EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Safety of the Food Chain
Pesticides and Biocides

COMMISSION WORKING DOCUMENT[1]

SANTE/10252/2021
23 February 2021

Magnitude of pesticides residues in fish

The contents of this working document have been finalised in the meeting of the Standing Committee on Plants, Animals, Food and Feed on 22/23 February 2021. It becomes only applicable upon its inclusion in the Commission Communications 2013/C 95/01[2] and 2013/C 95/02[3] and will then be completed with an implementation schedule.

1 This document has been conceived as Working Document of the Commission Services which was elaborated in co-operation with the Member States. It does not represent the official position of the Commission. It does not intend to produce legally binding effects. Only the European Court of Justice has jurisdiction to give preliminary rulings concerning the validity and interpretation of acts of the institutions of the EU pursuant to Article 267 of the Treaty.

2 Commission Communication in the framework of the implementation of Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

3 Commission Communication in the framework of the implementation of Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

CONTENTS

ABBREVIATIONS

BMF	Biomagnification factor
DM	Dry matter
FCR	Feed conversion ratio
GAP	Good agricultural practice
IUPAC	International Union of Pure and Applied Chemistry
log P_{OW}	Logarithm of the *n*-octanol/water partition coefficient
LOQ	Limit of quantification
MRBD	Maximum reasonably balanced diet
MRL	Maximum residue level
OECD	Organisation for Economic Co-operation and Development
SGR	Specific growth rate
TG	Test Guideline

INTRODUCTION

1. Uptake of pesticides by fish, leading to the occurrence of residues in fish products, can occur following ingestion of feed containing a pesticide residue. Consequently, transfer of pesticide residues into products of fish origin needs to be evaluated. Studies on the magnitude of residues in fish are conducted in order to quantify levels of residues in fillet (incl. skin), liver and carcass of fish. Carcass is needed to calculate the residues in the whole fish. This calculation is especially required for small fish which are usually consumed in total.

2. This Working Document focusses on residues originating from exposure via feed. Bioconcentration studies carried out in accordance to OECD TG 305 (OECD, 2012), exposing fish to the active substance in water, are in general not suitable as substitutes for feeding studies as different routes of ingestion may lead to different organ exposure and a potentially different metabolic pattern.

PURPOSE

3. The primary purposes of the studies on the magnitude of residues in fish are to determine the residue level which results from residues in feeding stuffs in fish products obtained from aquaculture and to provide the basis

- for conducting dietary intake assessments for consumer safety and
- for establishing maximum residue limits (MRLs).

GENERAL CONSIDERATIONS

4. Studies on the magnitude of residues in fish provide data on the quantitative transfer of residues from fish feed to fillet (incl. skin), liver and carcass.

5. Residue trials should be carried out with one fish species of a size appropriate to commercial food production. It is proposed but not mandatory to use the same species that was used in the study on the nature of pesticides residues in fish (European Commission, 2021).

6. Results gained on the magnitude of residues in freshwater species are extrapolated to marine species and vice versa.

7. Studies on the magnitude of residues in fish are only required

I. for active substances or relevant metabolites with a log Pow > 3 at one of the investigated pH values (independent of pH) and/or proven fat solubility (indicated by "F" in MRL Regulations for the active substance) and

II. if the active substance is used in crops whose products, also after processing, are fed to fish and where the total calculated fish dietary burden is ≥ 0.1 mg/kg feed DM (dry matter) and

III. if metabolism studies indicate that significant residues (> 0.01 mg/kg, based on the relevant residue definition) may occur in any edible fish commodity (muscle (fillet) including skin and liver tissue) at the 1X dose rate (calculated on the dry matter basis).

8. This Working Document should only be used in connection with residues in fish feed. Residues resulting from environmental contamination of waters with persistent chemicals (arising from historic pesticide use) or from the direct treatment of water bodies or from spray drift/run-off/drainage after treatment along water bodies are not within the scope of this Working Document and might require separate consideration.

9. Registrants are encouraged to consult national statutory requirements for animal protection and treatment, for sampling and, in particular, for sacrifice before commencing a study.

Situations in which a study may not be necessary

10. Studies on the *Magnitude of residues in fish* are not necessary when residue levels are below the limit of quantification in feed items obtained from crop field trials that reflect the critical GAP (good agricultural practice) of the pesticide (i.e., maximum application rate, maximum number of applications, minimum pre-harvest interval), unless there are indications of bioaccumulation of the pesticide and its metabolites in fish commodities. When quantifiable residues are present in the feed items that are relevant for fish feeding, it will be necessary to consider the anticipated dietary burden and the results of the *Nature of residues* study. Feeding studies shall not be required where intake is below 0.1 mg/kg feed DM, except in cases where indications are available that the residue tends to accumulate.

11. Where a *Nature of residues* study results in levels of the residue of concern ≤ 0.01 mg/kg in all edible commodities, taking into account the residue levels in potential feeding stuffs obtained at the 1X dose rate and calculated on the dry matter basis, no quantifiable residues would be anticipated in fish commodities as a result of the proposed use. In such situations, the *Nature of Residues* study can also serve as a feeding study provided that steady state conditions are reached, and regulatory authorities would consider the limit of quantification (LOQ) of a validated analytical method for determining residues in fillet (incl. skin), liver and carcass as the basis for conducting dietary intake assessments for consumer safety and/or for setting appropriate MRLs. Registrants therefore need to apply appropriate analytical methods for enforcement of residues in fish commodities (fillet incl. skin, liver and carcass). Where MRLs have to be established, the extraction efficiency of residue analytical methods has to be assessed.

12. The lipid content of the different fish tissues (fillet incl. skin, liver and carcass) needs to be determined to allow lipid normalisation of the measured residue levels (see para 37).

CONDUCT OF STUDIES

Nature of the test substance used for dosing

13. The test substance used in the study should be representative of the residue in the feed. Fish are dosed with the representative component(s) of the residue which might consist

of the parent compound and/or major plant metabolites of the parent compound, depending on the relevant residue expected in feed produced from plants/parts of plants. Generally, the feeding of mixtures is not recommended and needs a specific rationale.

Dose administration

14. Due to the practical difficulties associated with oral dosing of experimental fish, it is recommended that the test compound should be administered via commercial fish feed (floating and/or slow sinking pelletised diet) that has previously been fortified with the test substance (see following paragraph). The feed should have a uniform pellet size to increase the efficiency of the feed exposure and should be appropriate for the size of the fish used.

15. When spiking the feed with the test substance, homogeneity throughout the test feed should be achieved. Leaching losses should not exceed a maximum of 10 % prior to ingestion of the daily ration. If required the fortified feed pellets may be coated (e.g. corn oil or calcium alginate) to reduce leaching losses prior to the ingestion of the experimental diet (Goeritz et al., 2014). The methodology for preparing the dose and the extent of leaching from the treated pellets should be described in the report (or referenced if already available from the *Nature of residues study*).

16. Fish are fed at a fixed ration following the feeding recommendations provided by the feed manufacturer (e.g. 3 % of wet body weight per day). Feeding must be constantly observed to ensure that the fish are visibly consuming all of the feed presented. If any food remains in the tank, it should be removed as far as possible shortly after feeding (approx. after 10 minutes). The tank should be cleaned approx. 60 minutes after feeding to remove faeces as far as possible. The ration may be adjusted if the fish do not ingest the entire ration. If feed is consistently being left uneaten, it may be advisable to spread the dose over an extra feeding period in the feeding schedule (e.g. feeding half the amount twice daily). The feed ration should be adjusted on a weekly basis until the experimental period is completed to account for the expected growth increment of the animals. To calculate the average live weight, 5 representative fish from the experimental tank should be weighed weekly (see para 29). The acceptability of the experimental diet should be tested in advance to make sure it is palatable. The exact feeding rate and amount of feed ingested during the experiment must be recorded. Based on the growth increment of the experimental animals during the study, a feed conversion ratio (FCR) should be calculated which is a major indicator of feed efficiency in fish farming. The FCR is calculated as follows:

FCR = Feed given during experimental period / Animal weight gain during experimental period.

The same unit should be used for the numerator and denominator. There are no measurement units used together with the FCR. The lower the FCR, the higher the weight gain obtained from the feed. Typical FCRs for rainbow trout, Atlantic salmon and common carp raised using commercial feeds and intensive production methods are in the range of 1-2 (Fry et al. 2018). FCRs > 2 for dosed animals kept under optimal culture conditions may provide indications for an adverse influence of the applied dose level on the conversion of feed into animal biomass. This should be confirmed by comparison of the feeding behaviour during the acclimatisation and exposure period. The dose level should be adjusted if any adverse effects induced by the diet were observed during the *Nature of residues* study.

17. The specific growth rate (SGR) of the test animals is calculated based on the average live weight of fish collected from the experimental tank, using the following formula:

$SGR\ (\%) = [\ln (Wt_2) - \ln (Wt_1)] / (t_2-t_1) \times 100$

Wt_2 = mean live weight at the end of the experimental period (g)

Wt_1 = mean live weight at the start of the experimental period (g)

t_2 = end of the experimental period (d)

t_1 = start of the experimental period (d)

Dose levels

18. A *Magnitude of Residues in Fish* study comprises only one dose level, which usually represents the maximum expected dietary burden (1X dose). The dietary burden calculation for fish follows the principles of MRBD (maximum reasonably balanced diet) under consideration of the residues of the test compound (in mg/kg DM) in each potential feed ingredient (Schlechtriem et al., 2016). The calculation of the dietary burden is described in the Working Document on the Fish dietary burden calculator (European Commission, 2021a). The feeding study follows a kinetic approach. Considering the transfer kinetics observed in the *Nature of residues study*, higher dose levels might be selected to ensure detectable concentrations in the edible part of the fish during the exposure and depuration phase.

19. The single dose level in the study provides information on the relationship between the dose level and the resulting residue concentration in fish commodities and information on the uptake and elimination kinetics. Using kinetic transfer models allows the calculation of expected residue levels in fish for any dietary burden.

Test animals

20. It might be advantageous but is not mandatory to carry out the feeding studies with the same species as used for the *Nature of residues study*. Important aquaculture species reared for human consumption such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) or common carp (*Cyprinus carpio*) are the recommended species. These fresh water and marine species were chosen as they represent two different feeding habits (carnivorous vs. omnivorous), are easily reared under laboratory conditions and have been used widely as model species in research. Other species may however be used as well if justification is provided. The fish should be taken from a healthy stock free from observable diseases. The fish stock should not receive any disease treatment for one week preceding the test or during the test. Mortality in the fish stock should be <5% of the population in the preceding 7 days. The selected fish should be free of any visible injuries or abnormalities.

21. Fish selected for the study should have a minimum weight of 250 g at the onset of dosing to reach adult/marketable size at the end of the study. Individual fish should be of similar size/weight (± 20%) at the beginning of the dosing phase to ensure consistent feeding and minimise aggression.

Number of test animals

22. A group of 22 animals is required. With respect to animal welfare, a control group is not recommended. Any adverse effects should already be visible during the acclimatisation period. The two fish sampled at day 0 of the uptake phase prior to application serve for purposes of detecting potential background contaminations.

Condition of animals

23. The condition of the animals, both during the acclimatisation (see para 28) and dosing phases should be recorded throughout the study period, together with information on the age, body weights, daily observed feed consumption and behaviour. Weight should be recorded (see para 29) and feed conversion ratios be calculated.

24. The physical condition of the animals can provide important information on rates of absorption and depuration of the administered test substance. Any health problems, abnormal behaviour, low feed consumption or unusual treatment of the animals should be reported and the effect of these factors on the study results should be discussed where relevant. Guidance on recording sublethal clinical signs in fish is provided in OECD, 2019.

Housing conditions

25. A flow-through test system that provides a sufficient flow of water to the tanks should be used to avoid the accumulation of pesticide residues excreted by the test animals into the test water and to avoid that concentration of dissolved oxygen falls below 60 % saturation (OECD, 2012). The flow-rates should be recorded. Natural water is generally used in the test and should be obtained from uncontaminated and uniform quality source. The water should be characterised at least by pH, hardness, ammonium, nitrite and alkalinity (OECD, 2012).

26. The capacity of the tanks should be in compliance with a maximum loading rate of 5 g fish (wet weight) per litre of water per day (European Medicines Agency, 2009).

27. The water temperature should be in the range of 20 – 25°C for common carp and of 12 – 16 °C for rainbow trout and Atlantic salmon, respectively. A 12 to 16 hour photoperiod is recommended. Details of lighting should be reported. Partial tank coverings should be provided to cover sheltering areas which reduce stress for the test animals during the light photoperiod.

Performance of the study

28. Fish should be acclimatised to the experimental conditions for at least one week prior to dosing. During this period all fish will be maintained on the unfortified diet and monitored for health, feeding rate and growth rate. The two fish sampled at day 0 of the uptake phase serve for purposes of detecting potential background contaminations.

29. All animals should be individually weighed at the beginning of the acclimatisation period. Each fish which is sacrificed, should be individually weighed. Additionally, 5 representative fish from the test animals in the experimental tank should be weighed weekly to calculate the average live weight and adjust the feed ration if required (see para 16).

30. During the experimental phase, animals should be dosed daily to a minimum of 28 days, which is followed by an equal length of an elimination period in which the fish are fed a control (non-spiked) feed. A total of 5 time points in uptake and elimination period needs to be sampled to assess absorption and elimination kinetics, including half-life that is used to assess steady state levels. At each time point, four fish are sampled from the experimental tank. Details are provided in Table 1.

Water analysis

31. Water analysis for test substance concentrations should be carried out during the flow-through test on a weekly level. Measured concentrations in water may help to estimate the impact of bioconcentration processes on the magnitude of residues.

Sampling and sacrifice

32. An example of a sampling schedule is provided in Table 1.

Table 1. Example of a sampling schedule for a *Magnitude of residues* study with 28 day uptake and 28 day depuration phases

Sampling event	Day of phase	Number of fish to be collected
Uptake phase	0[#]	2
	28	4
Depuration phase	3	4
	7	4
	14	4
	28	4

[#] fish sampled prior to the first dosing

33. If analysis of the first sampling points during the depuration phase indicates a fast (or very slow) elimination of the residue, the sampling schedule should be adjusted accordingly (shorter or longer elimination phase with adjusted sampling time points, resulting in the same overall number of samplings).

34. Fish sampled at the end of the uptake phase should be sacrificed within 6 - 12 hours of administering the final dose. Details on how to sacrifice fish are provided elsewhere (OECD, 2019). When sacrificing animals, it should be ensured that blood, urine, other body fluids or faeces do not contaminate the tissue samples. Feed and faeces residues need to be completely removed from intestines before the carcass is analysed.

35. Tissues to be sampled and analysed include, as a minimum, fillet (incl. skin), liver and carcass. The tissues are dissected and processed individually as described in Table 2.

Table 2. Fish sampling

Sample Material	Sampling Method	Analytical Sample Preparation
Fillet + Skin	Collect the whole fillet from both body sides including the entire skin of the fillet. Take weight of individual samples	Homogenise each sample
Liver	Collect the entire organ (trout, Atlantic salmon) or the hepatopancreatic tissue (carp). Take weight of individual samples	Homogenise each sample
Carcass	Collect all remains, including feed and faeces free intestines and except fillet/skin and liver. Take weight of individual samples.	Homogenise each sample

Sample Analysis

36. The analytical method including sample extraction and clean-up procedures should be described in detail or referenced. Fortified samples should be run concurrently with those from the *Magnitude of residue* study to prove suitability and performance of the method.

37. The lipid content of homogenised tissue samples (sample aliquots) collected at the start of dosing, end of dosing and end of the depuration phase (Table 2) should be measured. Lipid analysis of the different tissues (fillet incl. skin, liver, carcass) should be carried out according to the method for lipid extraction recommended by TG OECD 305 (OECD, 2012).

Storage Stability Data

38. For samples not analysed within 30 days, storage stability data should be generated to provide sufficient evidence that no significant degradation of the residue occurs between sampling and analysis.

DATA REPORTING

39. The following elements should be considered during the design, conducting and reporting of the study.

Summary

- Summary of key results: residues transfer to fillet (incl. skin), liver, carcass and to the whole fish (calculated) from oral dosing at different sampling dates.
- Evaluation of these results.
- Noting of any anomalies and evaluation of their relevance with reference to the objective.

Objective

A description of the aims of the study has to be provided including the questions addressed in the study.

Test material

The test substance, which is fed, and those substances which are used as analytical standards should be specified by:

- Chemical name (IUPAC);
- Common name (ANSI, BSI, IS0) (if available);
- Chemical Abstracts Service (CAS) name and number;
- Source and purity of each compound;
- Chemical structure graphics.

If feeding compounds other than parent pesticide are used, a rationale should be given.

In-life part

- The conditions of the tanks should be described. Factors to consider include: Water source and chemical properties, water temperature, water exchange rate, dissolved oxygen, pH, and light regime (if any).
- Test animals:

(i) A description of the test animals should include: Species, breed, age, weight (including record of weight changes), general condition and health status.

(ii) Number of animals treated should be reported.

(iii) Body weights, growth rates and feed conversion ratios should be reported for the acclimatisation, uptake and depuration phase.

(iv) Any health problems, mortality, morbidity, abnormal behaviour, or unusual treatment of animals should be reported and their effect on study results should be discussed.

– Feed:

(i) The stability of fortified feeds after coating should be reported. A brief description of the method used to analyse fortified feeds and the results of such analyses (i.e. the extent of leaching losses) should be presented. These analyses should demonstrate that the pesticide was stable in the feed or dosing material throughout its entire storage period.

(ii) The diet of animals during acclimatisation, uptake and depuration phase should be described as to:

1. The types of feed (e.g. pellet size, commercial fish feed, fortification method);

2. The quantities provided.

(iii) Feed consumption should be reported on a mean basis.

(iv) Dosing:

1. The preparation of the dose should be described (spiking of the feed with the test substance, coating-method, etc.). The level of the test substance in the total diet in parts per million (mg/kg feed) is needed. The dose calculation should be reported and justified (incl. output from the dietary burden calculation). The recommended dose is the 1X dose, additional doses or deviations should be justified.

2. The date of dose preparation should be specified along with the storage conditions prior to its administration.

3. A brief description of the method used to analyse fortified feeds and the results of such analyses should be presented. These analyses should demonstrate that the pesticide was stable in the feed or dosing material throughout its entire storage period.

4. The dates of the initial and final doses or applications (or the total length of the dosing period) should be indicated. Dose rates should be reported as mg/kg diet.

Post-sacrifice sampling

(i) The mode of sacrifice and the time interval in hours between time of sacrifice and the administration of the last dose or application of last treatment should be specified. An explanation of intervals longer than 12 hours after the sampling time

point at the end of the uptake phase should be presented along with a discussion of their effect on residues.

(ii) The tissues taken after sacrifice, their type (e.g., fillet (incl. skin), liver and carcass), and their weights should be listed.

Sample handling and storage stability

(i) The storage and handling of tissues between sample collection and analysis should be described. Factors to be considered are:

1. Sample preparation (e.g., homogenisation) prior to storage;
2. Containers;
3. Time interval between sampling, storage, and analysis;
4. Storage temperature;
5. Duration of storage (dates of collection, shipping, analysis, etc.);
6. Mode of shipping, if applicable.

(ii) Where samples are not analysed within 30 days, evidence should be presented showing that the storage did not affect the results of the study, i.e. demonstrate or reference adequate storage stability.

Extraction, clean-up, determination, evaluation

A description of the method used to prepare and measure the samples, identification of the residue levels in tissues and the methods used to assess the results are to be reported.

Analysis of samples

(i) A detailed description of the analytical method employed (including method validation data, recovery and method sensitivity) to measure residues is required along with a statement as to which substances were measured (parent pesticide, metabolites). When the method has been submitted as a separate report in the total data package, it may be referenced. Preparation and handling of the sample throughout the method should be described in detail. Note that methods for metabolites may also be needed.

(ii) Raw data such as sample weights, final volumes of extracts, and peak heights/areas should be furnished for all feed and fish samples (including those for storage stability data) to support reported residue values and recoveries.

(iii) Instrumentation, equipment and reagents used and the operating conditions of the instrumentation should be reported. If the extraction/clean-up procedure is complex, a flow diagram should be submitted.

(iv) Recovery data should be obtained concurrently with the residue analyses to validate the method and establish its sensitivity (lowest reliable limit of quantification). The experimental design of these validation studies should be described including:

1. Identity of the test compounds and substrates;

2. Magnitudes of fortification levels;

3. Number of replicates per test compound per fortification level.

(v) Dates of sample fortification, extraction, and analysis of extracts should be listed. If extracts are not analysed on the day of preparation, storage conditions should be described.

(vi) Analytical responses of standards (calibration curves), copies of representative chromatograms should be supplied for feed samples (control, fortified) and fish samples (feed, fillet (incl. skin), liver and carcass) along with at least one sample calculation of residue levels and percent recoveries using the raw data. Examples of calibration curves of analytical standards should also be provided.

(vii) The lipid content of homogenised tissue samples (fillet incl. skin, liver, carcass) collected at the start of dosing, end of dosing and end of the depuration phase should be determined.

Calculations

Calculations are carried out as described by Goeritz et al. 2013 and Berntssen et al. 2008. The carry-over of residues from the feed to the fillet, liver and whole fish (integrating fillet, liver and carcass residue concentrations) under fish culture conditions is evaluated. Absorption and elimination kinetics are determined to assess steady-state tissue concentrations.

Biomagnification factors of the test item in fillet incl. skin, liver and carcass are determined as a dynamic (kinetic) value derived from the assimilation efficiency of the test substance in the fish feed (α), the food ingestion rate (I), and the depuration (elimination) rate constant (k_2). The constant k_2 is determined by fitting tissue concentration data to a first-order decay curve. Elimination half-lives ($t_{1/2}$) are determined as ln 2/k_2.

The assimilation efficiency *(α)* of the test item is calculated as:

$$\alpha = \frac{C_{0,\mathrm{d}} \cdot k_2}{I \cdot C_{food}} \cdot \left(\frac{1}{1 - e^{(-k_2 \cdot t)}} \right)$$

Once the assimilation efficiency has been obtained, the BMF can be calculated by multiplying it with the feeding rate constant *I* and dividing the product by the overall depuration rate constant k_2.

$$BMF = \frac{I \cdot \alpha}{k_2}$$

BMFs can be used to calculate tissue concentrations under steady-state conditions for any dose rate. It is suggested that calculations are made for the 1X, 3X and 10X rate. Fillet (incl. skin), liver and carcass concentrations can be modelled over time by using a simple model based on one compartment first-order rate kinetics originally described by Sijm et al. 1993.

$$C_{tissue}(t) = \frac{I * \alpha}{k_2} C_{food}(1 - e^{-(k_2)t}) + C_{0,d}e^{-(k_2)t}$$

α = assimilation efficiency (absorption of test substance across the gut)

$C_{0,d}$ = concentration in fish tissue at time zero of the depuration phase (mg/kg)

k_2 = overall (not growth-corrected) depuration rate constant (day^{-1})

I = food ingestion rate (g food/g fish/day)

C_{food} = concentration in fish feed (mg/kg feed)

t = duration of the feeding period (d)

$C_{tissue}\ (t)$ = concentration in fish tissue over time (mg/kg)

Advanced dietary transfer models are developed which can be used to predict levels of pesticide residues in the edible part of farmed fish exposed to contaminated diets over prolonged periods (e.g. Berntssen et al. 2018; Larisch and Goss 2018). The evaluation of the feed-to-fillet transfer carried out as part of the *Magnitude of residue study* can be supplemented by the use of such models.

Calculations should refer to the residue of concern, i.e. the residue definition(s) for monitoring and risk assessment. Concentrations of major metabolites of the test item identified during the *Nature of residues* study should be covered.

Results and discussion

This section of the report discusses and evaluates the reported measurements in the light of the questions outlined in the objective section. The relevance of results should be discussed in relation to the proposed uses of the pesticide.

(i) Recovery percentages (all values, not just averages or ranges) for the pesticide and/or its metabolites should be reported for tissues fortified with these compounds.

(ii) Storage stability data showing the behaviour of residues as a function of time in tissues should be submitted or referenced when required. Storage duration and temperature of these samples should be specified.

(iii) Levels of the residue of concern should be reported for each sample. The tissues recommended for analysis include fillet (incl. skin), liver and carcass. The individual values should be listed for all samples of feeds and tissues (not merely averages or ranges). From the results in fillet (incl. skin), liver and carcass additionally the residues in the whole fish should be calculated. It should be clearly indicated whether residue values have been corrected for recoveries. If the residue of concern consists of several compounds, the residues of each should be reported, as far as analytically achievable.

(iv) Discussion should be presented concerning the transfer to tissues and the tendency to accumulate in certain tissues.

(v) The lipid content of homogenised tissue samples (fillet incl. skin, liver, carcass) should be reported.

(vi) BMF values under steady-state conditions should be calculated for the 1X, 3X and 10X dose rate.

Conclusion

A conclusion should be reached as to whether residues of the pesticide transfer from feed items into fish commodities. If so, the extent of transfer should be discussed. The results are preferably summarised in a table showing the ranges and maximum residues in each sample type for each dose level.

Study Report

The study report should at least contain all information mentioned in the Data reporting section.

LITERATURE

Berntssen, M.H.G., Glover, C.N., Robb, D.H.F., Jakobsen, J.V., and Petri, D., 2008. Accumulation and elimination kinetics of dietary endosulfan in Atlantic salmon (*Salmo salar*). Aquatic Toxicology 86, 104-111

Berntssen, M.H.G., Hannisdal, R., Buttle, L., Hoogenveen, R., Mengelers, M., Bokkers, B.G.H., and Zellmaker, M.J., 2018. Modelling the long-term feed-to-fillet transfer of leuco crystal violet and leuco malachite green in Atlantic salmon (*Salmo salar*). Food Additives & Contaminants: Part A. 35, 1484-1496

European Commission, 2021. Nature of pesticides residues in fish. Commission Working Document SANTE/10254/2021, 23 February 2021

European Commission, 2021a. Fish dietary burden calculator. Commission Working Document SANTE/10250/2021, 23 February 2021

European Medicines Agency, 2009. Guideline on demonstration of target animal safety and efficacy of veterinary medicinal products intended for use in farmed finfish. Committee for Medical Products for Veterinary Use (CVMP), 19 October 2009.

Fry, J.P., Mailloux, N.A., Love, D.C., Milli, M.C., Cao, L., 2018. Feed conversion efficiency in aquaculture: do we measure it correctly? Environmental Research Letters 13. DOI: https://doi.org/10.1088/1748-9326/aaa273

Goeritz, I., Falk, S., Stahl, T., Schäfers, C., and Schlechtriem, C., 2013. Biomagnification and Tissue Distribution of perfluoroalkyl substances (PFASs) in market size rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry. 32:2078–2088

Goeritz, I., Whalley, P., Seymour, P., Atorf, C., Klein, M. and Schlechtriem, C., 2014. Investigation into feed preparation for regulatory fish metabolism studies. Journal of the Science of Food and Agriculture 94, 438-44

Larisch, W. and Goss, G-U., 2018. Modelling oral up-take of hydrophobic and superhydrophobic chemicals in fish. Environ. Sci.-Proc. Imp. 20, 98 - 104

Organisation for Economic Co-operation and Development, 2007. Residues in Livestock. OECD Guideline for the Testing of Chemicals No 505, Paris, January 2007

Organisation for Economic Co-operation and Development, 2012. Bioaccumulation in fish: Aqueous and dietary exposure. OECD Guideline for the Testing of Chemicals No 305, Paris, October 2012

Organisation for Economic Co-operation and Development, 2013. Guidance Document on Residues in Livestock. OECD Environment, Health and Safety Publications, Series on Pesticides No. 73, ENV/JM/MONO(2013)8

Organisation for Economic Co-operation and Development, 2019. Fish, Acute Toxicity Testing. OECD Guideline for the Testing of Chemicals No. 203, Paris, 18 June 2019

Schlechtriem, C., Fliedner, A., and Schäfers, C., 2012. Determination of lipid content in fish samples from bioaccumulation studies: Contributions to the revision of guideline OECD 305. Environmental Sciences Europe 24, 13

Schlechtriem, C., Bischof, I., Atorf, C., Bergendahl, E., Seymour, P., Whalley, P., 2015. Development of a regulatory testing procedure to study the metabolism of pesticides in farmed fish. Pest Management Science 72, 362-370

Schlechtriem, C., Pucher, J., and Michalski, B., 2016. Dietary burden calculation for fish metabolism studies. Journal of the Science of Food and Agriculture 96, 1415-1419