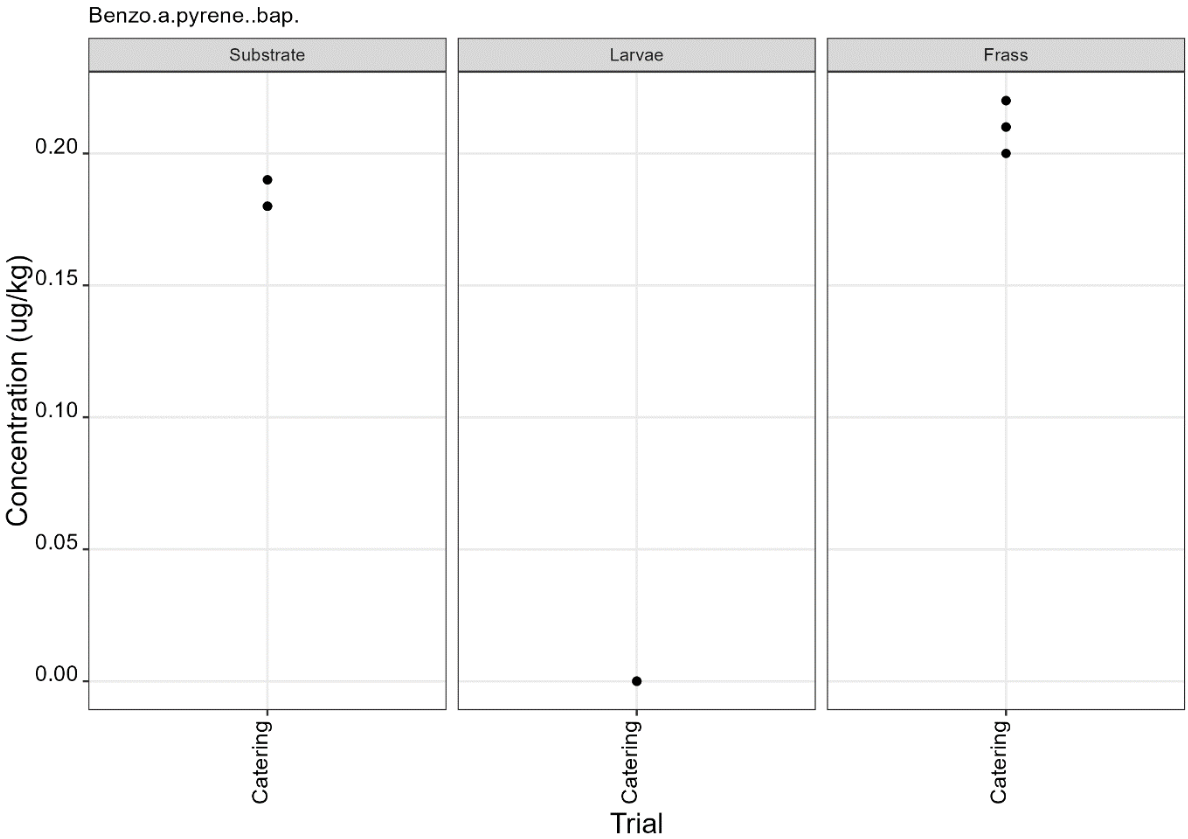
12.


Benzo.j.fluoranthene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.06
0.04
0.02
0.00
Catering
Catering
Catering
Trial

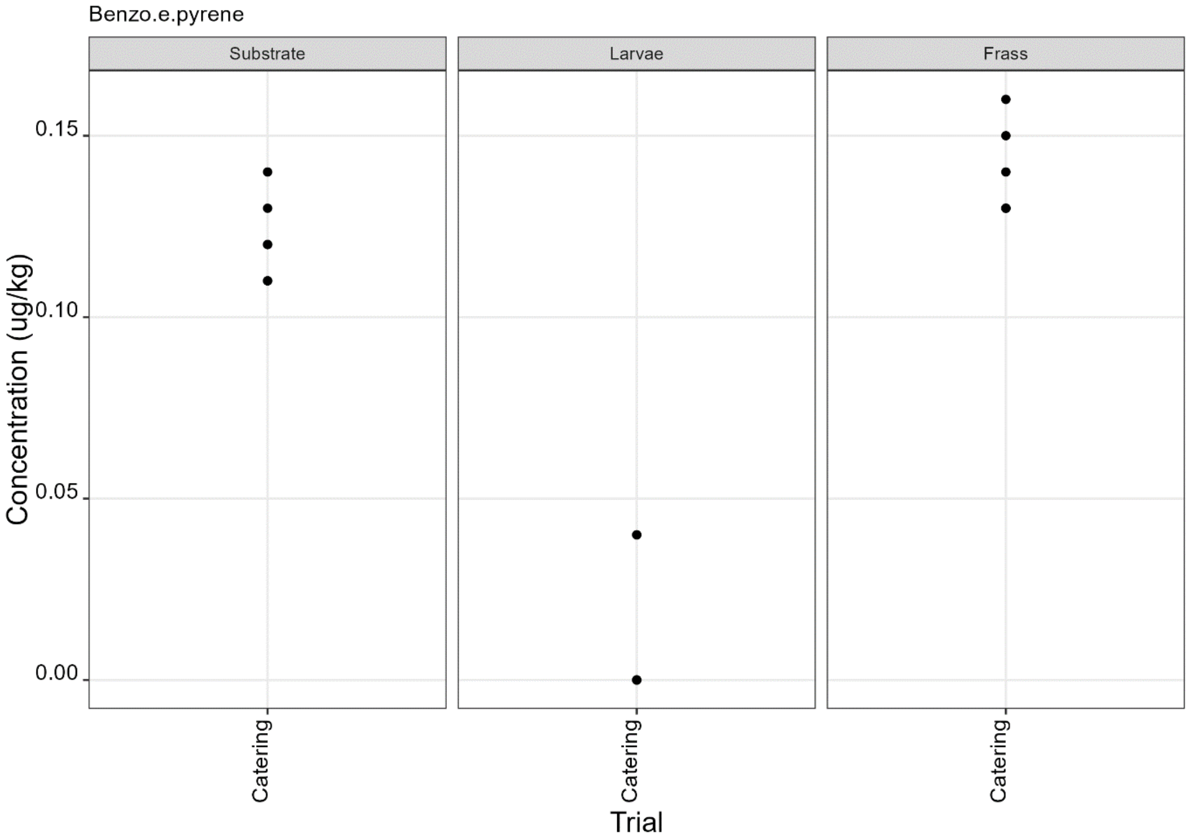
13.


Benzo.k.fluoranthene
Substrate
Larvae
Frass
0.100
0.075
0.050
0.025
0.000
Concentration (ug/kg)
Catering
Catering
Catering
Trial

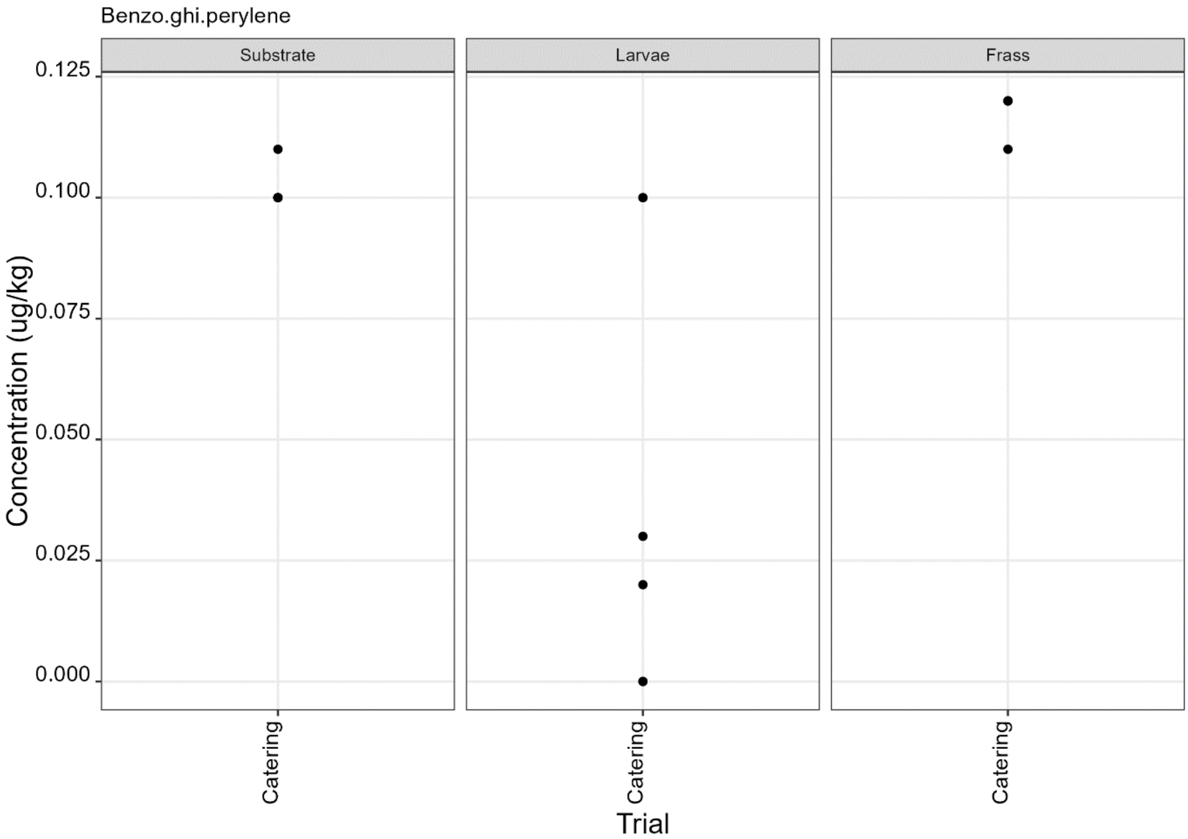
14.


Benzo.a.pyrene..bap.
Substrate
Larvae
Frass
0.20
0.15
0.10
0.05
0.00
Concentration (ug/kg)
Catering
Catering
Catering
Trial

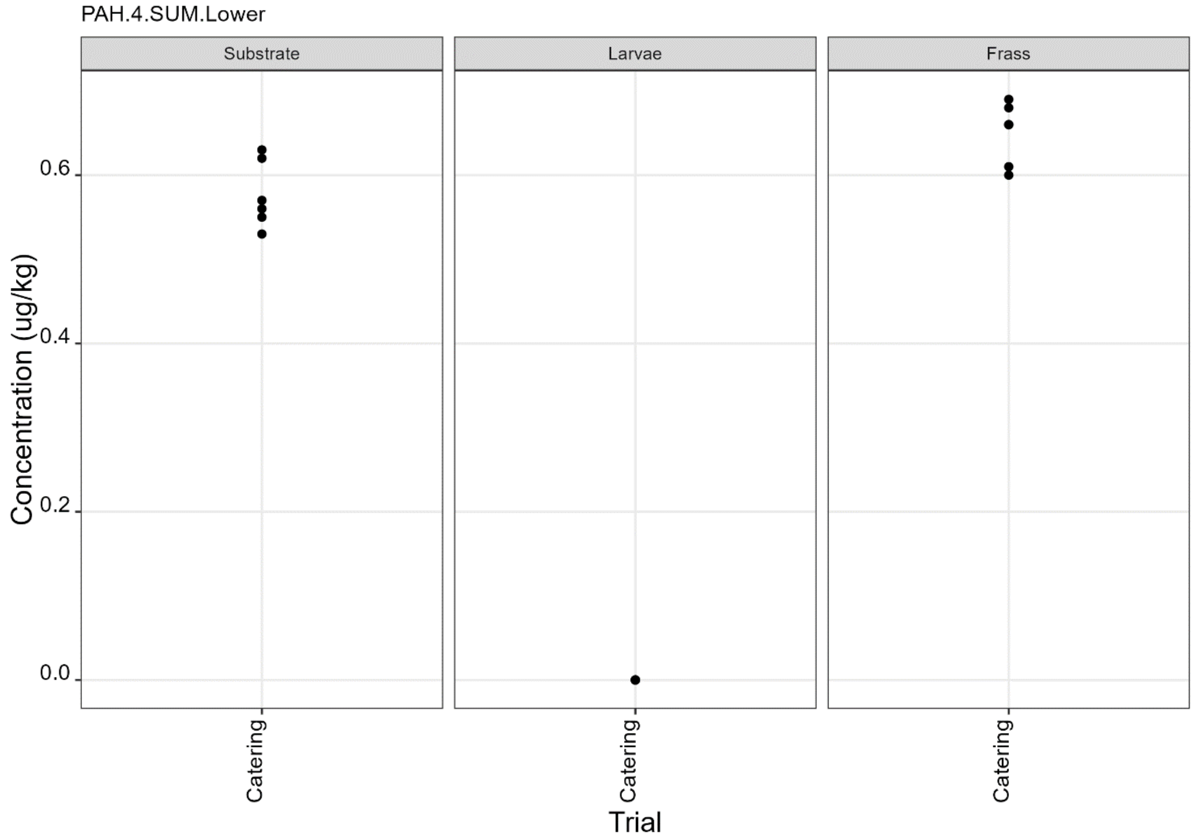
15.


Benzo.e.pyrene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.15
0.10
0.05
0.00
Catering
Catering
Catering
Trial

16.


Benzo.ghi.perylene
Substrate
Larvae
Frass
Concentration (ug/kg)
0.125
0.100
0.075
0.050
0.025
0.000
Catering
Catering
Catering
Trial

17.



18.

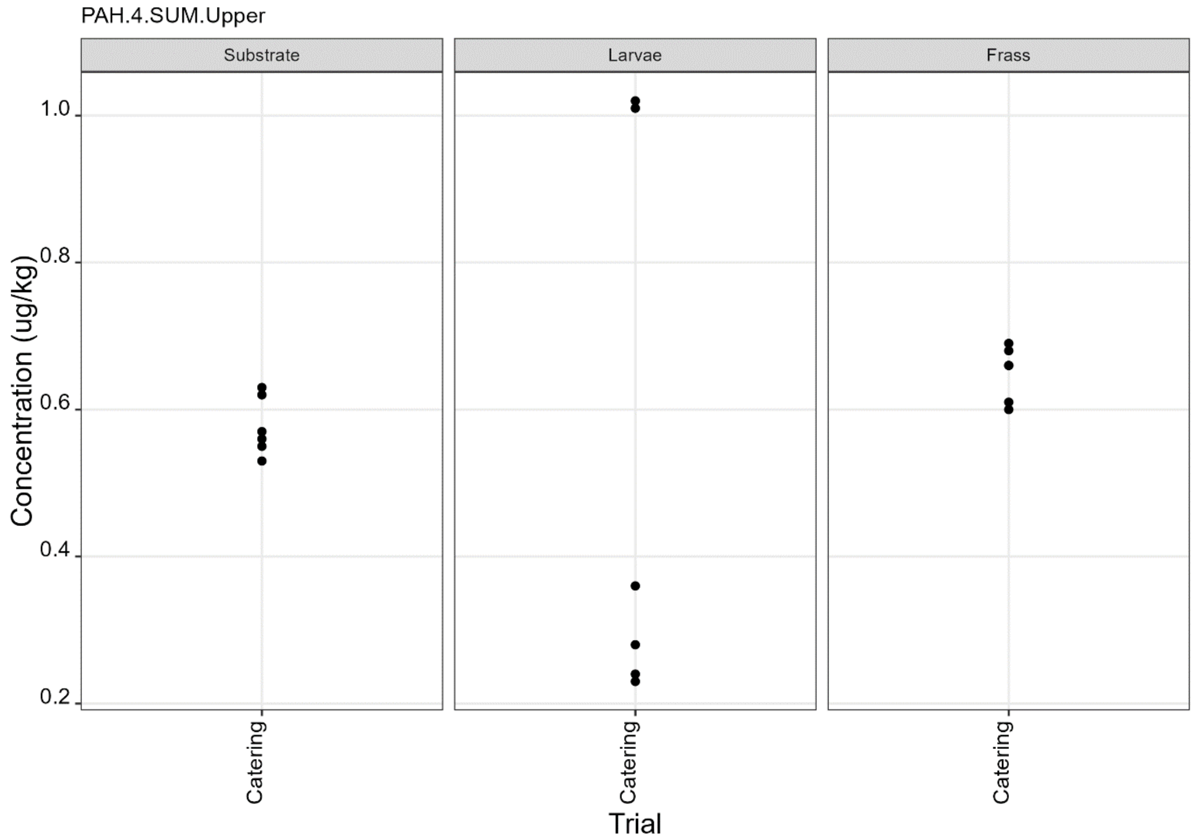


Figure V (1 – 18). Concentration (µg/kg) of PAHs analysed in substrate rearing, larvae and frass from catering.

Table IX. Calculated quantities of PAHs.

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Substrate	Acenaphthene	6	5	0.28	0.39	NA	0.03571	-0.6429
Catering	Larvae	Acenaphthene	6	1	0.35	1.4	1.25	0	2
Catering	Frass	Acenaphthene	6	6	0.3363	0.38	1.201	0.1041	-0.0669
Catering	Substrate	Fluorene	6	6	4.238	4.91	NA	-0.1416	0.2065
Catering	Larvae	Fluorene	6	2	0.7512	3.07	0.1773	-4	-2
Catering	Frass	Fluorene	6	6	3.599	5.73	0.8492	-0.4654	0.3647
Catering	Substrate	Phenanthrene	6	5	3.736	4.48	NA	0.5929	0.2161
Catering	Larvae	Phenanthrene	6	6	3.481	5.08	0.9317	0.6076	-0.265
Catering	Frass	Phenanthrene	6	6	4.27	4.99	1.143	-0.2225	-0.06557
Catering	Substrate	Fluoranthene	6	6	1.661	2.33	NA	0.1776	0.6668
Catering	Larvae	Fluoranthene	6	4	0.64	0.99	0.3853	-0.9375	1.062
Catering	Frass	Fluoranthene	6	6	1.119	1.21	0.6737	-0.1296	-0.02458
Catering	Substrate	Benzo.c.fluorene	6	6	0.0275	0.04	NA	-0.3636	-0.1818
Catering	Larvae	Benzo.c.fluorene	6	0	0	0	0	NA	NA
Catering	Frass	Benzo.c.fluorene	6	6	0.0225	0.03	0.8182	0.4444	-0.2222
Catering	Substrate	Pyrene	6	5	2.234	3.18	NA	0.8393	0.8024
Catering	Larvae	Pyrene	6	4	1.651	2.65	0.739	-0.7965	1.204
Catering	Frass	Pyrene	6	6	1.439	1.8	0.6441	0.7401	0.2519

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Substrate	Benzo.a.anthracene	6	6	0.09625	0.12	NA	-0.3636	0.3896
Catering	Larvae	Benzo.a.anthracene	6	0	0	0	0	NA	NA
Catering	Frass	Benzo.a.anthracene	6	6	0.1112	0.12	1.155	-0.1349	0.06745
Catering	Substrate	Cyclopenta.cd.pyrene	6	6	0.06375	0.07	NA	0.07843	0.03922
Catering	Larvae	Cyclopenta.cd.pyrene	6	0	0	0	0	NA	NA
Catering	Frass	Cyclopenta.cd.pyrene	6	6	0.05625	0.06	0.8824	-0.08889	0.1333
Catering	Substrate	Chrysene	6	6	0.1312	0.16	NA	0.03811	0.362
Catering	Larvae	Chrysene	6	0	0	0	0	NA	NA
Catering	Frass	Chrysene	6	6	0.1288	0.14	0.9817	-0.1941	0.09705
Catering	Substrate	X5.methylchrysene	6	1	0.0075	0.03	NA	0	2
Catering	Larvae	X5.methylchrysene	6	0	0	0	0	NA	NA
Catering	Frass	X5.methylchrysene	6	0	0	0	0	NA	NA
Catering	Substrate	Benzo.b.fluoranthene	6	6	0.1788	0.19	NA	0.02796	-0.04195
Catering	Larvae	Benzo.b.fluoranthene	6	0	0	0	0	NA	NA

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Frass	Benzo.b.fluoranthene	6	6	0.2037	0.21	1.139	-0.07364	0.06136
Catering	Substrate	Benzo.j.fluoranthene	6	6	0.05	0.05	NA	0	0
Catering	Larvae	Benzo.j.fluoranthene	6	0	0	0	0	NA	NA
Catering	Frass	Benzo.j.fluoranthene	6	6	0.05375	0.06	1.075	-0.2791	0.04651
Catering	Substrate	Benzo.k.fluoranthene	6	6	0.06625	0.08	NA	-0.07547	-0.03774
Catering	Larvae	Benzo.k.fluoranthene	6	0	0	0	0	NA	NA
Catering	Frass	Benzo.k.fluoranthene	6	6	0.0825	0.1	1.245	-0.1212	0.303
Catering	Substrate	Benzo.a.pyrene..bap.	6	6	0.1825	0.19	NA	0	-0.0274
Catering	Larvae	Benzo.a.pyrene..bap.	6	0	0	0	0	NA	NA
Catering	Frass	Benzo.a.pyrene..bap.	6	6	0.2125	0.22	1.164	-0.04706	0.02353
Catering	Substrate	Benzo.e.pyrene	6	6	0.1212	0.14	NA	0.2063	-0.02063
Catering	Larvae	Benzo.e.pyrene	6	2	0.02	0.04	0.165	0	2
Catering	Frass	Benzo.e.pyrene	6	6	0.1388	0.16	1.145	-0.1801	-0.05403
Catering	Substrate	Benzo.ghi.perylene	6	6	0.1013	0.11	NA	0.04936	-0.02468

Trial	Sample Type	PAH	Number of injections	Detected	Mean (ug/kg)	Max (ug/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Benzo.ghi.perylene	6	4	0.05625	0.1	0.5553	-0.4444	1.556
Catering	Frass	Benzo.ghi.perylene	6	6	0.1188	0.12	1.173	-0.04209	0.02104
Catering	Substrate	PAH.4.SUM.Lower	6	6	0.5888	0.63	NA	-0.04246	0.1231
Catering	Larvae	PAH.4.SUM.Lower	6	0	0	0	0	NA	NA
Catering	Frass	PAH.4.SUM.Lower	6	6	0.6562	0.69	1.114	-0.09906	0.05715
Catering	Substrate	PAH.4.SUM.Upper	6	6	0.5888	0.63	NA	-0.04246	0.1231
Catering	Larvae	PAH.4.SUM.Upper	6	6	0.4725	1.02	0.8025	1.651	-0.6455
Catering	Frass	PAH.4.SUM.Upper	6	6	0.6562	0.69	1.114	-0.09906	0.05715

Inf = inferred, NA = not applicable

Appendix G - Nitrates and nitrites

The aim of the analysis was to

- estimate the quantity of each analyte in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of bioaccumulation from substrate into larvae and frass

Duplicate or single injections were undertaken on samples (replicates A, B1 and B2) and the average concentration for each replicate was estimated as the average of injections, then the average for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

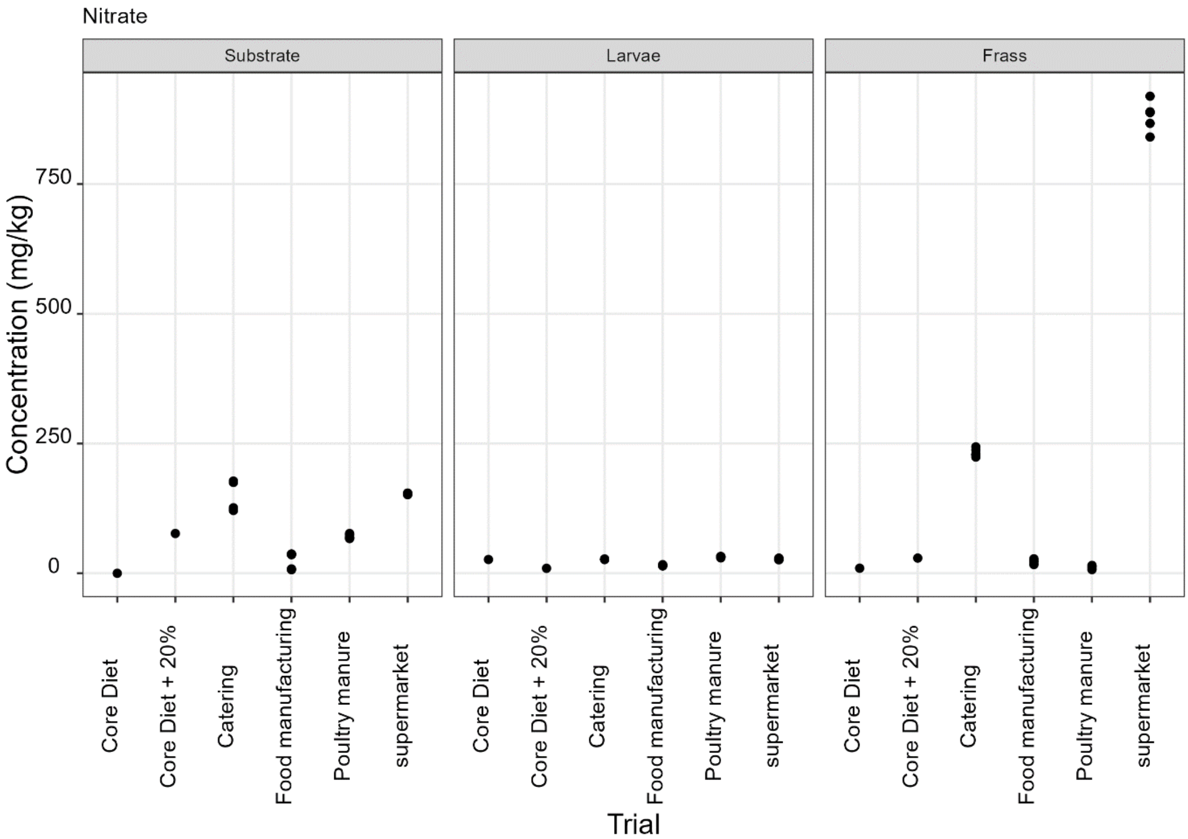
An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$ each divided by the average for the type and source.

Results that were reported as less than the limit of detection were treated as zero.

Individual results are shown in the figures and the table shows the calculated quantities.

The reporting limit for nitrate and nitrite was 1 mg/kg.

1.


Nitrate
Substrate
Larvae
Frass
Concentration (mg/kg)
750
500
250
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

2.

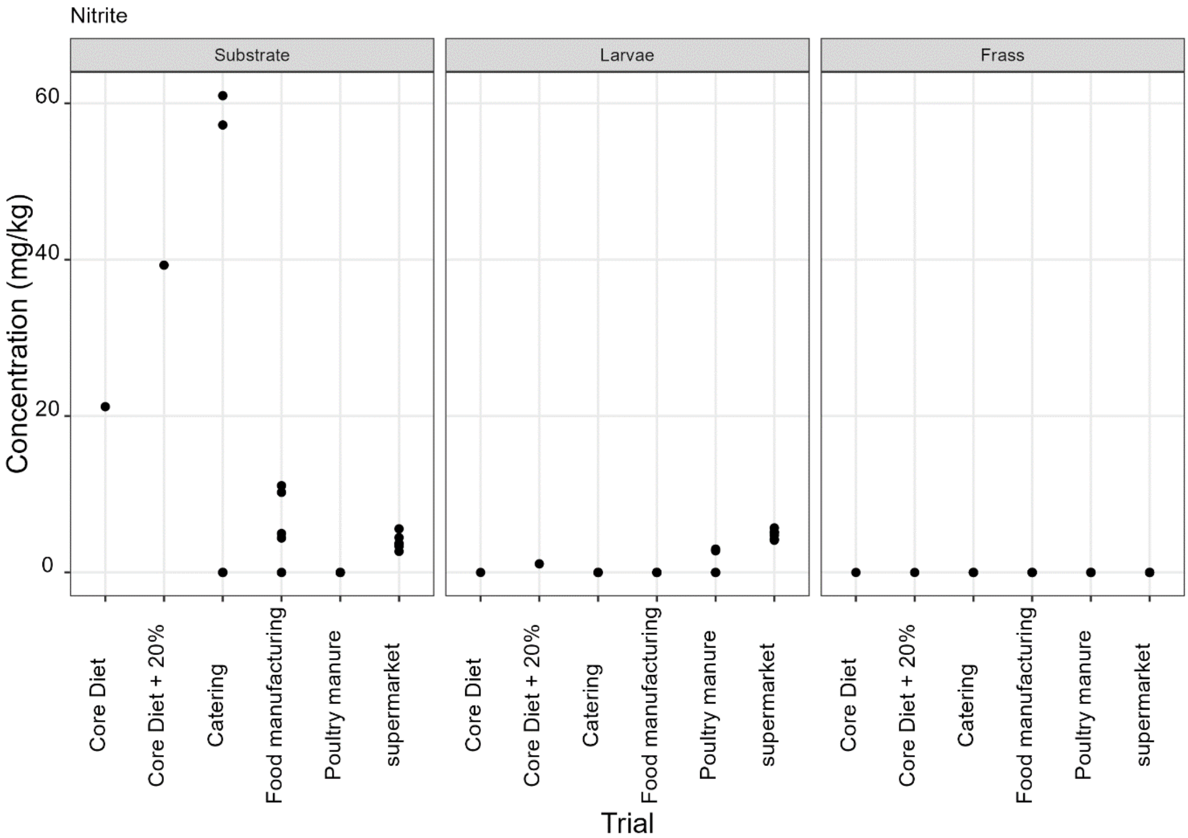

Nitrite
Substrate
Larvae
Frass
Concentration (mg/kg)
60
40
20
0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

Figure VI (1 – 2). Concentration (mg/kg) of nitrates (1) and nitrites (2) analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table X. Calculated quantities of nitrates and nitrites.

Trial	Sample Type	Analyte	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
core diet	Substrate	Nitrite	1	1	21.2	21.2	NA	NA	NA
core diet	Larvae	Nitrite	1	0	0	0	0	NA	NA
core diet	Frass	Nitrite	1	0	0	0	0	NA	NA
core diet	Substrate	Nitrate	1	0	0	0	NA	NA	NA
core diet	Larvae	Nitrate	1	1	26.6	26.6	Inf	NA	NA
core diet	Frass	Nitrate	1	1	9.8	9.8	Inf	NA	NA
core diet + 20%	Substrate	Nitrite	1	1	39.3	39.3	NA	NA	NA
core diet + 20%	Larvae	Nitrite	1	1	1.1	1.1	0.02799	NA	NA
core diet + 20%	Frass	Nitrite	1	0	0	0	0	NA	NA
core diet + 20%	Substrate	Nitrate	1	1	76.7	76.7	NA	NA	NA
core diet + 20%	Larvae	Nitrate	1	1	9.6	9.6	0.1252	NA	NA
core diet + 20%	Frass	Nitrate	1	1	29.3	29.3	0.382	NA	NA
Catering	Substrate	Nitrite	6	2	29.55	60.98	NA	0	2

Trial	Sample Type	Analyte	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Catering	Larvae	Nitrite	6	0	0	0	0	NA	NA
Catering	Frass	Nitrite	6	0	0	0	0	NA	NA
Catering	Substrate	Nitrate	6	6	149.9	177.7	NA	0.001447	0.3527
Catering	Larvae	Nitrate	6	6	26.98	27.49	0.18	-0.02964	-0.00769
Catering	Frass	Nitrate	6	6	234.1	243.5	1.562	-0.01278	0.05585
Manufacturing	Substrate	Nitrite	6	4	5.014	11.1	NA	2.128	-0.1285
Manufacturing	Larvae	Nitrite	6	0	0	0	0	NA	NA
Manufacturing	Frass	Nitrite	6	0	0	0	0	NA	NA
Manufacturing	Substrate	Nitrate	6	6	22.1	37.17	NA	-0.02296	-1.308
Manufacturing	Larvae	Nitrate	6	6	15.48	16.58	0.7005	-0.04703	0.05446
Manufacturing	Frass	Nitrate	6	6	23.26	27.95	1.052	-0.2231	0.3095
Poultry manure	Substrate	Nitrite	6	3	0	0	NA	NA	NA
Poultry manure	Larvae	Nitrite	6	3	1.094	2.989	Inf	1.266	0.7331
Poultry manure	Frass	Nitrite	6	3	0	0	NA	NA	NA
Poultry manure	Substrate	Nitrate	6	3	71.59	76.72	NA	-0.01292	0.1004

Trial	Sample Type	Analyte	Number of injections	Detected	Mean (mg/kg)	Max (mg/kg)	Accumulation	Within replicate difference	Between replicate difference
Poultry manure	Larvae	Nitrate	6	5	31.26	32.55	0.4367	-0.0212	-0.05423
Poultry manure	Frass	Nitrate	6	3	10.48	15.21	0.1464	-0.2045	-0.5543
supermarket	Substrate	Nitrite	6	6	3.796	5.574	NA	-0.01199	-0.1661
supermarket	Larvae	Nitrite	6	6	4.743	5.697	1.249	0.1413	-0.1303
supermarket	Frass	Nitrite	6	3	0	0	0	NA	NA
supermarket	Substrate	Nitrate	6	6	152.9	154.6	NA	0.01397	0.000861
supermarket	Larvae	Nitrate	6	6	27.77	29.1	0.1816	-0.02059	0.09413
supermarket	Frass	Nitrate	6	3	875	919.3	5.723	-0.01855	-0.04845

Inf = inferred, NA = not applicable

Appendix H - PFAS

The aim of the analysis was to

- estimate the amount of bioaccumulation from substrate into larvae and frass

Results that were reported as less than the limit of detection were treated as zero.

Substances which were not detected in any extracts from any samples are not reported here. They are: PFUdA, PFBS, PFDS, 11Cl.PF3OuDS, 9Cl.PF3ONS, N.MeFOSAA, N.EtFOSAA, 6_2.FTS, 8_2.FTS, FHxSA, PFDoA, PFTrDA and PFTeDA.

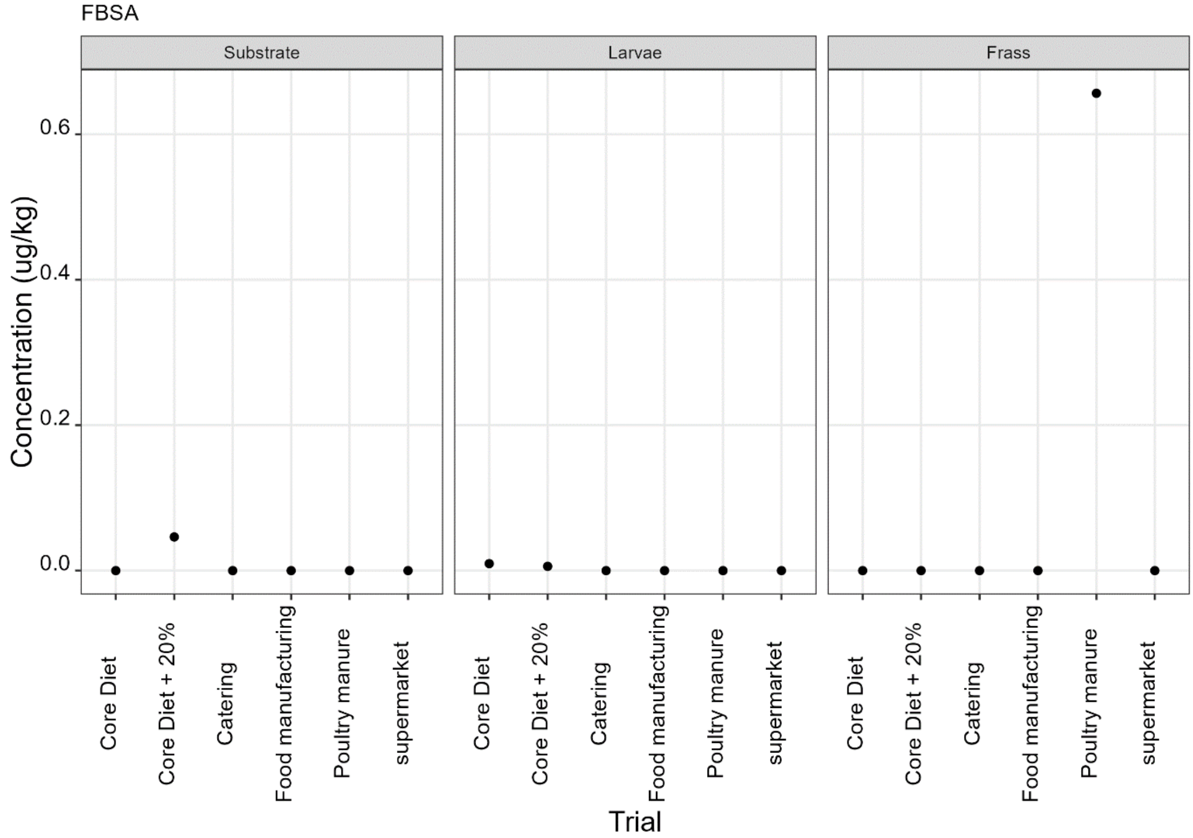
The reporting limits are shown below:

Table XI. Reporting limits for PFAS analysis.

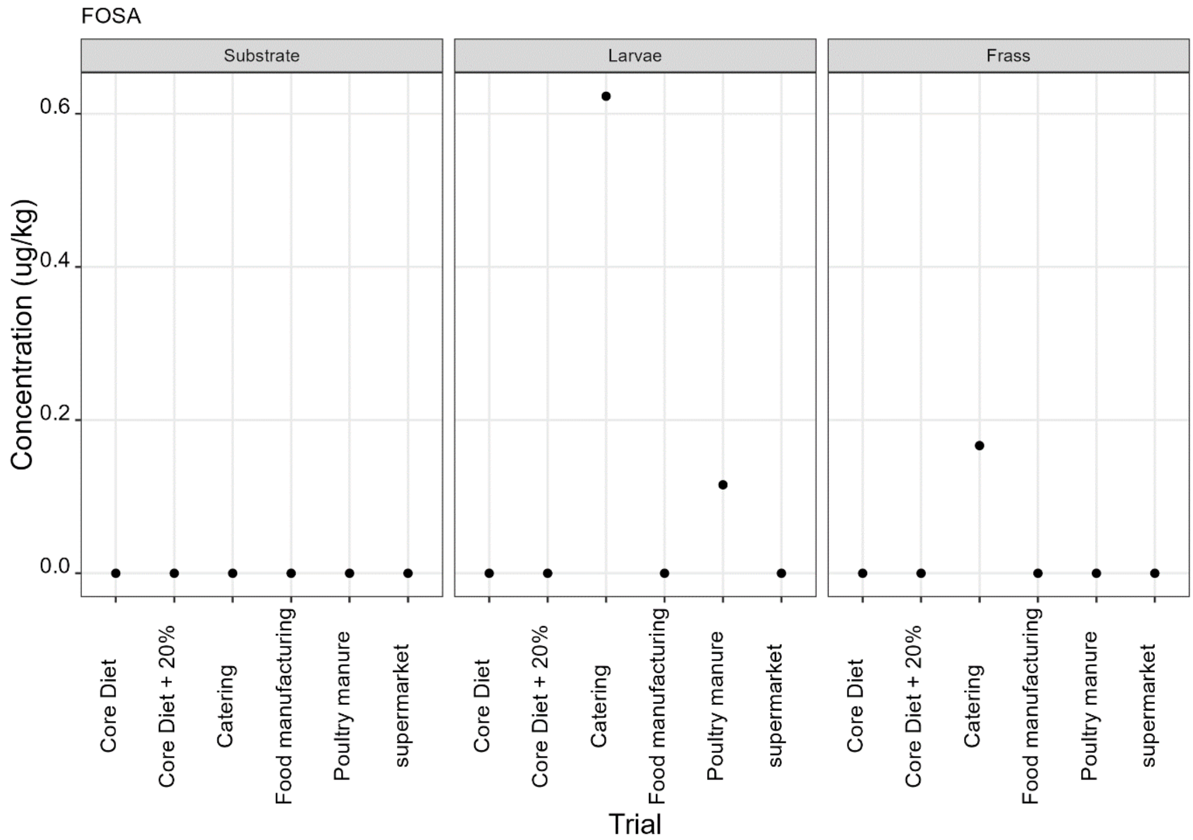
PFAS	Reporting limit (µg/kg)	PFAS	Reporting limit (µg/kg)
L-PFHxS	0.1	PFDS	0.1
Br-PFHxS	0.1	PFPeS	0.1
Total PFHxS	0.2	NaDONA	0.1
PFOA	0.1	HFPO-DA	0.005
PFNA	0.1	11Cl-PF3OuDS	0.1
L-PFOS	0.1	9Cl-PF3ONS	0.005
Br_PFOS	0.1	N-MeFOSAA	0.1
Total PFOS	0.2	N-EtFOSAA	0.1
PFBA	0.5	4_2 FTS	0.1
PFHxA	0.1	6_2 FTS	0.1
PFHpA	0.1	8_2 FTS	0.1
PFDA	0.1	FOSA	0.1
PFUdA	0.1	FHxSA	0.005
PFPeA	0.005	FBSA	0.005
PFBS	0.1	PFDoA	ND
PFHpS	0.1	PFTrDA	ND
PFNS	0	PFTeDA	ND

ND = not detected

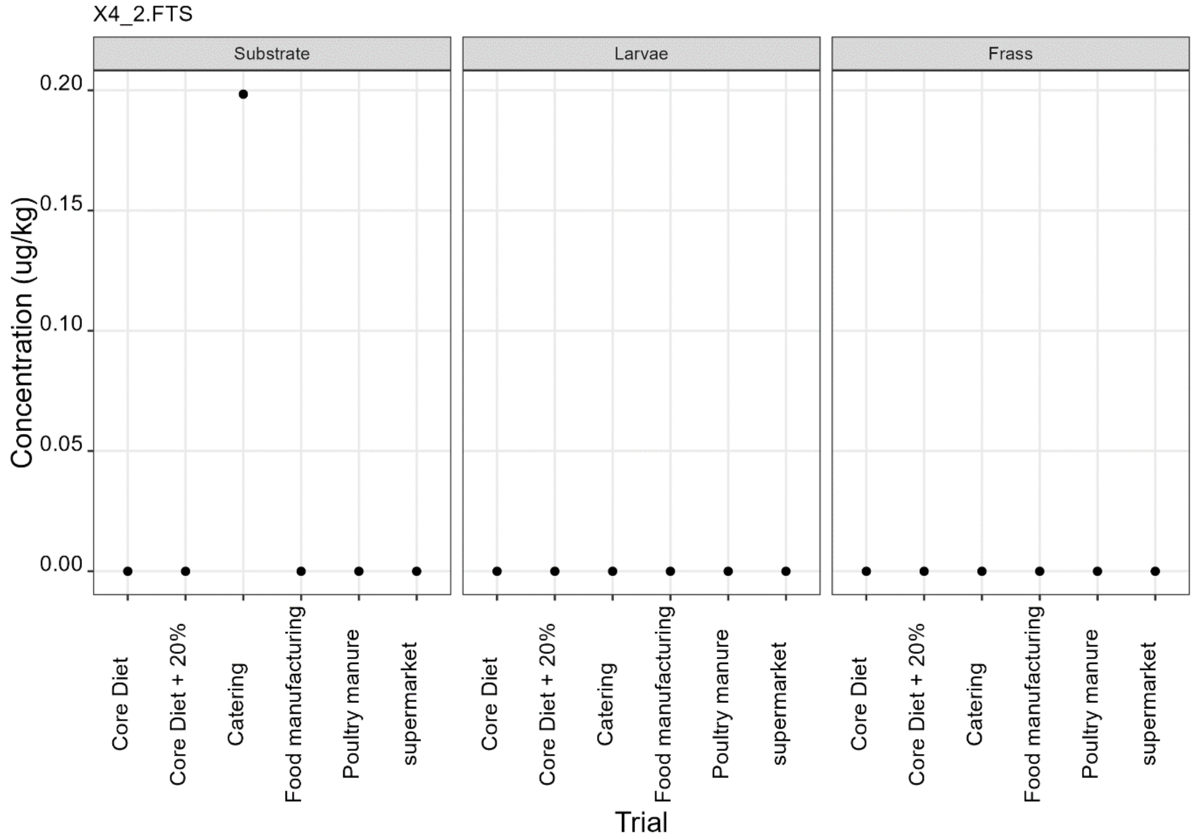
1.


FBSA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

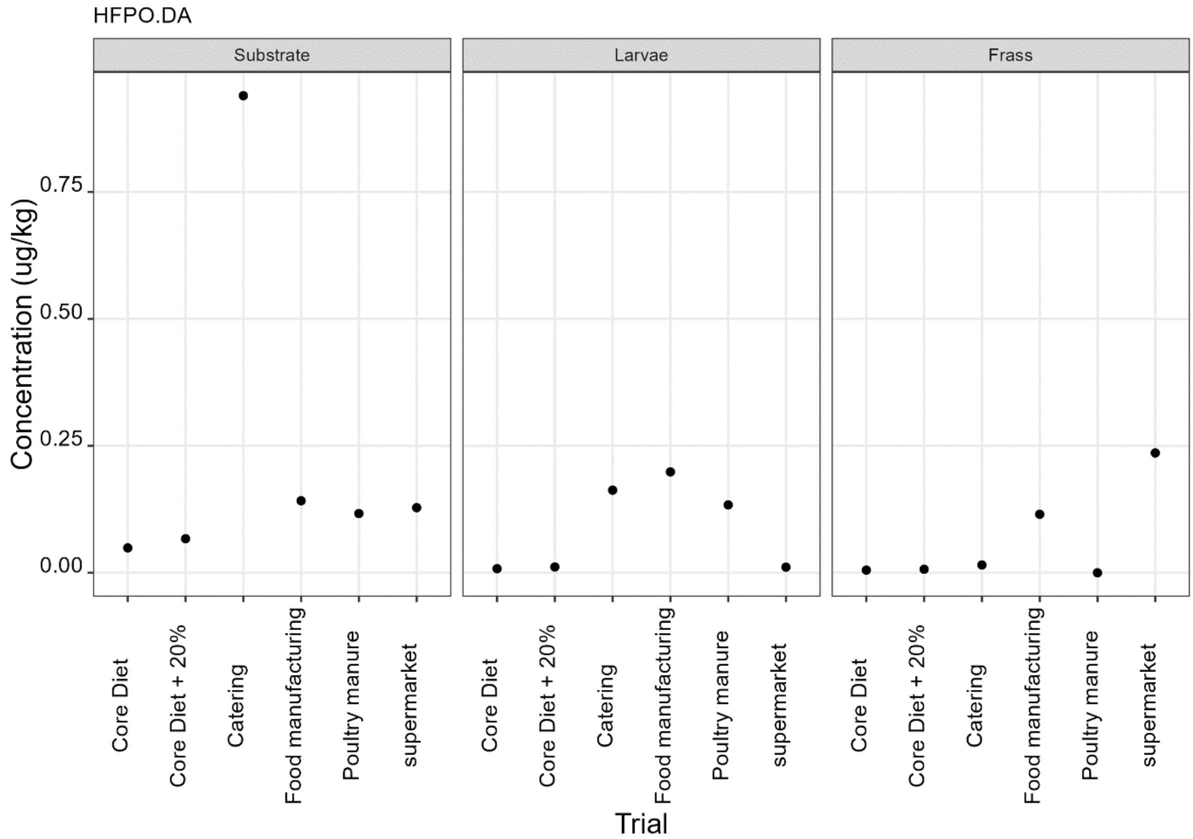
2.


FOSA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

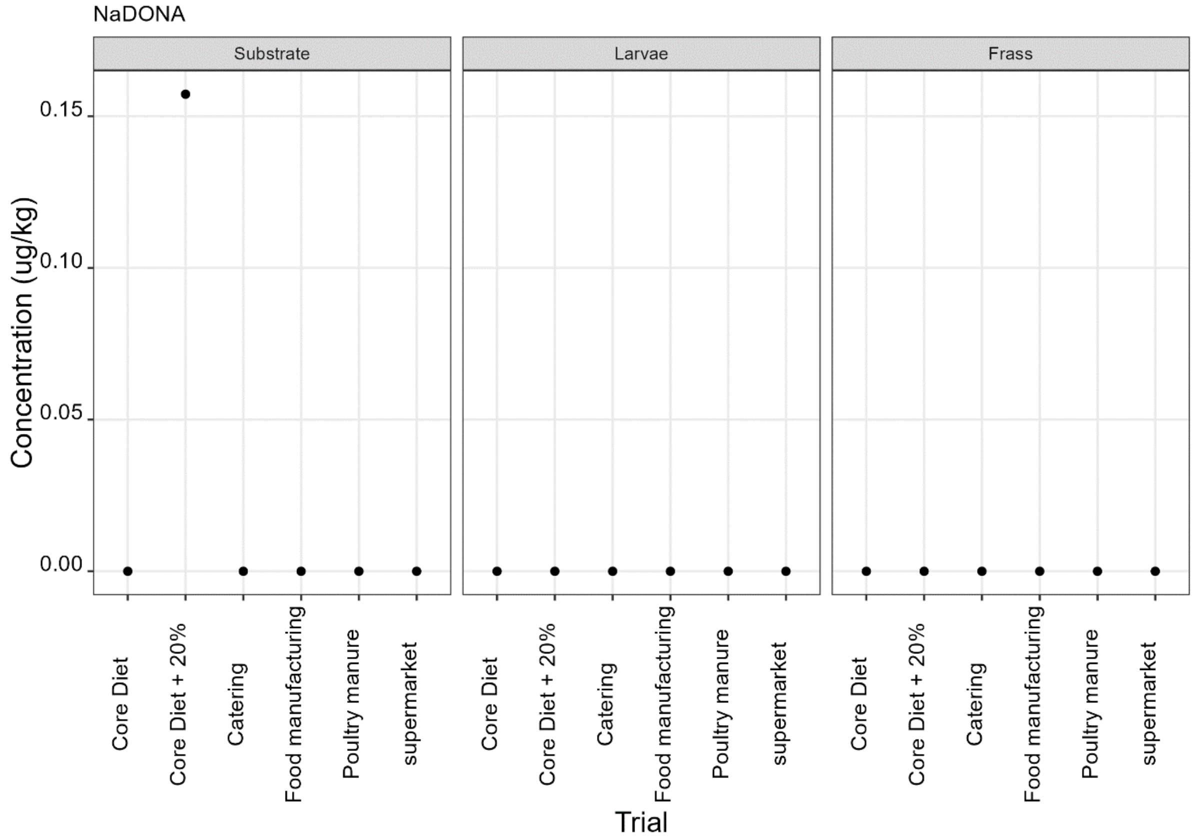
3.


X4_2.FTS
Substrate
Larvae
Frass
0.20
0.15
0.10
0.05
0.00
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

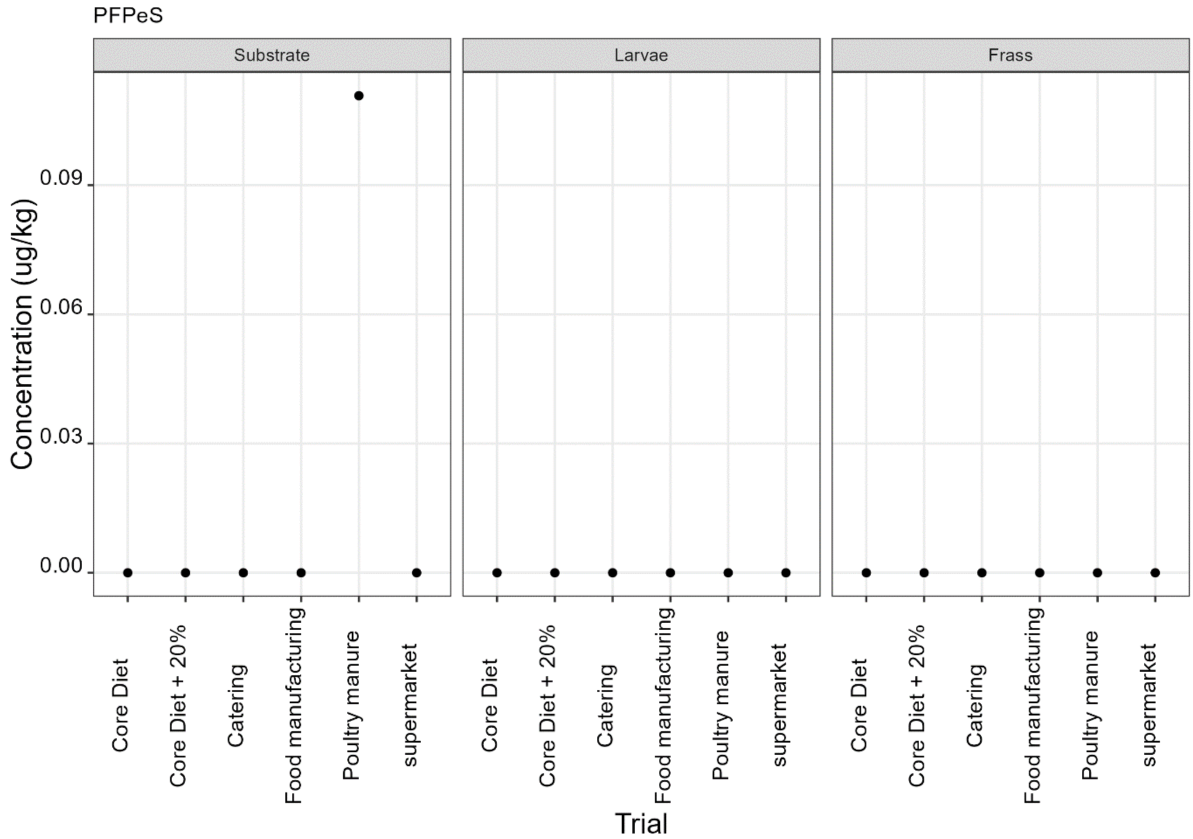
4.


HFPO.DA
Substrate
Larvae
Frass
0.75
0.50
0.25
0.00
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

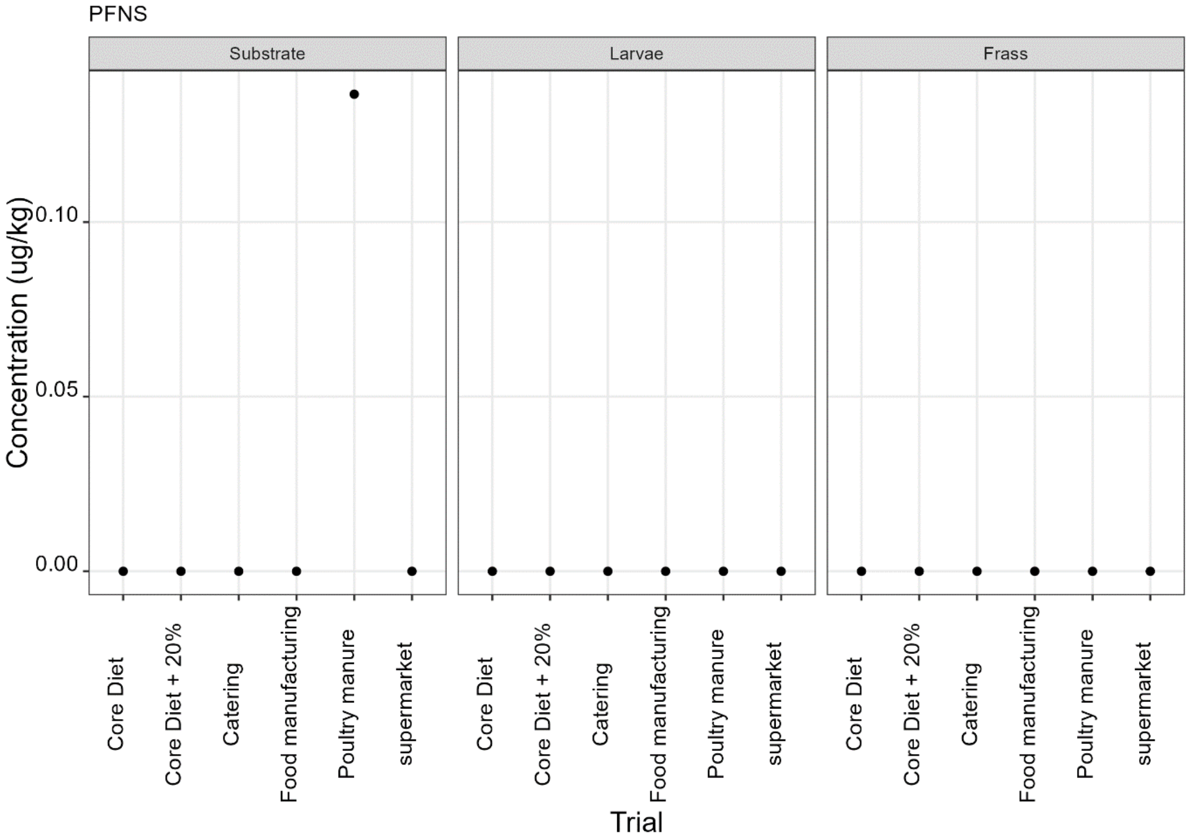
5.


NaDONA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

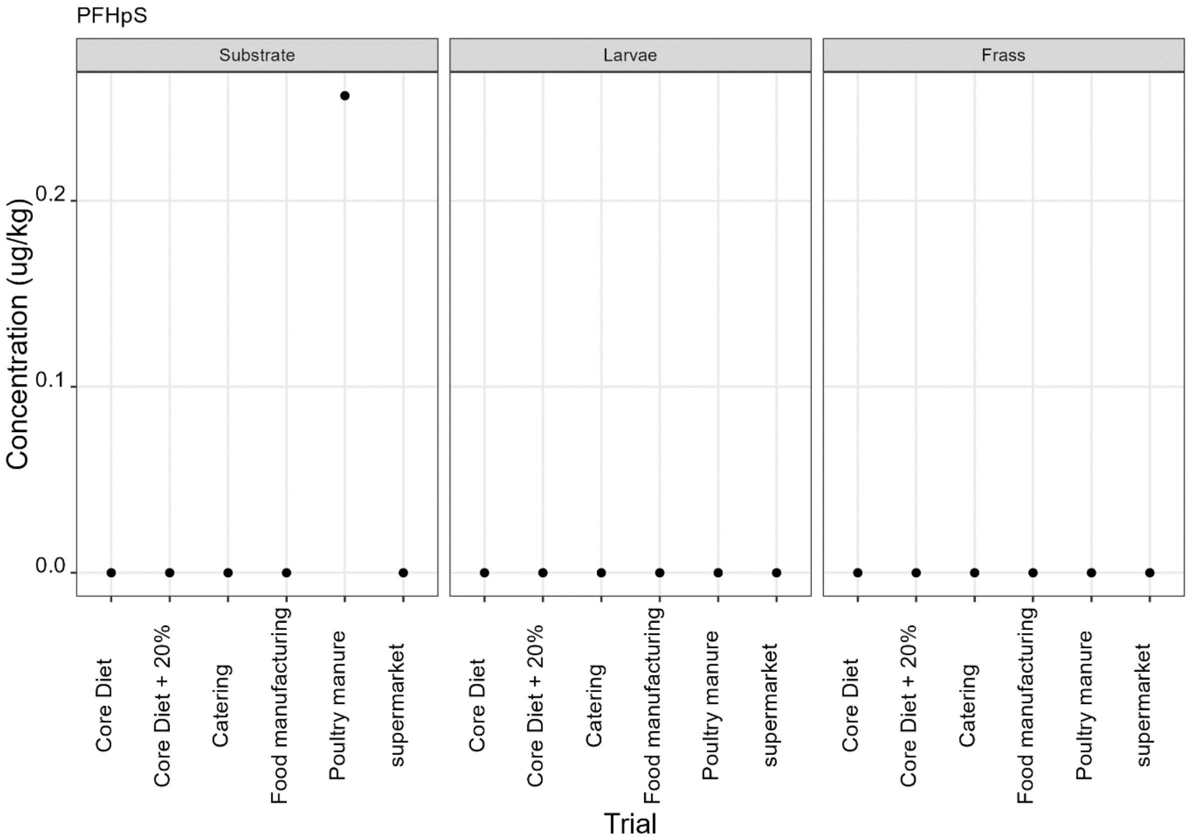
6.


PFPeS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.09
0.06
0.03
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

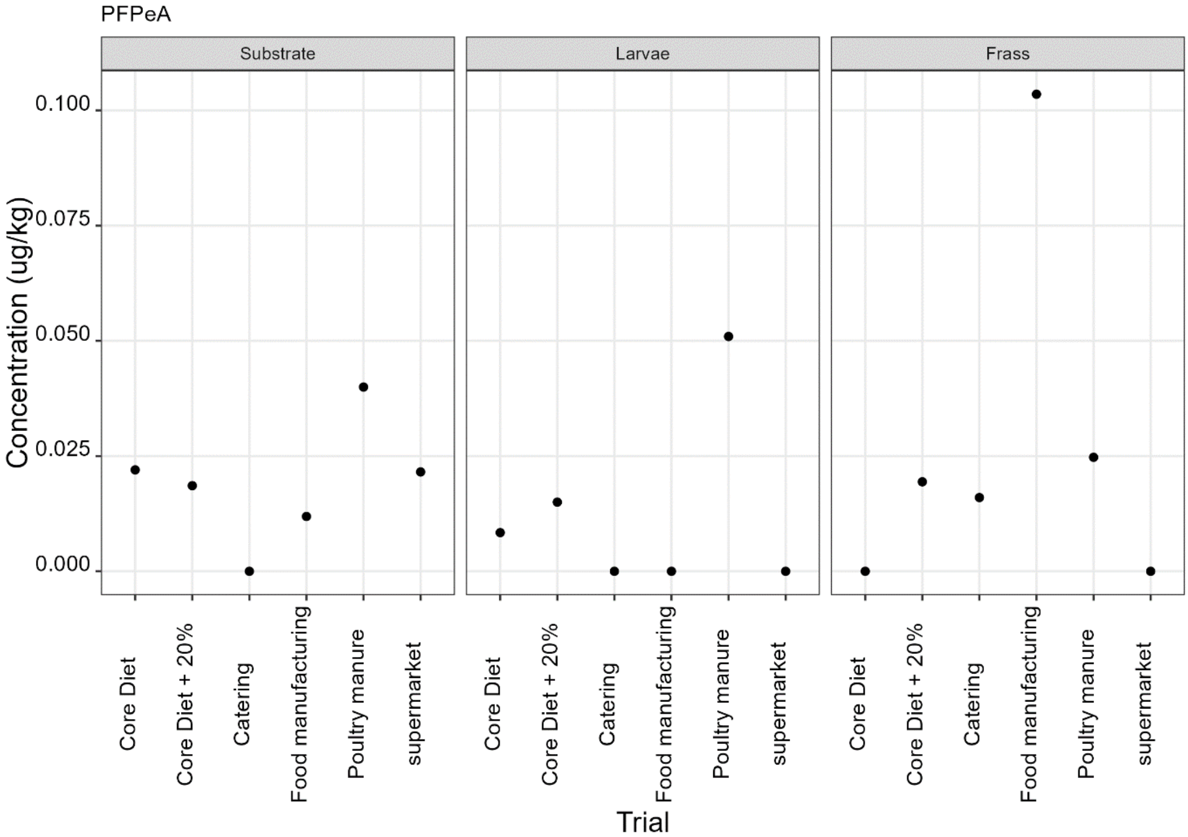
7.


PFNS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

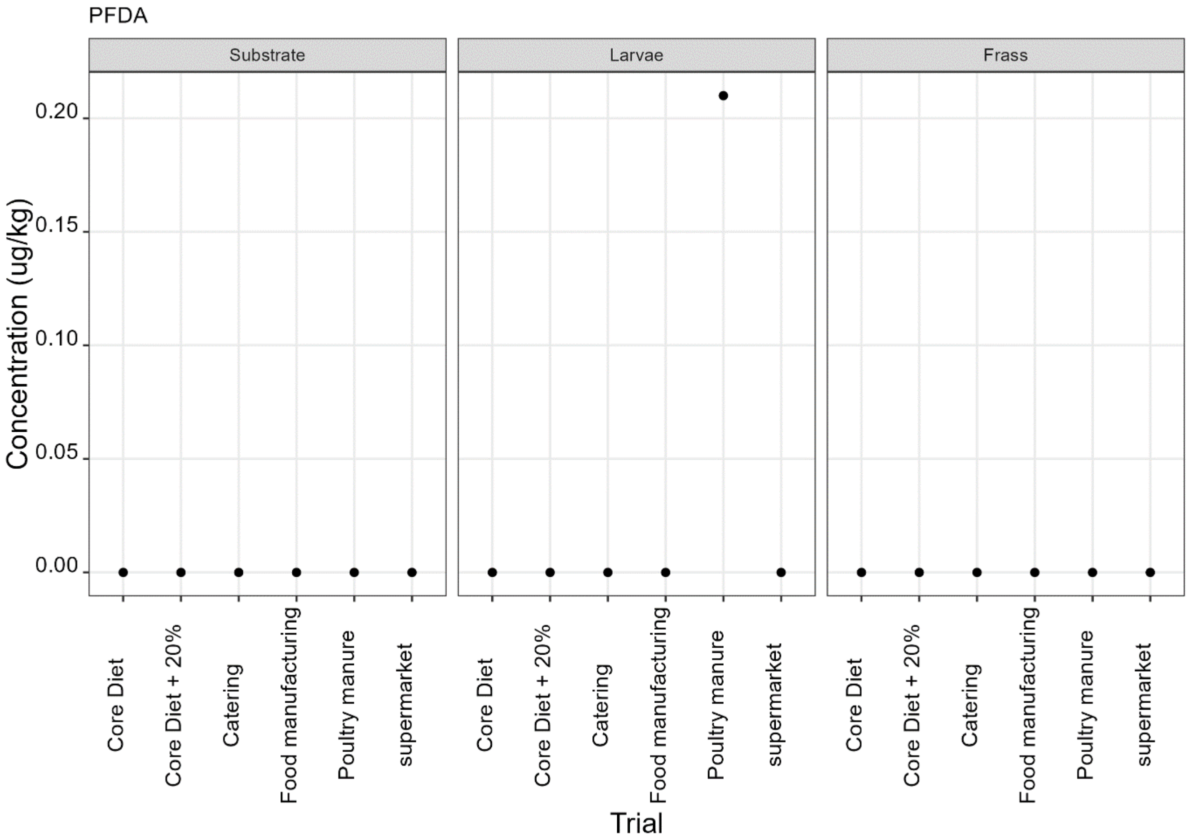
8.


PFHpS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

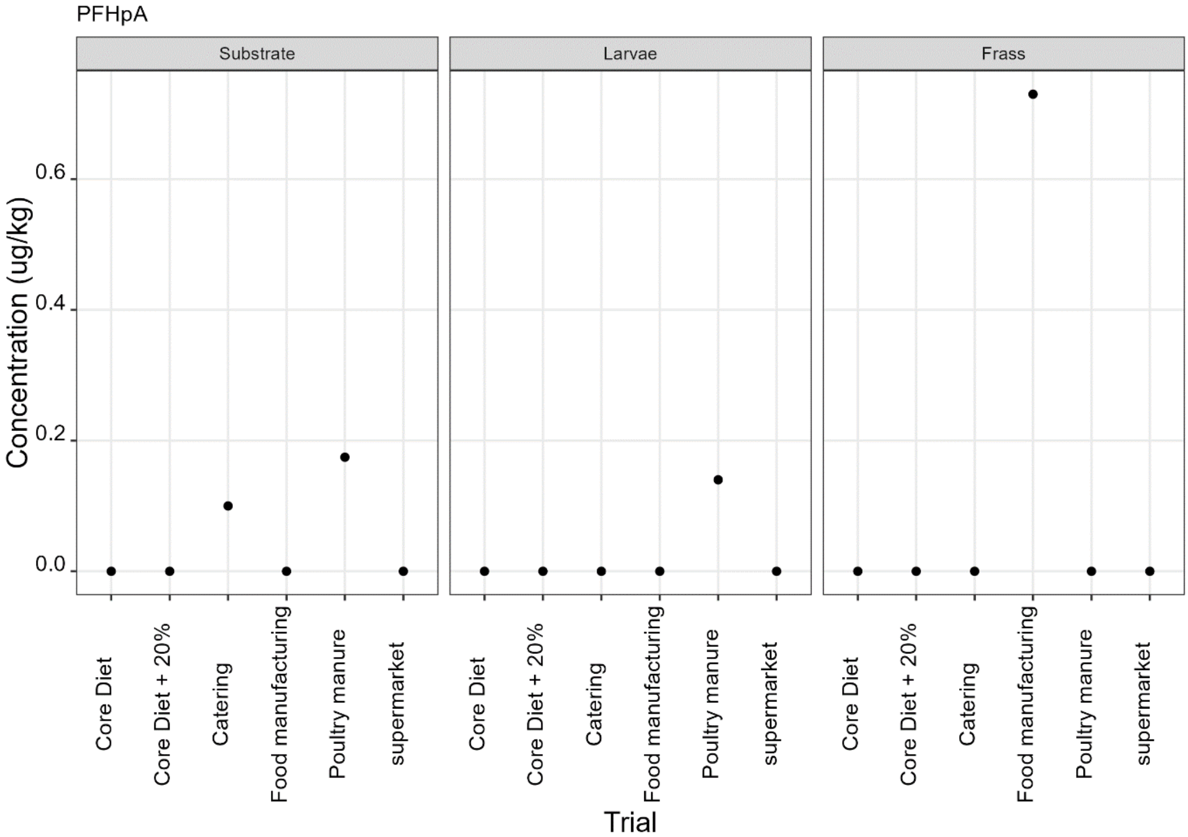
9.


PFPeA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.100
0.075
0.050
0.025
0.000
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

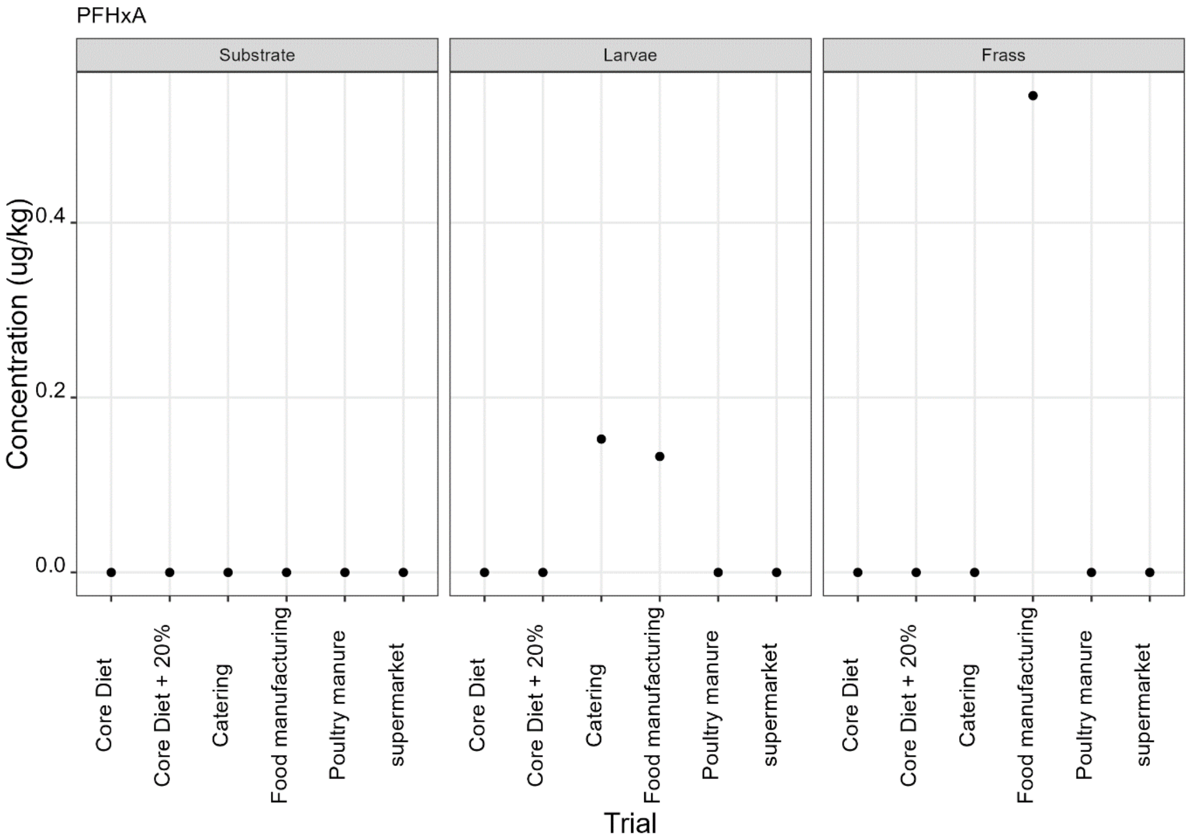
10.


PFDA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.20
0.15
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

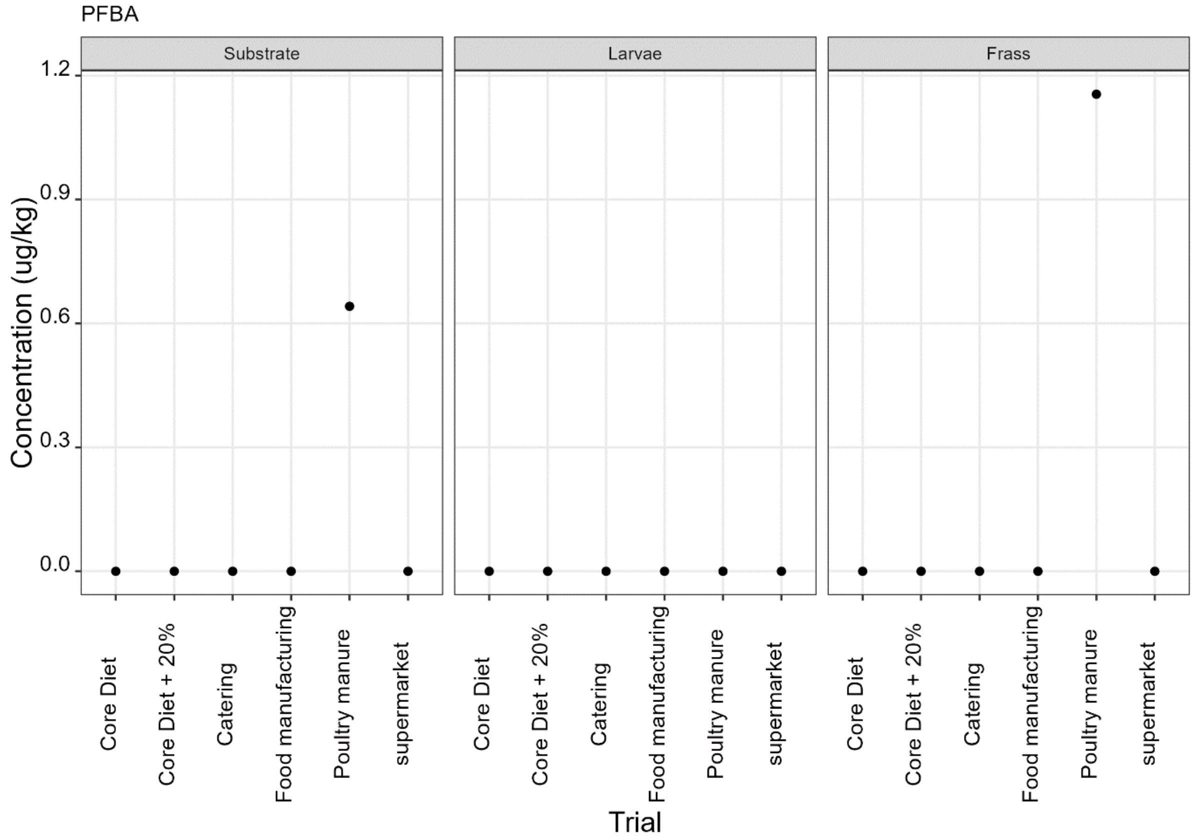
11.


PFHpA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.6
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

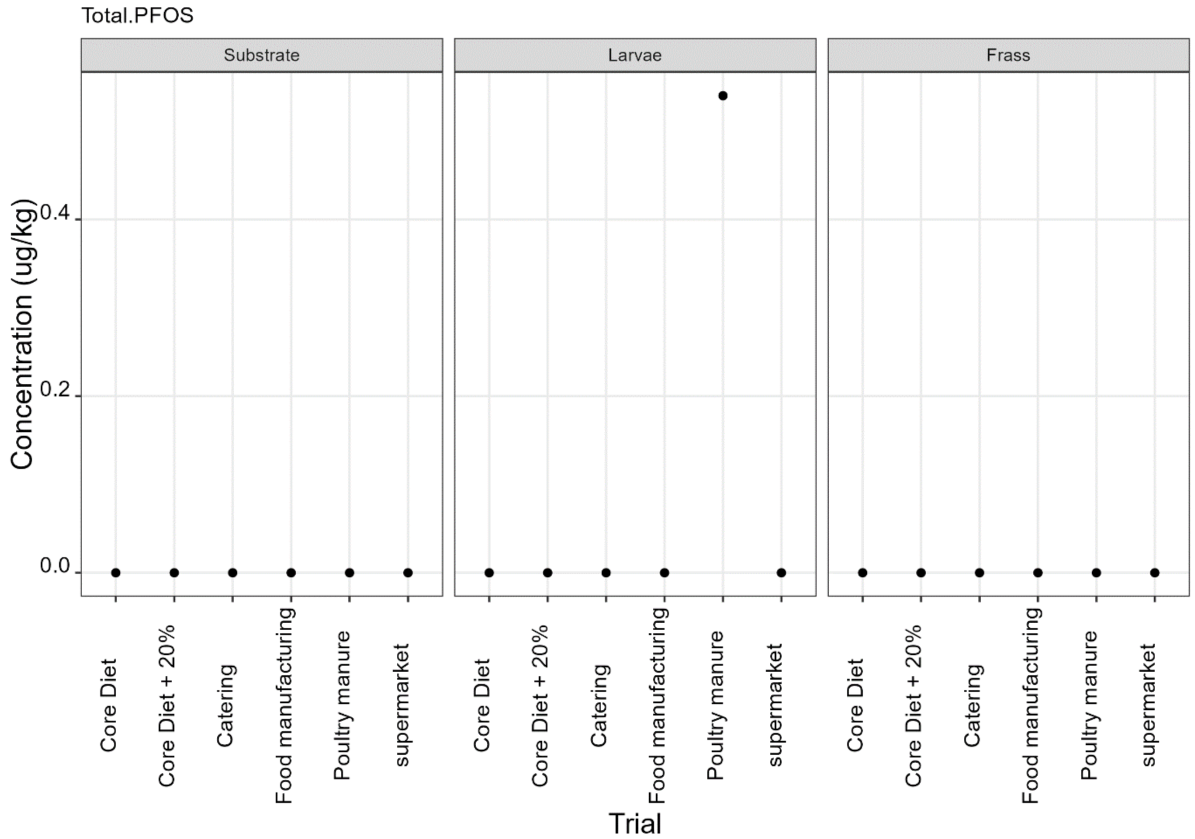
12.


PFHxA
Substrate
Larvae
Frass
Concentration (ug/kg)
0.4
0.2
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

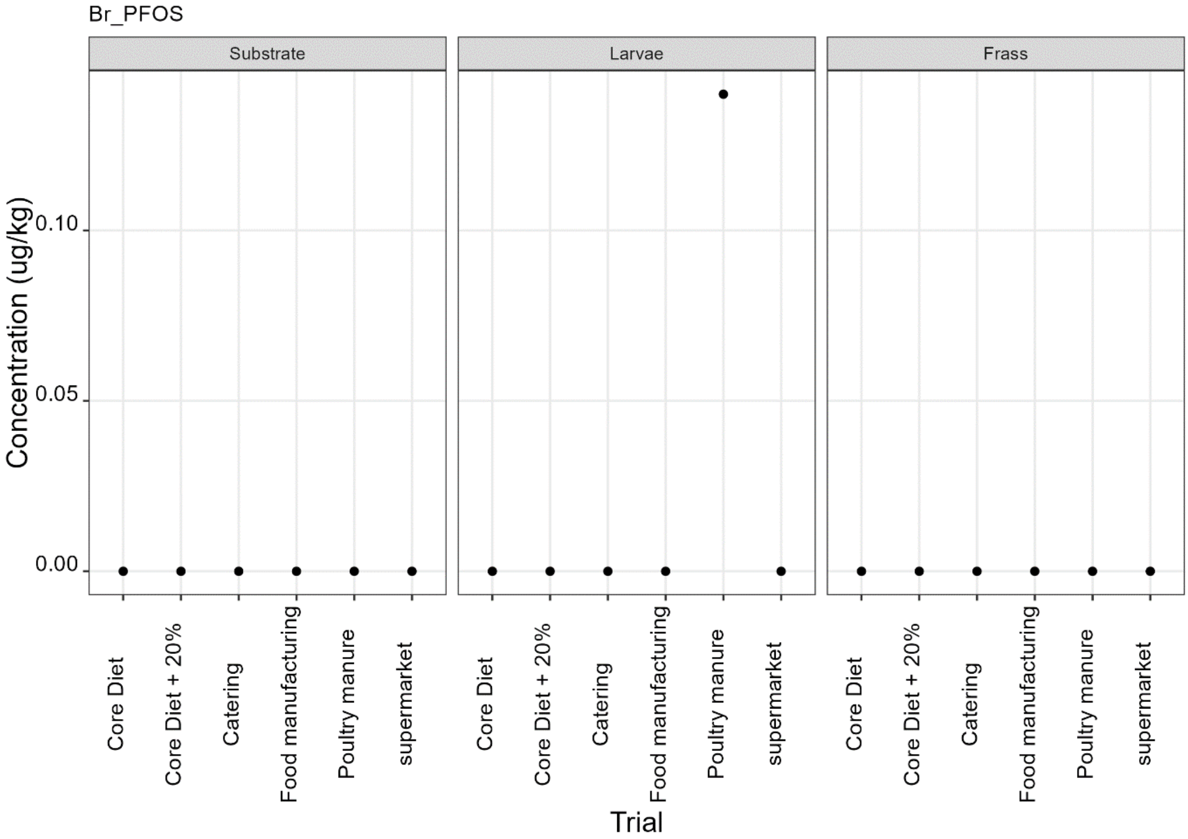
13.


PFBA
Substrate
Larvae
Frass
1.2
0.9
0.6
0.3
0.0
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

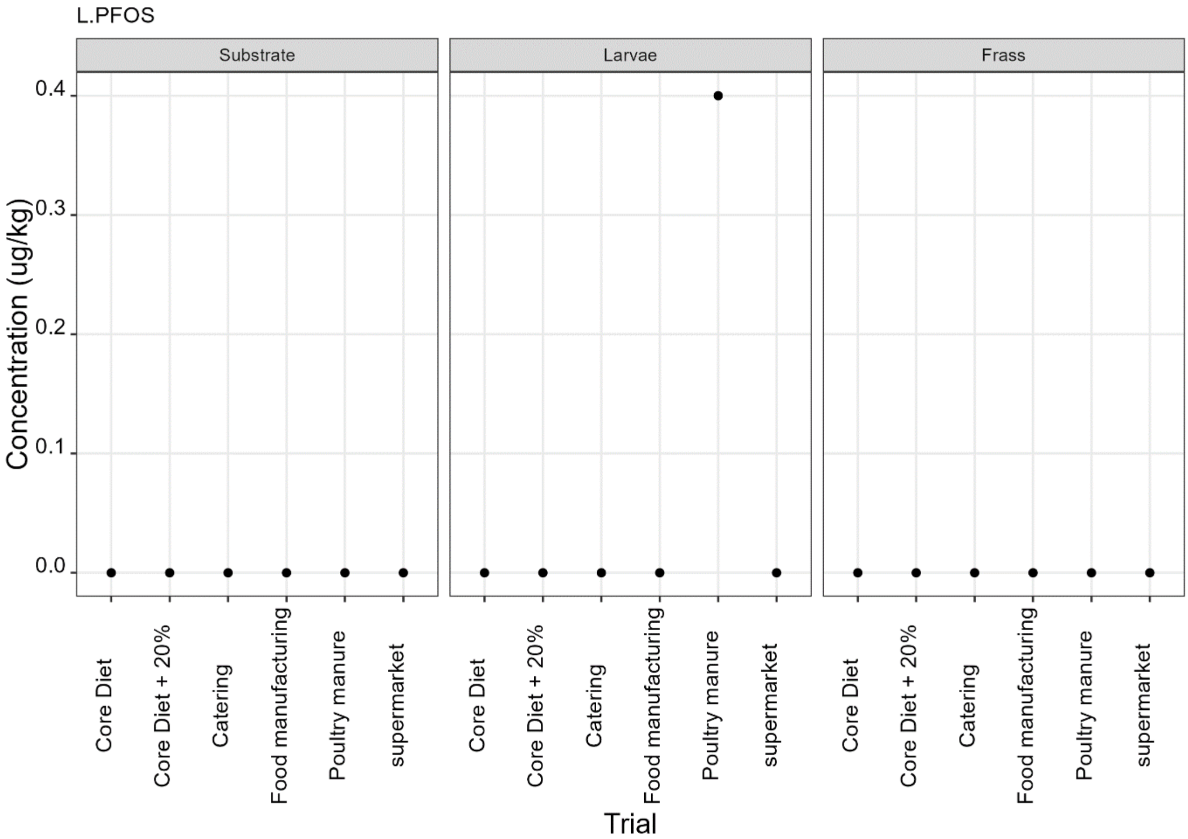
14.


Total.PFOS
Substrate
Larvae
Frass
0.4
0.2
0.0
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

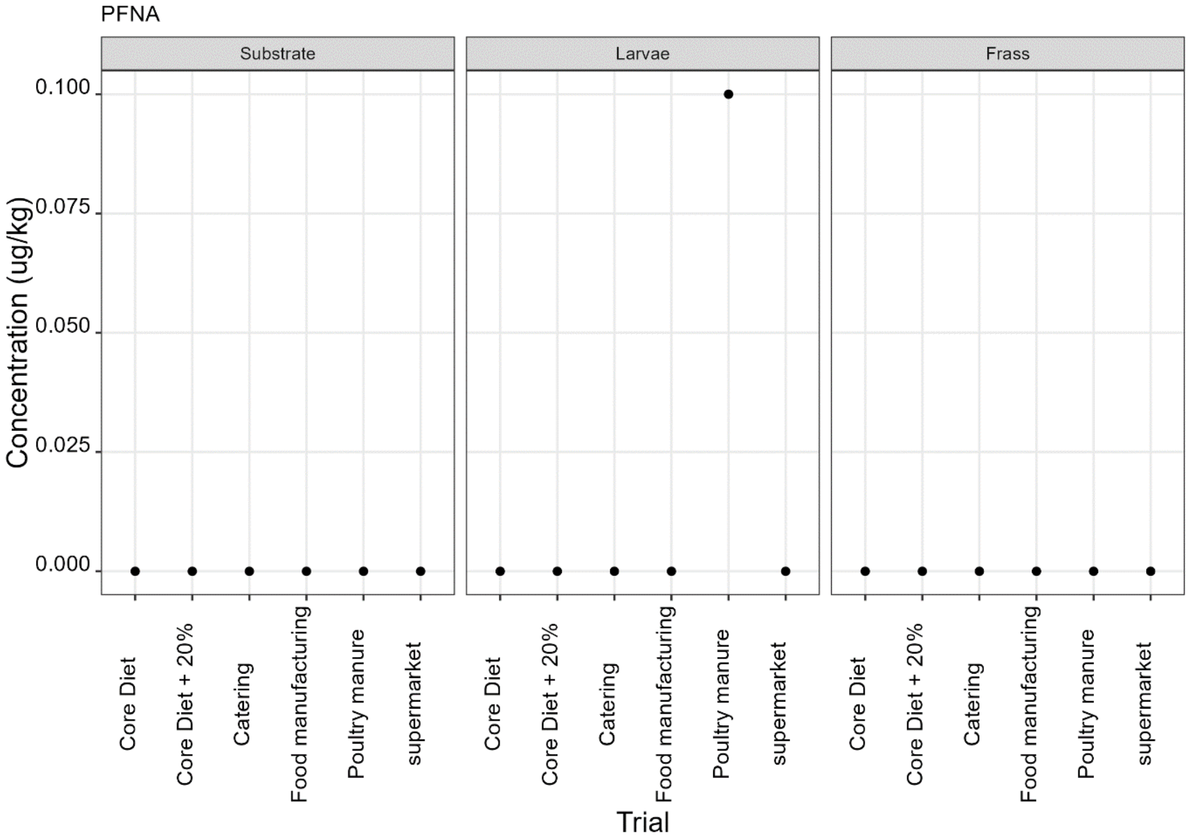
15.


Br_PFOS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.10
0.05
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

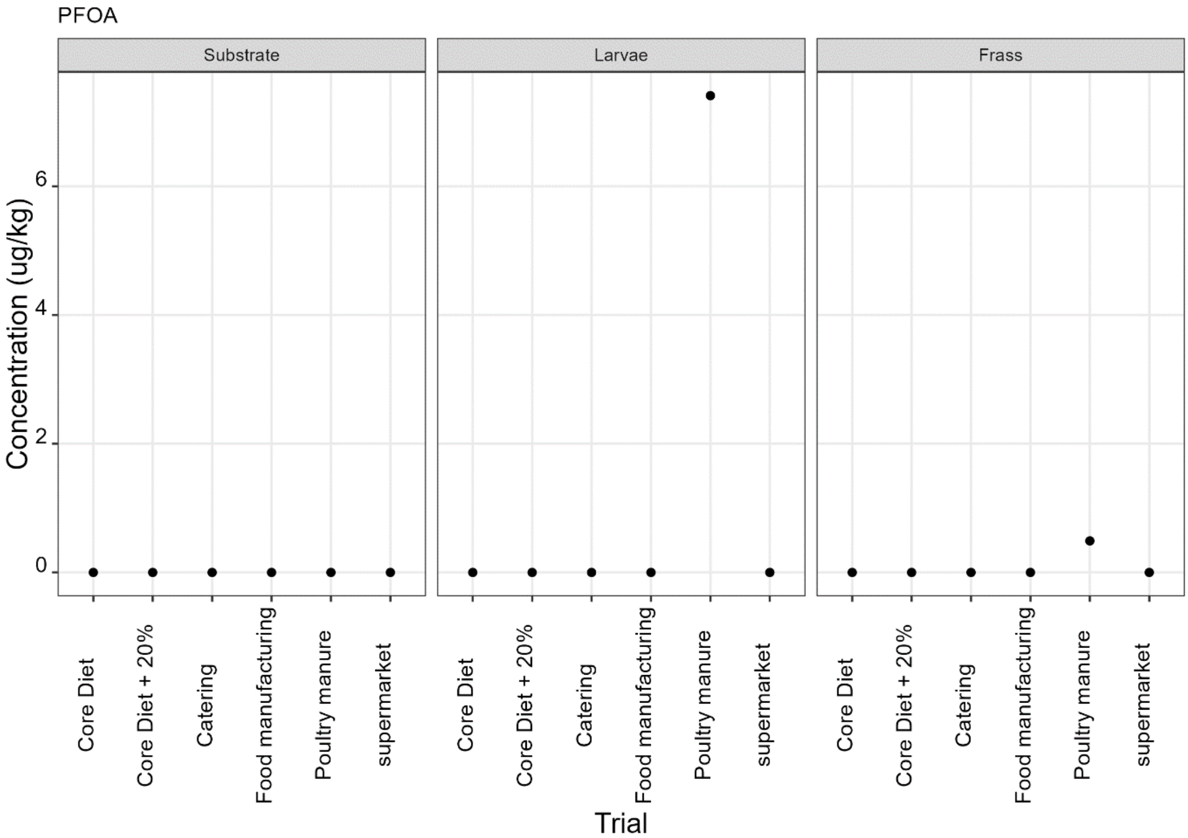
16.


L.PFOS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.4
0.3
0.2
0.1
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

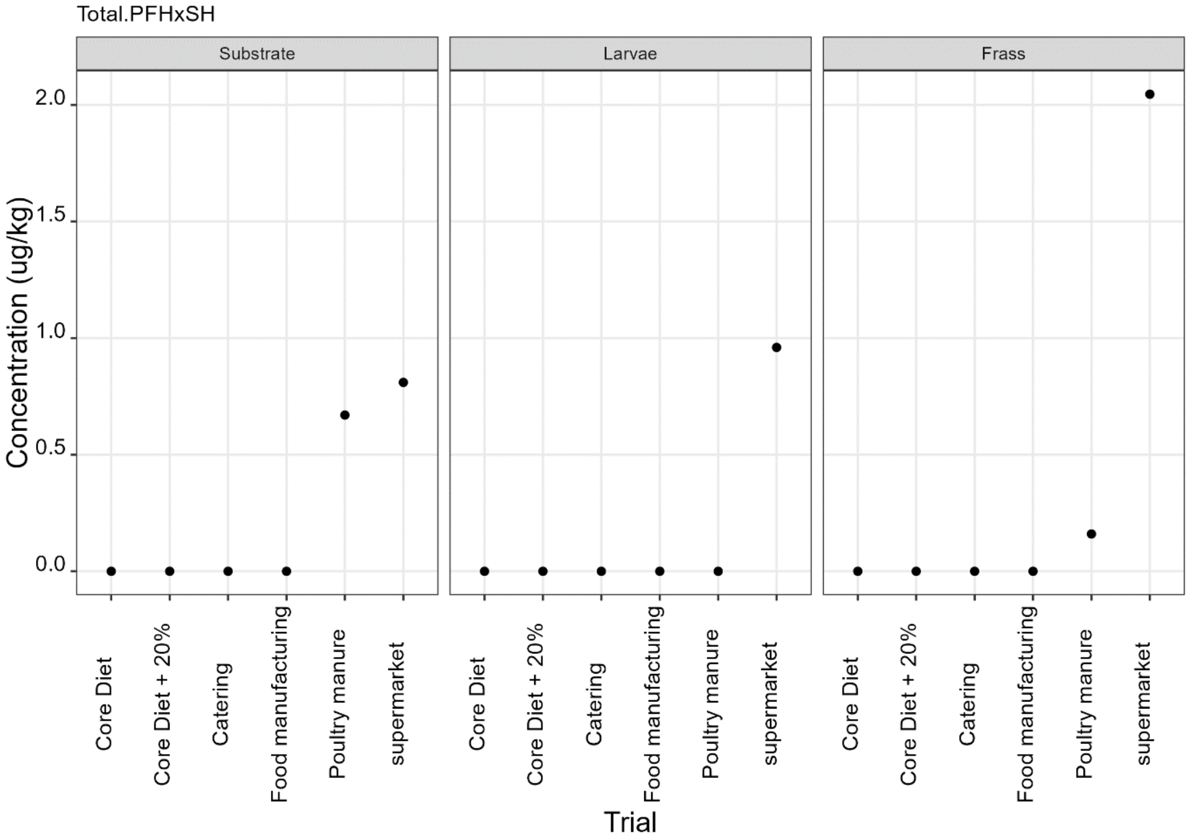
17.


PFNA
Substrate
Larvae
Frass
0.100
0.075
0.050
0.025
0.000
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

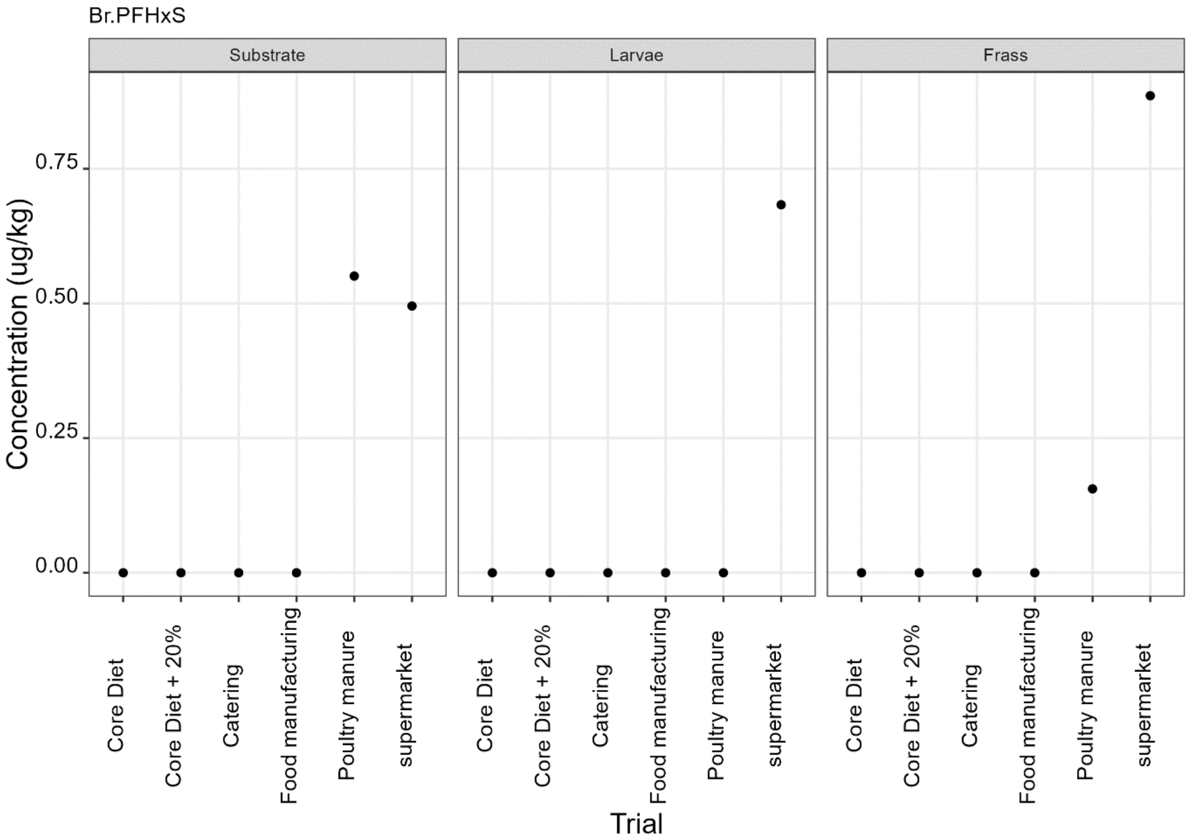
18.


PFOA
Substrate
Larvae
Frass
6
4
2
0
Concentration (ug/kg)
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

19.


Total.PFHxSH
Substrate
Larvae
Frass
Concentration (ug/kg)
2.0
1.5
1.0
0.5
0.0
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

20.


Br.PFHxS
Substrate
Larvae
Frass
Concentration (ug/kg)
0.75
0.50
0.25
0.00
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
supermarket
Trial

21.

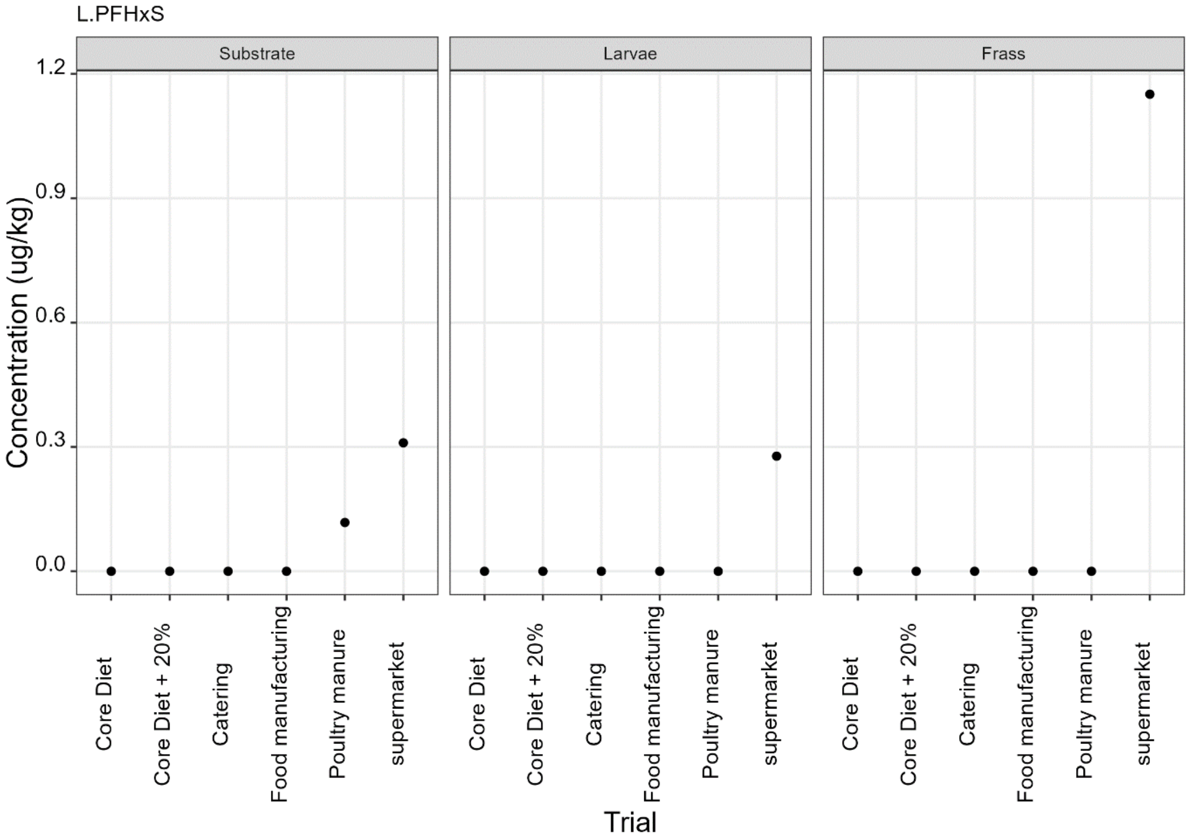


Figure VII (1 – 21). Concentration (μg/kg) of PFAS analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table XII. Calculated quantities of PFAS.

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
core diet	Substrate	L.PFHxS	1	0	0	NA
core diet	Larvae	L.PFHxS	1	0	0	NA
core diet	Frass	L.PFHxS	1	0	0	NA
core diet	Substrate	Br.PFHxS	1	0	0	NA
core diet	Larvae	Br.PFHxS	1	0	0	NA
core diet	Frass	Br.PFHxS	1	0	0	NA
core diet	Substrate	Total.PFHxSH	1	0	0	NA
core diet	Larvae	Total.PFHxSH	1	0	0	NA
core diet	Frass	Total.PFHxSH	1	0	0	NA
core diet	Substrate	PFOA	1	0	0	NA
core diet	Larvae	PFOA	1	0	0	NA
core diet	Frass	PFOA	1	0	0	NA
core diet	Substrate	PFNA	1	0	0	NA
core diet	Larvae	PFNA	1	0	0	NA
core diet	Frass	PFNA	1	0	0	NA
core diet	Substrate	L.PFOS	1	0	0	NA
core diet	Larvae	L.PFOS	1	0	0	NA
core diet	Frass	L.PFOS	1	0	0	NA
core diet	Substrate	Br_PFOS	1	0	0	NA
core diet	Larvae	Br_PFOS	1	0	0	NA
core diet	Frass	Br_PFOS	1	0	0	NA
core diet	Substrate	Total.PFOS	1	0	0	NA
core diet	Larvae	Total.PFOS	1	0	0	NA
core diet	Frass	Total.PFOS	1	0	0	NA
core diet	Substrate	PFBA	1	0	0	NA
core diet	Larvae	PFBA	1	0	0	NA
core diet	Frass	PFBA	1	0	0	NA
core diet	Substrate	PFHxA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
core diet	Larvae	PFHxA	1	0	0	NA
core diet	Frass	PFHxA	1	0	0	NA
core diet	Substrate	PFHpA	1	0	0	NA
core diet	Larvae	PFHpA	1	0	0	NA
core diet	Frass	PFHpA	1	0	0	NA
core diet	Substrate	PFDA	1	0	0	NA
core diet	Larvae	PFDA	1	0	0	NA
core diet	Frass	PFDA	1	0	0	NA
core diet	Substrate	PFPeA	1	1	0.022003	NA
core diet	Larvae	PFPeA	1	1	0.008391	0.3813
core diet	Frass	PFPeA	1	0	0	0
core diet	Substrate	PFHpS	1	0	0	NA
core diet	Larvae	PFHpS	1	0	0	NA
core diet	Frass	PFHpS	1	0	0	NA
core diet	Substrate	PFNS	1	0	0	NA
core diet	Larvae	PFNS	1	0	0	NA
core diet	Frass	PFNS	1	0	0	NA
core diet	Substrate	PFPeS	1	0	0	NA
core diet	Larvae	PFPeS	1	0	0	NA
core diet	Frass	PFPeS	1	0	0	NA
core diet	Substrate	NaDONA	1	0	0	NA
core diet	Larvae	NaDONA	1	0	0	NA
core diet	Frass	NaDONA	1	0	0	NA
core diet	Substrate	HFPO.DA	1	1	0.049028	NA
core diet	Larvae	HFPO.DA	1	1	0.00813	0.1658
core diet	Frass	HFPO.DA	1	1	0.005312	0.1084
core diet	Substrate	X4_2.FTS	1	0	0	NA
core diet	Larvae	X4_2.FTS	1	0	0	NA
core diet	Frass	X4_2.FTS	1	0	0	NA
core diet	Substrate	FOSA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
core diet	Larvae	FOSA	1	0	0	NA
core diet	Frass	FOSA	1	0	0	NA
core diet	Substrate	FBSA	1	0	0	NA
core diet	Larvae	FBSA	1	1	0.009628	Inf
core diet	Frass	FBSA	1	0	0	NA
core diet + 20%	Substrate	L.PFHxS	1	0	0	NA
core diet + 20%	Larvae	L.PFHxS	1	0	0	NA
core diet + 20%	Frass	L.PFHxS	1	0	0	NA
core diet + 20%	Substrate	Br.PFHxS	1	0	0	NA
core diet + 20%	Larvae	Br.PFHxS	1	0	0	NA
core diet + 20%	Frass	Br.PFHxS	1	0	0	NA
core diet + 20%	Substrate	Total.PFHxSH	1	0	0	NA
core diet + 20%	Larvae	Total.PFHxSH	1	0	0	NA
core diet + 20%	Frass	Total.PFHxSH	1	0	0	NA
core diet + 20%	Substrate	PFOA	1	0	0	NA
core diet + 20%	Larvae	PFOA	1	0	0	NA
core diet + 20%	Frass	PFOA	1	0	0	NA
core diet + 20%	Substrate	PFNA	1	0	0	NA
core diet + 20%	Larvae	PFNA	1	0	0	NA
core diet + 20%	Frass	PFNA	1	0	0	NA
core diet + 20%	Substrate	L.PFOS	1	0	0	NA
core diet + 20%	Larvae	L.PFOS	1	0	0	NA
core diet + 20%	Frass	L.PFOS	1	0	0	NA
core diet + 20%	Substrate	Br_PFOS	1	0	0	NA
core diet + 20%	Larvae	Br_PFOS	1	0	0	NA
core diet + 20%	Frass	Br_PFOS	1	0	0	NA
core diet + 20%	Substrate	Total.PFOS	1	0	0	NA
core diet + 20%	Larvae	Total.PFOS	1	0	0	NA
core diet + 20%	Frass	Total.PFOS	1	0	0	NA
core diet + 20%	Substrate	PFBA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
core diet + 20%	Larvae	PFBA	1	0	0	NA
core diet + 20%	Frass	PFBA	1	0	0	NA
core diet + 20%	Substrate	PFHxA	1	0	0	NA
core diet + 20%	Larvae	PFHxA	1	0	0	NA
core diet + 20%	Frass	PFHxA	1	0	0	NA
core diet + 20%	Substrate	PFHpA	1	0	0	NA
core diet + 20%	Larvae	PFHpA	1	0	0	NA
core diet + 20%	Frass	PFHpA	1	0	0	NA
core diet + 20%	Substrate	PFDA	1	0	0	NA
core diet + 20%	Larvae	PFDA	1	0	0	NA
core diet + 20%	Frass	PFDA	1	0	0	NA
core diet + 20%	Substrate	PFPeA	1	1	0.018577	NA
core diet + 20%	Larvae	PFPeA	1	1	0.015	0.8075
core diet + 20%	Frass	PFPeA	1	1	0.019415	1.045
core diet + 20%	Substrate	PFHpS	1	0	0	NA
core diet + 20%	Larvae	PFHpS	1	0	0	NA
core diet + 20%	Frass	PFHpS	1	0	0	NA
core diet + 20%	Substrate	PFNS	1	0	0	NA
core diet + 20%	Larvae	PFNS	1	0	0	NA
core diet + 20%	Frass	PFNS	1	0	0	NA
core diet + 20%	Substrate	PFPeS	1	0	0	NA
core diet + 20%	Larvae	PFPeS	1	0	0	NA
core diet + 20%	Frass	PFPeS	1	0	0	NA
core diet + 20%	Substrate	NaDONA	1	1	0.15725	NA
core diet + 20%	Larvae	NaDONA	1	0	0	0
core diet + 20%	Frass	NaDONA	1	0	0	0
core diet + 20%	Substrate	HFPO.DA	1	1	0.067096	NA
core diet + 20%	Larvae	HFPO.DA	1	1	0.01149	0.1712
core diet + 20%	Frass	HFPO.DA	1	1	0.006996	0.1043
core diet + 20%	Substrate	X4_2.FTS	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
core diet + 20%	Larvae	X4_2.FTS	1	0	0	NA
core diet + 20%	Frass	X4_2.FTS	1	0	0	NA
core diet + 20%	Substrate	FOSA	1	0	0	NA
core diet + 20%	Larvae	FOSA	1	0	0	NA
core diet + 20%	Frass	FOSA	1	0	0	NA
core diet + 20%	Substrate	FBSA	1	1	0.046322	NA
core diet + 20%	Larvae	FBSA	1	1	0.005872	0.1268
core diet + 20%	Frass	FBSA	1	0	0	0
Catering	Substrate	L.PFHxS	1	0	0	NA
Catering	Larvae	L.PFHxS	1	0	0	NA
Catering	Frass	L.PFHxS	1	0	0	NA
Catering	Substrate	Br.PFHxS	1	0	0	NA
Catering	Larvae	Br.PFHxS	1	0	0	NA
Catering	Frass	Br.PFHxS	1	0	0	NA
Catering	Substrate	Total.PFHxSH	1	0	0	NA
Catering	Larvae	Total.PFHxSH	1	0	0	NA
Catering	Frass	Total.PFHxSH	1	0	0	NA
Catering	Substrate	PFOA	1	0	0	NA
Catering	Larvae	PFOA	1	0	0	NA
Catering	Frass	PFOA	1	0	0	NA
Catering	Substrate	PFNA	1	0	0	NA
Catering	Larvae	PFNA	1	0	0	NA
Catering	Frass	PFNA	1	0	0	NA
Catering	Substrate	L.PFOS	1	0	0	NA
Catering	Larvae	L.PFOS	1	0	0	NA
Catering	Frass	L.PFOS	1	0	0	NA
Catering	Substrate	Br_PFOS	1	0	0	NA
Catering	Larvae	Br_PFOS	1	0	0	NA
Catering	Frass	Br_PFOS	1	0	0	NA
Catering	Substrate	Total.PFOS	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Catering	Larvae	Total.PFOS	1	0	0	NA
Catering	Frass	Total.PFOS	1	0	0	NA
Catering	Substrate	PFBA	1	0	0	NA
Catering	Larvae	PFBA	1	0	0	NA
Catering	Frass	PFBA	1	0	0	NA
Catering	Substrate	PFHxA	1	0	0	NA
Catering	Larvae	PFHxA	1	1	0.15252	Inf
Catering	Frass	PFHxA	1	0	0	NA
Catering	Substrate	PFHpA	1	1	0.1	NA
Catering	Larvae	PFHpA	1	0	0	0
Catering	Frass	PFHpA	1	0	0	0
Catering	Substrate	PFDA	1	0	0	NA
Catering	Larvae	PFDA	1	0	0	NA
Catering	Frass	PFDA	1	0	0	NA
Catering	Substrate	PFPeA	1	0	0	NA
Catering	Larvae	PFPeA	1	0	0	NA
Catering	Frass	PFPeA	1	1	0.016	Inf
Catering	Substrate	PFHpS	1	0	0	NA
Catering	Larvae	PFHpS	1	0	0	NA
Catering	Frass	PFHpS	1	0	0	NA
Catering	Substrate	PFNS	1	0	0	NA
Catering	Larvae	PFNS	1	0	0	NA
Catering	Frass	PFNS	1	0	0	NA
Catering	Substrate	PFPeS	1	0	0	NA
Catering	Larvae	PFPeS	1	0	0	NA
Catering	Frass	PFPeS	1	0	0	NA
Catering	Substrate	NaDONA	1	0	0	NA
Catering	Larvae	NaDONA	1	0	0	NA
Catering	Frass	NaDONA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Catering	Substrate	HFPO.DA	1	1	0.93935	NA
Catering	Larvae	HFPO.DA	1	1	0.16269	0.1732
Catering	Frass	HFPO.DA	1	1	0.0154	0.01639
Catering	Substrate	X4_2.FTS	1	1	0.19841	NA
Catering	Larvae	X4_2.FTS	1	0	0	0
Catering	Frass	X4_2.FTS	1	0	0	0
Catering	Substrate	FOSA	1	0	0	NA
Catering	Larvae	FOSA	1	1	0.62277	Inf
Catering	Frass	FOSA	1	1	0.16667	Inf
Catering	Substrate	FBSA	1	0	0	NA
Catering	Larvae	FBSA	1	0	0	NA
Catering	Frass	FBSA	1	0	0	NA
Manufacturing	Substrate	L.PFHxS	1	0	0	NA
Manufacturing	Larvae	L.PFHxS	1	0	0	NA
Manufacturing	Frass	L.PFHxS	1	0	0	NA
Manufacturing	Substrate	Br.PFHxS	1	0	0	NA
Manufacturing	Larvae	Br.PFHxS	1	0	0	NA
Manufacturing	Frass	Br.PFHxS	1	0	0	NA
Manufacturing	Substrate	Total.PFHxSH	1	0	0	NA
Manufacturing	Larvae	Total.PFHxSH	1	0	0	NA
Manufacturing	Frass	Total.PFHxSH	1	0	0	NA
Manufacturing	Substrate	PFOA	1	0	0	NA
Manufacturing	Larvae	PFOA	1	0	0	NA
Manufacturing	Frass	PFOA	1	0	0	NA
Manufacturing	Substrate	PFNA	1	0	0	NA
Manufacturing	Larvae	PFNA	1	0	0	NA
Manufacturing	Frass	PFNA	1	0	0	NA
Manufacturing	Substrate	L.PFOS	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Manufacturing	Larvae	L.PFOS	1	0	0	NA
Manufacturing	Frass	L.PFOS	1	0	0	NA
Manufacturing	Substrate	Br_PFOS	1	0	0	NA
Manufacturing	Larvae	Br_PFOS	1	0	0	NA
Manufacturing	Frass	Br_PFOS	1	0	0	NA
Manufacturing	Substrate	Total.PFOS	1	0	0	NA
Manufacturing	Larvae	Total.PFOS	1	0	0	NA
Manufacturing	Frass	Total.PFOS	1	0	0	NA
Manufacturing	Substrate	PFBA	1	0	0	NA
Manufacturing	Larvae	PFBA	1	0	0	NA
Manufacturing	Frass	PFBA	1	0	0	NA
Manufacturing	Substrate	PFHxA	1	0	0	NA
Manufacturing	Larvae	PFHxA	1	1	0.13266	Inf
Manufacturing	Frass	PFHxA	1	1	0.54536	Inf
Manufacturing	Substrate	PFHpA	1	0	0	NA
Manufacturing	Larvae	PFHpA	1	0	0	NA
Manufacturing	Frass	PFHpA	1	1	0.73	Inf
Manufacturing	Substrate	PFDA	1	0	0	NA
Manufacturing	Larvae	PFDA	1	0	0	NA
Manufacturing	Frass	PFDA	1	0	0	NA
Manufacturing	Substrate	PFPeA	1	1	0.011898	NA
Manufacturing	Larvae	PFPeA	1	0	0	0
Manufacturing	Frass	PFPeA	1	1	0.10351	8.7
Manufacturing	Substrate	PFHpS	1	0	0	NA
Manufacturing	Larvae	PFHpS	1	0	0	NA
Manufacturing	Frass	PFHpS	1	0	0	NA
Manufacturing	Substrate	PFNS	1	0	0	NA
Manufacturing	Larvae	PFNS	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Manufacturing	Frass	PFNS	1	0	0	NA
Manufacturing	Substrate	PFPeS	1	0	0	NA
Manufacturing	Larvae	PFPeS	1	0	0	NA
Manufacturing	Frass	PFPeS	1	0	0	NA
Manufacturing	Substrate	NaDONA	1	0	0	NA
Manufacturing	Larvae	NaDONA	1	0	0	NA
Manufacturing	Frass	NaDONA	1	0	0	NA
Manufacturing	Substrate	HFPO.DA	1	1	0.14182	NA
Manufacturing	Larvae	HFPO.DA	1	1	0.19864	1.401
Manufacturing	Frass	HFPO.DA	1	1	0.11518	0.8122
Manufacturing	Substrate	X4_2.FTS	1	0	0	NA
Manufacturing	Larvae	X4_2.FTS	1	0	0	NA
Manufacturing	Frass	X4_2.FTS	1	0	0	NA
Manufacturing	Substrate	FOSA	1	0	0	NA
Manufacturing	Larvae	FOSA	1	0	0	NA
Manufacturing	Frass	FOSA	1	0	0	NA
Manufacturing	Substrate	FBSA	1	0	0	NA
Manufacturing	Larvae	FBSA	1	0	0	NA
Manufacturing	Frass	FBSA	1	0	0	NA
Poultry manure	Substrate	L.PFHxS	1	1	0.11775	NA
Poultry manure	Larvae	L.PFHxS	1	0	0	0
Poultry manure	Frass	L.PFHxS	1	0	0	0
Poultry manure	Substrate	Br.PFHxS	1	1	0.55079	NA
Poultry manure	Larvae	Br.PFHxS	1	0	0	0
Poultry manure	Frass	Br.PFHxS	1	1	0.15575	0.2828
Poultry manure	Substrate	Total.PFHxSH	1	1	0.67	NA
Poultry manure	Larvae	Total.PFHxSH	1	0	0	0
Poultry manure	Frass	Total.PFHxSH	1	1	0.16	0.2388

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Poultry manure	Substrate	PFOA	1	0	0	NA
Poultry manure	Larvae	PFOA	1	1	7.4083	Inf
Poultry manure	Frass	PFOA	1	1	0.49	Inf
Poultry manure	Substrate	PFNA	1	0	0	NA
Poultry manure	Larvae	PFNA	1	1	0.1	Inf
Poultry manure	Frass	PFNA	1	0	0	NA
Poultry manure	Substrate	L.PFOS	1	0	0	NA
Poultry manure	Larvae	L.PFOS	1	1	0.4	Inf
Poultry manure	Frass	L.PFOS	1	0	0	NA
Poultry manure	Substrate	Br_PFOS	1	0	0	NA
Poultry manure	Larvae	Br_PFOS	1	1	0.14	Inf
Poultry manure	Frass	Br_PFOS	1	0	0	NA
Poultry manure	Substrate	Total.PFOS	1	0	0	NA
Poultry manure	Larvae	Total.PFOS	1	1	0.54	Inf
Poultry manure	Frass	Total.PFOS	1	0	0	NA
Poultry manure	Substrate	PFBA	1	1	0.64146	NA
Poultry manure	Larvae	PFBA	1	0	0	0
Poultry manure	Frass	PFBA	1	1	1.1549	1.8
Poultry manure	Substrate	PFHxA	1	0	0	NA
Poultry manure	Larvae	PFHxA	1	0	0	NA
Poultry manure	Frass	PFHxA	1	0	0	NA
Poultry manure	Substrate	PFHpA	1	1	0.17458	NA
Poultry manure	Larvae	PFHpA	1	1	0.14	0.8019
Poultry manure	Frass	PFHpA	1	0	0	0
Poultry manure	Substrate	PFDA	1	0	0	NA
Poultry manure	Larvae	PFDA	1	1	0.21	Inf
Poultry manure	Frass	PFDA	1	0	0	NA
Poultry manure	Substrate	PFPeA	1	1	0.039976	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
Poultry manure	Larvae	PFPeA	1	1	0.050946	1.274
Poultry manure	Frass	PFPeA	1	1	0.024732	0.6187
Poultry manure	Substrate	PFHpS	1	1	0.25647	NA
Poultry manure	Larvae	PFHpS	1	0	0	0
Poultry manure	Frass	PFHpS	1	0	0	0
Poultry manure	Substrate	PFNS	1	1	0.1366	NA
Poultry manure	Larvae	PFNS	1	0	0	0
Poultry manure	Frass	PFNS	1	0	0	0
Poultry manure	Substrate	PFPeS	1	1	0.11076	NA
Poultry manure	Larvae	PFPeS	1	0	0	0
Poultry manure	Frass	PFPeS	1	0	0	0
Poultry manure	Substrate	NaDONA	1	0	0	NA
Poultry manure	Larvae	NaDONA	1	0	0	NA
Poultry manure	Frass	NaDONA	1	0	0	NA
Poultry manure	Substrate	HFPO.DA	1	1	0.1167	NA
Poultry manure	Larvae	HFPO.DA	1	1	0.13368	1.146
Poultry manure	Frass	HFPO.DA	1	0	0	0
Poultry manure	Substrate	X4_2.FTS	1	0	0	NA
Poultry manure	Larvae	X4_2.FTS	1	0	0	NA
Poultry manure	Frass	X4_2.FTS	1	0	0	NA
Poultry manure	Substrate	FOSA	1	0	0	NA
Poultry manure	Larvae	FOSA	1	1	0.1155	Inf
Poultry manure	Frass	FOSA	1	0	0	NA
Poultry manure	Substrate	FBSA	1	0	0	NA
Poultry manure	Larvae	FBSA	1	0	0	NA
Poultry manure	Frass	FBSA	1	1	0.65657	Inf
supermarket	Substrate	L.PFHxS	1	1	0.30996	NA
supermarket	Larvae	L.PFHxS	1	1	0.27777	0.8961

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
supermarket	Frass	L.PFHxS	1	1	1.151	3.713
supermarket	Substrate	Br.PFHxS	1	1	0.49517	NA
supermarket	Larvae	Br.PFHxS	1	1	0.68318	1.38
supermarket	Frass	Br.PFHxS	1	1	0.88557	1.788
supermarket	Substrate	Total.PFHxSH	1	1	0.81	NA
supermarket	Larvae	Total.PFHxSH	1	1	0.96	1.185
supermarket	Frass	Total.PFHxSH	1	1	2.046	2.526
supermarket	Substrate	PFOA	1	0	0	NA
supermarket	Larvae	PFOA	1	0	0	NA
supermarket	Frass	PFOA	1	0	0	NA
supermarket	Substrate	PFNA	1	0	0	NA
supermarket	Larvae	PFNA	1	0	0	NA
supermarket	Frass	PFNA	1	0	0	NA
supermarket	Substrate	L.PFOS	1	0	0	NA
supermarket	Larvae	L.PFOS	1	0	0	NA
supermarket	Frass	L.PFOS	1	0	0	NA
supermarket	Substrate	Br_PFOS	1	0	0	NA
supermarket	Larvae	Br_PFOS	1	0	0	NA
supermarket	Frass	Br_PFOS	1	0	0	NA
supermarket	Substrate	Total.PFOS	1	0	0	NA
supermarket	Larvae	Total.PFOS	1	0	0	NA
supermarket	Frass	Total.PFOS	1	0	0	NA
supermarket	Substrate	PFBA	1	0	0	NA
supermarket	Larvae	PFBA	1	0	0	NA
supermarket	Frass	PFBA	1	0	0	NA
supermarket	Substrate	PFHxA	1	0	0	NA
supermarket	Larvae	PFHxA	1	0	0	NA
supermarket	Frass	PFHxA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
supermarket	Substrate	PFHpA	1	0	0	NA
supermarket	Larvae	PFHpA	1	0	0	NA
supermarket	Frass	PFHpA	1	0	0	NA
supermarket	Substrate	PFDA	1	0	0	NA
supermarket	Larvae	PFDA	1	0	0	NA
supermarket	Frass	PFDA	1	0	0	NA
supermarket	Substrate	PFPeA	1	1	0.021558	NA
supermarket	Larvae	PFPeA	1	0	0	0
supermarket	Frass	PFPeA	1	0	0	0
supermarket	Substrate	PFHpS	1	0	0	NA
supermarket	Larvae	PFHpS	1	0	0	NA
supermarket	Frass	PFHpS	1	0	0	NA
supermarket	Substrate	PFNS	1	0	0	NA
supermarket	Larvae	PFNS	1	0	0	NA
supermarket	Frass	PFNS	1	0	0	NA
supermarket	Substrate	PFPeS	1	0	0	NA
supermarket	Larvae	PFPeS	1	0	0	NA
supermarket	Frass	PFPeS	1	0	0	NA
supermarket	Substrate	NaDONA	1	0	0	NA
supermarket	Larvae	NaDONA	1	0	0	NA
supermarket	Frass	NaDONA	1	0	0	NA
supermarket	Substrate	HFPO.DA	1	1	0.12825	NA
supermarket	Larvae	HFPO.DA	1	1	0.011105	0.08659
supermarket	Frass	HFPO.DA	1	1	0.23599	1.84
supermarket	Substrate	X4_2.FTS	1	0	0	NA
supermarket	Larvae	X4_2.FTS	1	0	0	NA
supermarket	Frass	X4_2.FTS	1	0	0	NA
supermarket	Substrate	FOSA	1	0	0	NA

Trial	Sample Type	PFAS	Number of injections	Detected	Mean (ug/kg)	Accumulation
supermarket	Larvae	FOSA	1	0	0	NA
supermarket	Frass	FOSA	1	0	0	NA
supermarket	Substrate	FBSA	1	0	0	NA
supermarket	Larvae	FBSA	1	0	0	NA
supermarket	Frass	FBSA	1	0	0	NA

Inf = inferred, NA = not applicable

Appendix I - Microbiology

The aim of the analysis was to

- estimate the quantity of organism in each sample type from each source
- examine how variable results were in replicate samples
- estimate the amount of growth from substrate into larvae and frass

Duplicate or single plates were undertaken on samples (replicates A, B1 and B2) and the average $\log_{10}$ concentration for a replicate was estimated as the average of each $\log_{10}$ plate observation, then the average $\log_{10}$ for the sample type and source was estimated as:

$$\text{Average for type and source} = (A + ((B1 + B2)/2))/2$$

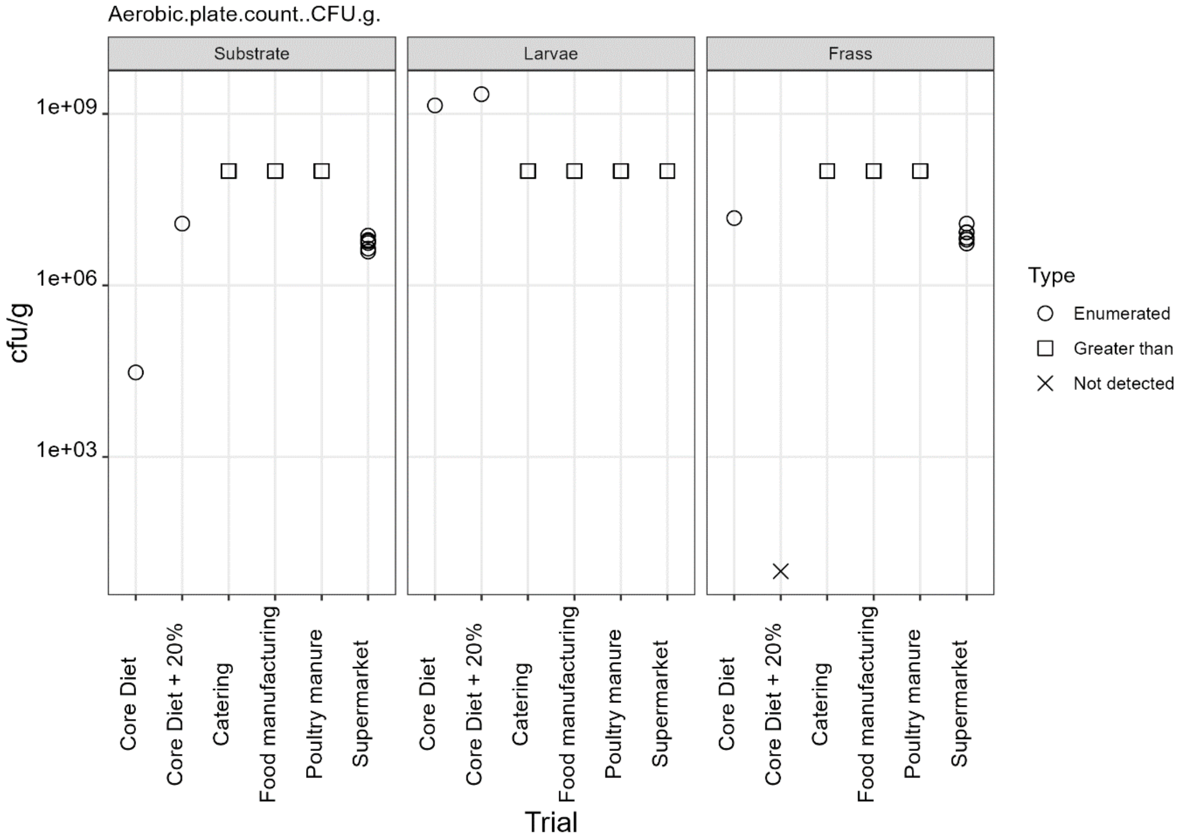
An indication of within replicate variation was given by B1−B2, of between replicate variation by $A - \frac{B1+B2}{2}$.

Results that were reported as less than the limit of detection were treated as being at the limit of detection. Results reported as being above a value were treated as being at that value.

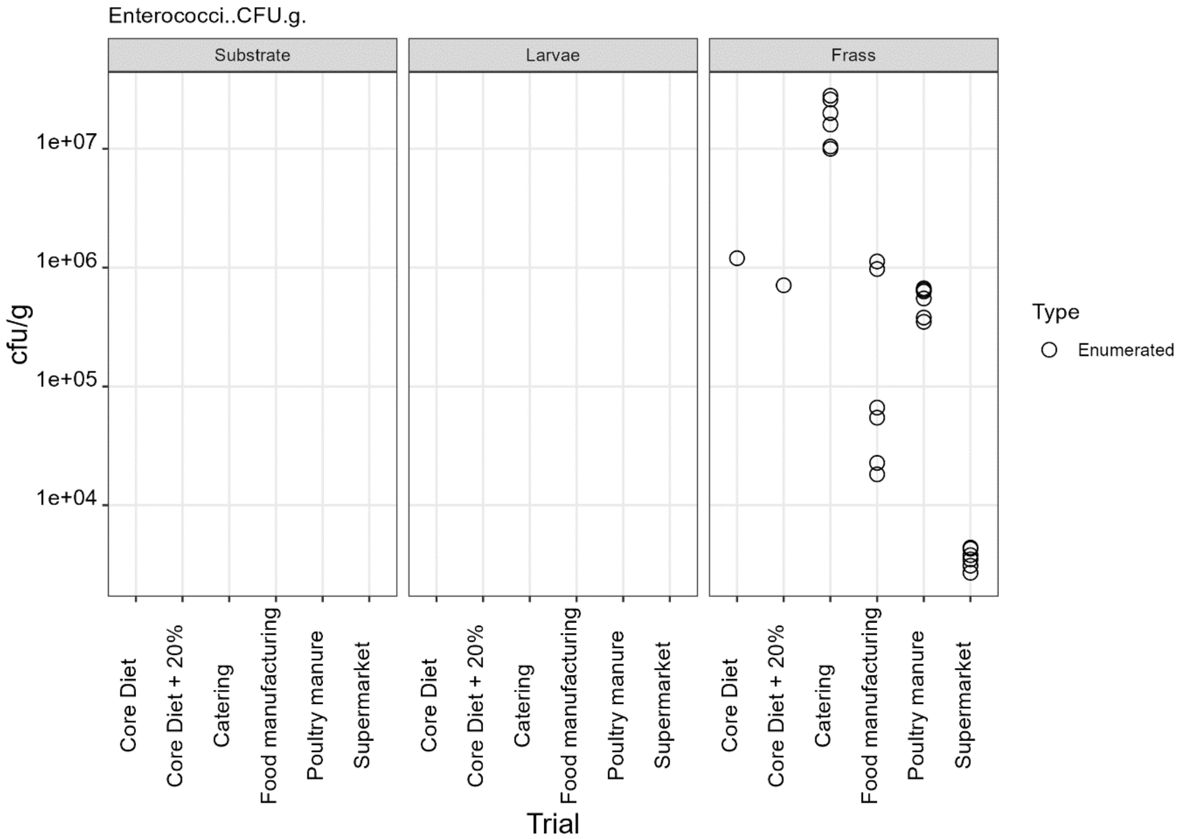
Individual results are shown in the figures and the table shows the calculated quantities.

Organisms that were not detected in any extracts from any samples are not reported here. They are: *Salmonella*, Campylobacter and ESBL *E. coli*.

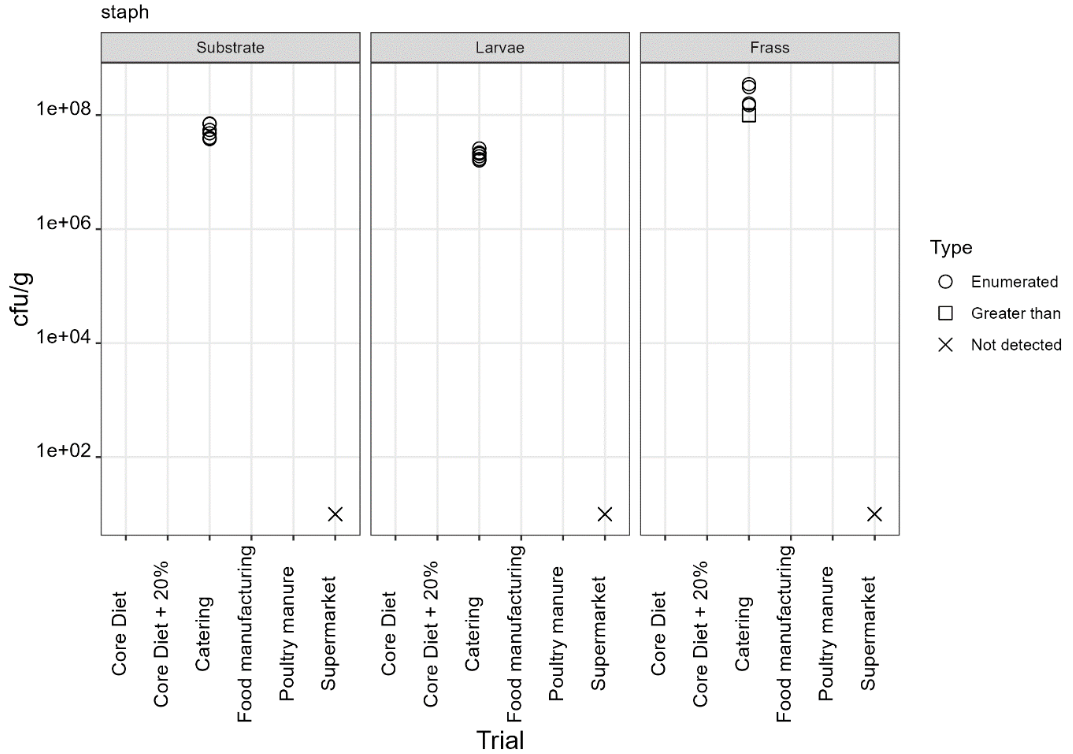
1.


Aerobic.plate.count..CFU.g.
Substrate
Larvae
Frass
1e+09
1e+06
1e+03
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

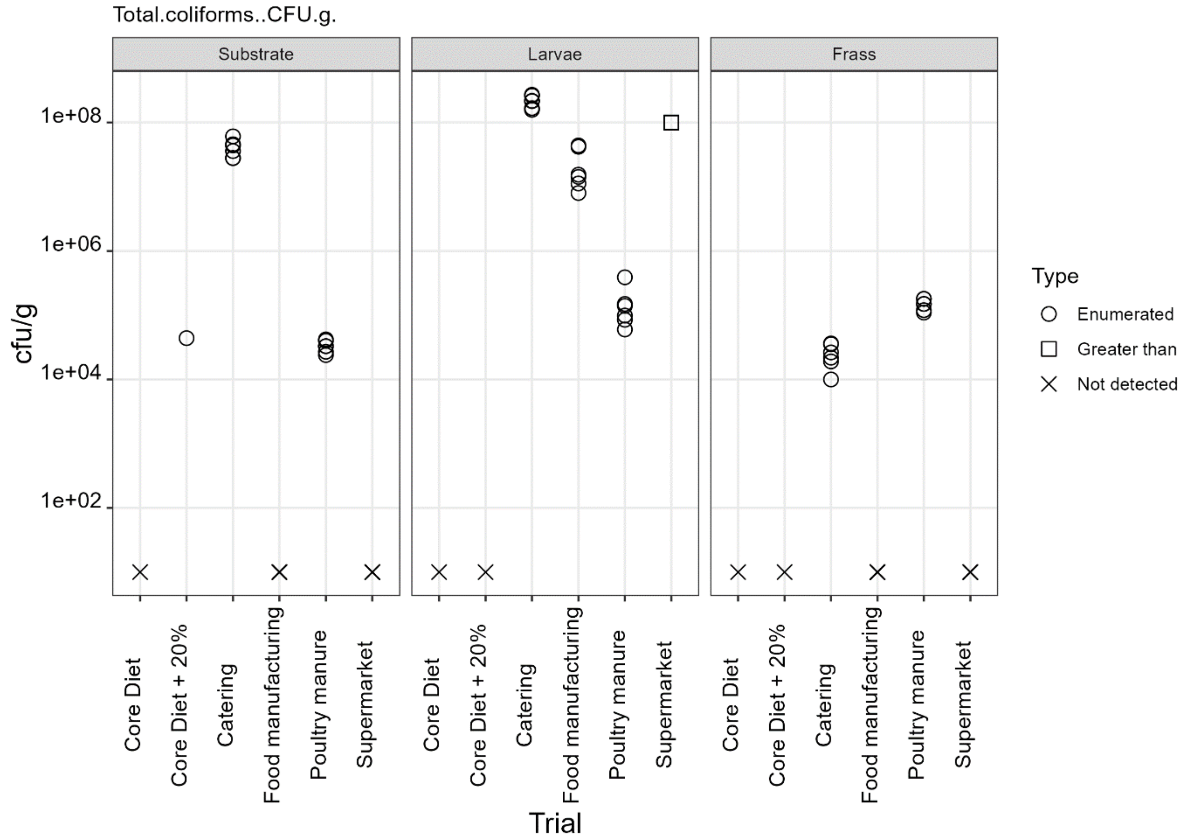
2.


Enterococci..CFU.g.
Substrate
Larvae
Frass
1e+07
1e+06
1e+05
1e+04
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated

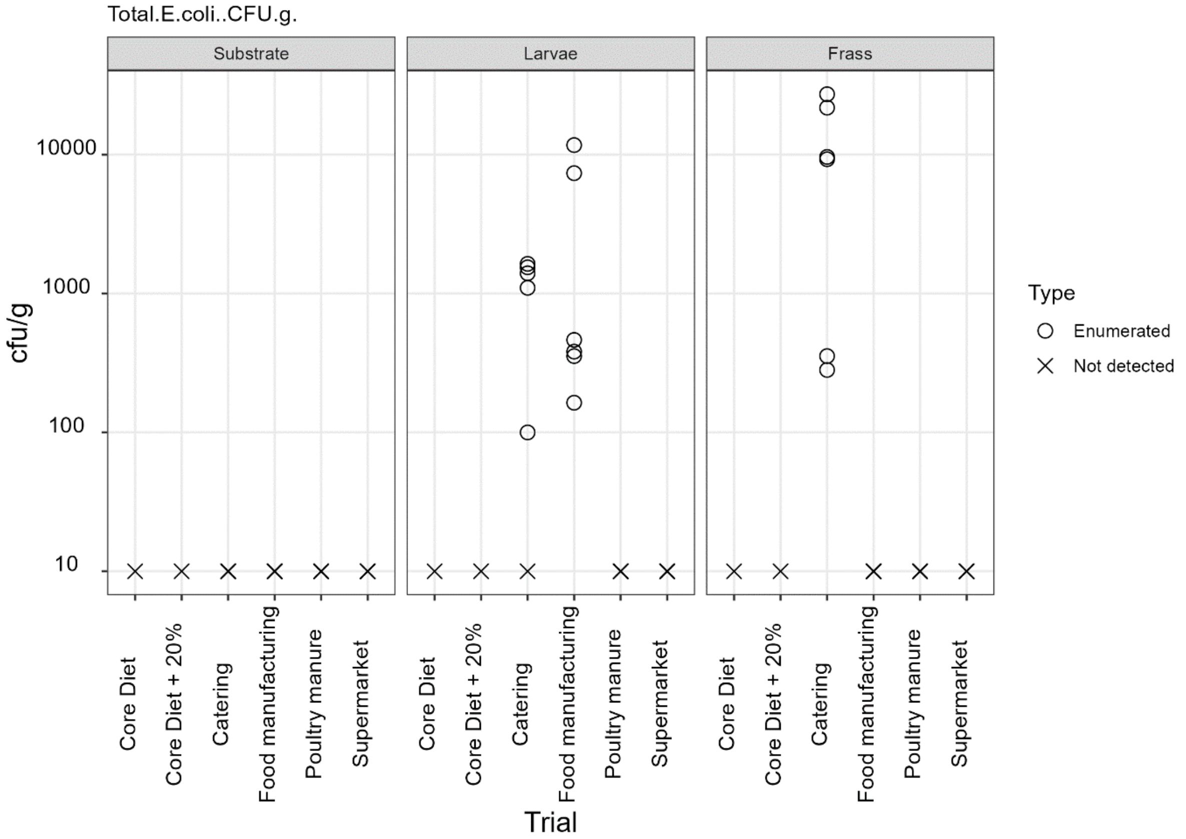
3.


staph
Substrate
Larvae
Frass
1e+08
1e+06
1e+04
1e+02
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

4.

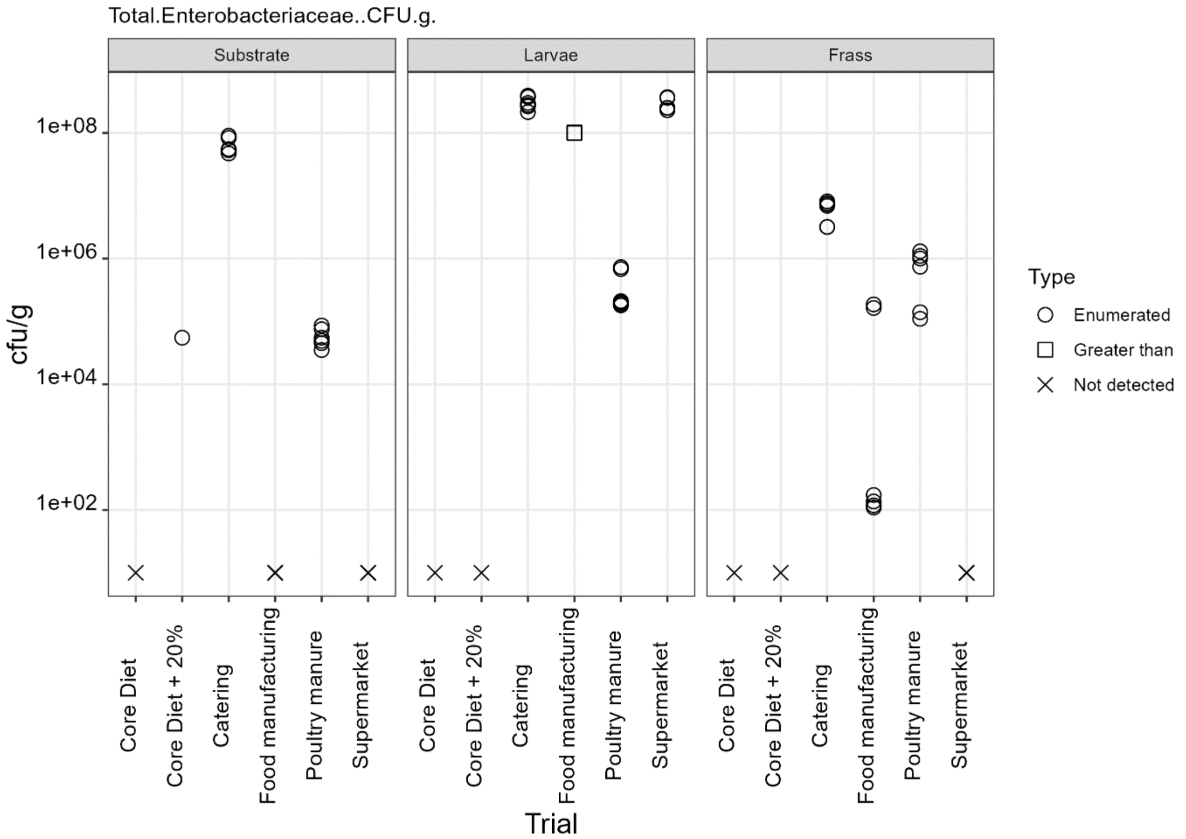

Total.coliforms..CFU.g.
Substrate
Larvae
Frass
1e+08
1e+06
1e+04
1e+02
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

5.



Total.E.coli..CFU.g.
Substrate
Larvae
Frass
10000
1000
100
10
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Not detected

6.



Total.Enterobacteriaceae..CFU.g.
Substrate
Larvae
Frass
1e+08
1e+06
1e+04
1e+02
cfu/g
Core Diet
Core Diet + 20%
Catering
Food manufacturing
Poultry manure
Supermarket
Trial
Type
Enumerated
Greater than
Not detected

Figure VIII (1 – 6). Concentration (cfu/g) of organisms analysed in substrate rearing, larvae and frass from core diet, core diet + 20%, catering, food manufacturing, poultry manure and supermarket.

Table XIII. Calculated quantities of microorganisms.

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
core diet	Substrate	Aerobic.plate.count	1	1	4.477	4.477	NA	NA	NA
core diet	Larvae	Aerobic.plate.count	1	1	9.146	9.146	4.669	NA	NA
core diet	Frass	Aerobic.plate.count	1	1	7.176	7.176	2.699	NA	NA
core diet	Substrate	Total.Enterobacteriaceae	1	0	1.000	1.000	NA	NA	NA
core diet	Larvae	Total.Enterobacteriaceae	1	0	1.000	1.000	0	NA	NA
core diet	Frass	Total.Enterobacteriaceae	1	0	1.000	1.000	0	NA	NA
core diet	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
core diet	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
core diet	Frass	Enterococci	1	1	6.079	6.079	NA	NA	NA
core diet	Substrate	Total.coliforms	1	0	1.000	1.000	NA	NA	NA
core diet	Larvae	Total.coliforms	1	0	1.000	1.000	0	NA	NA
core diet	Frass	Total.coliforms	1	0	1.000	1.000	0	NA	NA
core diet	Substrate	Total.E.coli	1	0	1.000	1.000	NA	NA	NA
core diet	Larvae	Total.E.coli	1	0	1.000	1.000	0	NA	NA
core diet	Frass	Total.E.coli	1	0	1.000	1.000	0	NA	NA
core diet	Substrate	staph	0	0	NA	NA	NA	NA	NA
core diet	Larvae	staph	0	0	NA	NA	NA	NA	NA

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
core diet	Frass	staph	0	0	NA	NA	NA	NA	NA
core diet + 20%	Substrate	Aerobic.plate.count	1	1	7.079	7.079	NA	NA	NA
core diet + 20%	Larvae	Aerobic.plate.count	1	1	9.342	9.342	2.263	NA	NA
core diet + 20%	Frass	Aerobic.plate.count	1	0	1.000	1.000	-6.079	NA	NA
core diet + 20%	Substrate	Total.Enterobacteriaceae	1	1	4.740	4.740	NA	NA	NA
core diet + 20%	Larvae	Total.Enterobacteriaceae	1	0	1.000	1.000	-3.74	NA	NA
core diet + 20%	Frass	Total.Enterobacteriaceae	1	0	1.000	1.000	-3.74	NA	NA
core diet + 20%	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
core diet + 20%	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
core diet + 20%	Frass	Enterococci	1	1	5.851	5.851	NA	NA	NA
core diet + 20%	Substrate	Total.coliforms	1	1	4.643	4.643	NA	NA	NA
core diet + 20%	Larvae	Total.coliforms	1	0	1.000	1.000	-3.643	NA	NA
core diet + 20%	Frass	Total.coliforms	1	0	1.000	1.000	-3.643	NA	NA
core diet + 20%	Substrate	Total.E.coli	1	0	1.000	1.000	NA	NA	NA

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
core diet + 20%	Larvae	Total.E.coli	1	0	1.000	1.000	0	NA	NA
core diet + 20%	Frass	Total.E.coli	1	0	1.000	1.000	0	NA	NA
core diet + 20%	Substrate	staph	0	0	NA	NA	NA	NA	NA
core diet + 20%	Larvae	staph	0	0	NA	NA	NA	NA	NA
core diet + 20%	Frass	staph	0	0	NA	NA	NA	NA	NA
Catering	Substrate	Aerobic.plate.count	6	6	8.000	8.000	NA	0.000	0.000
Catering	Larvae	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
Catering	Frass	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
Catering	Substrate	Total.Enterobacteriaceae	6	6	7.827	7.954	NA	0.219	-0.038
Catering	Larvae	Total.Enterobacteriaceae	6	6	8.462	8.591	0.635	-0.060	-0.177
Catering	Frass	Total.Enterobacteriaceae	6	6	6.780	6.908	-1.047	0.184	-0.371
Catering	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
Catering	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
Catering	Frass	Enterococci	6	6	7.236	7.447	NA	0.032	-0.421
Catering	Substrate	Total.coliforms	6	6	7.621	7.785	NA	0.186	0.053
Catering	Larvae	Total.coliforms	6	6	8.297	8.433	0.676	-0.169	-0.089

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
Catering	Frass	Total.coliforms	6	6	4.304	4.561	-3.317	-0.327	0.036
Catering	Substrate	Total.E.coli	6	0	1.000	1.000	NA	0.000	0.000
Catering	Larvae	Total.E.coli	6	5	2.324	3.214	1.324	-1.648	-0.108
Catering	Frass	Total.E.coli	6	6	3.710	4.436	2.71	0.532	1.887
Catering	Substrate	staph	6	6	7.715	7.852	NA	-0.009	-0.258
Catering	Larvae	staph	6	6	7.288	7.415	-0.427	-0.066	-0.023
Catering	Frass	staph	6	6	8.224	8.544	0.509	-0.069	-0.518
Manufacturing	Substrate	Aerobic.plate.count	6	6	8.000	8.000	NA	0.000	0.000
Manufacturing	Larvae	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
Manufacturing	Frass	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
Manufacturing	Substrate	Total.Enterobacteriaceae	6	0	1.000	1.000	NA	0.000	0.000
Manufacturing	Larvae	Total.Enterobacteriaceae	6	6	8.000	8.000	7	0.000	0.000
Manufacturing	Frass	Total.Enterobacteriaceae	6	6	2.885	5.270	1.885	-1.659	3.056
Manufacturing	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
Manufacturing	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
Manufacturing	Frass	Enterococci	6	6	4.854	6.052	NA	-1.092	1.241

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
Manufacturing	Substrate	Total.coliforms	6	0	1.000	1.000	NA	0.000	0.000
Manufacturing	Larvae	Total.coliforms	6	6	7.190	7.643	6.19	-0.425	-0.462
Manufacturing	Frass	Total.coliforms	6	0	1.000	1.000	0	0.000	0.000
Manufacturing	Substrate	Total.E.coli	6	0	1.000	1.000	NA	0.000	0.000
Manufacturing	Larvae	Total.E.coli	6	6	3.236	4.069	2.236	1.465	0.210
Manufacturing	Frass	Total.E.coli	6	0	1.000	1.000	0	0.000	0.000
Manufacturing	Substrate	staph	0	0	NA	NA	NA	NA	NA
Manufacturing	Larvae	staph	0	0	NA	NA	NA	NA	NA
Manufacturing	Frass	staph	0	0	NA	NA	NA	NA	NA
chicken waste	Substrate	Aerobic.plate.count	6	6	8.000	8.000	NA	0.000	0.000
chicken waste	Larvae	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
chicken waste	Frass	Aerobic.plate.count	6	6	8.000	8.000	0	0.000	0.000
chicken waste	Substrate	Total.Enterobacteriaceae	6	6	4.709	4.934	NA	-0.184	-0.208

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
chicken waste	Larvae	Total.Enterobacteriaceae	6	6	5.432	5.863	0.723	-0.263	0.570
chicken waste	Frass	Total.Enterobacteriaceae	6	6	5.550	6.114	0.841	-0.912	0.102
chicken waste	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
chicken waste	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
chicken waste	Frass	Enterococci	6	6	5.678	5.826	NA	-0.233	-0.036
chicken waste	Substrate	Total.coliforms	6	6	4.510	4.623	NA	-0.016	0.085
chicken waste	Larvae	Total.coliforms	6	6	5.117	5.591	0.607	0.088	0.222
chicken waste	Frass	Total.coliforms	6	6	5.167	5.255	0.657	0.018	0.195
chicken waste	Substrate	Total.E.coli	6	0	1.000	1.000	NA	0.000	0.000
chicken waste	Larvae	Total.E.coli	6	0	1.000	1.000	0	0.000	0.000
chicken waste	Frass	Total.E.coli	6	0	1.000	1.000	0	0.000	0.000
chicken waste	Substrate	staph	0	0	NA	NA	NA	NA	NA
chicken waste	Larvae	staph	0	0	NA	NA	NA	NA	NA

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
chicken waste	Frass	staph	0	0	NA	NA	NA	NA	NA
supermarket	Substrate	Aerobic.plate.count	6	6	6.705	6.869	NA	-0.176	-0.075
supermarket	Larvae	Aerobic.plate.count	6	6	8.000	8.000	1.295	0.000	0.000
supermarket	Frass	Aerobic.plate.count	6	6	6.870	7.079	0.165	-0.078	-0.186
supermarket	Substrate	Total.Enterobacteriaceae	6	0	1.000	1.000	NA	0.000	0.000
supermarket	Larvae	Total.Enterobacteriaceae	6	6	8.425	8.568	7.425	-0.091	-0.182
supermarket	Frass	Total.Enterobacteriaceae	6	0	1.000	1.000	0	0.000	0.000
supermarket	Substrate	Enterococci	0	0	NA	NA	NA	NA	NA
supermarket	Larvae	Enterococci	0	0	NA	NA	NA	NA	NA
supermarket	Frass	Enterococci	6	6	3.545	3.643	NA	-0.054	-0.079
supermarket	Substrate	Total.coliforms	6	0	1.000	1.000	NA	0.000	0.000
supermarket	Larvae	Total.coliforms	6	6	8.000	8.000	7	0.000	0.000
supermarket	Frass	Total.coliforms	6	0	1.000	1.000	0	0.000	0.000
supermarket	Substrate	Total.E.coli	6	0	1.000	1.000	NA	0.000	0.000
supermarket	Larvae	Total.E.coli	6	0	1.000	1.000	0	0.000	0.000
supermarket	Frass	Total.E.coli	6	0	1.000	1.000	0	0.000	0.000
supermarket	Substrate	Staph	6	0	1.000	1.000	NA	0.000	0.000
supermarket	Larvae	Staph	6	0	1.000	1.000	0	0.000	0.000

Trial	Sample Type	Organism	Number of injections	Detected	Mean (Log_{10} cfu/g)	Max (Log_{10} cfu/g)	Accumulation (Log_{10})	Within replicate difference	Between replicate difference
supermarket	Frass	staph	6	0	1.000	1.000	0	0.000	0.000

Inf = inferred, NA = not applicable