COMMISSION IMPLEMENTING REGULATION (EU) 2022/893

of 7 June 2022

amending Annex VI to Regulation (EC) No 152/2009 as regards the methods of analysis for the detection of constituents of terrestrial invertebrates for the official control of feed

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation) (¹), and in particular Article 34(6), first subparagraph, point (a), thereof,

Whereas:

- (1) Commission Regulation (EC) No 152/2009 (²) establishes testing methods used to support official controls to enforce the ban on the use of processed animal protein in feed for food producing animals. This includes methods of analysis for the determination of constituents of animal origin for the official control of feed, which are described in Annex VI to that Regulation and performed by light microscopy or polymerase chain reaction (PCR).
- (2) The use of processed animal protein derived from farmed insects has been authorised in the feed of aquaculture animals by Commission Regulation (EU) 2017/893 (³), and in the feed of porcine animals and poultry by Commission Regulation (EU) 2021/1372 (⁴), but is still prohibited under Regulation (EC) No 999/2001 of the European Parliament and of the Council (⁵) in certain feed, notably in the feed of ruminants.
- (3) The European Union reference laboratory for animal proteins in feedingstuffs has developed and validated a special protocol, including a double sedimentation step, which ensures the detection of particles from terrestrial invertebrates, including insects, if present in feed materials, compound feed and premixtures submitted to laboratory testing. With this additional step, that protocol should be used in the framework of official controls to verify the correct enforcement of the ban on the use of processed animal protein of insects in certain feed for food producing animals.

⁽¹⁾ OJ L 95, 7.4.2017, p. 1.

^{(&}lt;sup>2</sup>) Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (OJ L 54, 26.2.2009, p. 1).

^{(&}lt;sup>3</sup>) Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein (OJ L 138, 25.5.2017, p. 92).

^(*) Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals (OJ L 295, 18.8.2021, p. 1).

⁽i) Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (OJ L 147, 31.5.2001, p. 1).

- (4) The description of the light microscopy method set out in Annex VI to Regulation (EC) No 152/2009 should therefore be adjusted in order to include a double sedimentation step in the protocol for the preparation of samples to be tested for detecting constituents of terrestrial invertebrates.
- (5) Annex VI to Regulation (EC) No 152/2009 should therefore be amended accordingly.
- (6) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

EN

Article 1

Annex VI to Regulation (EC) No 152/2009 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the Official Journal of the European Union.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 7 June 2022.

For the Commission The President Ursula VON DER LEYEN

ANNEX

Annex VI to Regulation (EC) No 152/2009 is amended as follows:

- (1) point 1 is replaced by the following:
 - '1. PURPOSE AND SCOPE

The determination of constituents of animal origin in feed shall be performed by light microscopy or polymerase chain reaction (PCR) in accordance with the provisions laid down in this Annex.

These two methods make it possible to detect the presence of constituents of animal origin in premixtures, feed materials and compound feed. However, they do not make it possible to calculate the amount of such constituents in premixtures, feed materials and compound feed. Both methods have a limit of detection below 0,1 % (w/w).

The PCR method makes it possible to identify the taxonomic group of constituents of animal origin present in premixtures, feed materials and compound feed.

These methods shall apply for the control of the application of the prohibitions laid down in Article 7(1) of Regulation (EC) No 999/2001 of the European Parliament and of the Council (*), Annex IV to that Regulation and Article 11(1) of Regulation (EC) No 1069/2009 of the European Parliament and of the Council (**).

Depending on the type of feed being tested, these methods may be used, within one single operational protocol, either on their own or combined together in accordance with the standard operating procedures ("SOPs") established by the EU reference laboratory for animal proteins in feedingstuffs (EURL-AP) and published on its website (***).

- (*) Regulation (EC) No 999/2001 of the European Parliament and of the Council of 22 May 2001 laying down rules for the prevention, control and eradication of certain transmissible spongiform encephalopathies (OJ L 147, 31.5.2001, p. 1).
- (**) Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009, p. 1).
- (***) https://www.eurl.craw.eu/legal-sources-and-sops/method-of-reference-and-sops/;
- (2) point 2.1 is replaced by the following:

^{2.1.} Light microscopy

2.1.1. Principle

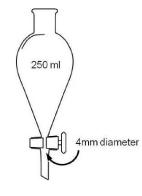
The constituents of animal origin which may be present in premixtures, feed materials and compound feed sent for analysis are identified on the basis of typical and microscopically identifiable characteristics such as muscle fibres and other meat particles, cartilage, bones, horn, hair, bristles, invertebrates cuticular fragments, insect tracheal structures, blood products, milk globules, lactose crystals, feathers, egg shells, fish bones and scales.

Microscopic examinations shall be performed after preparation of samples by sedimentation.

Samples shall be subject to a sedimentation step as follows:

- (a) for the detection of constituents of animal origin other than terrestrial invertebrates, a single Tetrachloroethylene (TCE) sedimentation step as detailed in point 2.1.3.4.3;
- (b) for the detection of constituents of terrestrial invertebrates, a double Petroleum ether/Tetrachloroethylene (PE/TCE) sedimentation step as detailed in point 2.1.3.4.4.
- 2.1.2. Reagents and equipment
- 2.1.2.1. Reagents
- 2.1.2.1.1. Concentrating agent
 - Tetrachloroethylene (specific gravity 1,62).
 - Petroleum ether (PE) boiling point 40-60 °C (specific gravity 0,65).

- 2.1.2.1.2. Staining reagent
 - Alizarin Red solution (dilute 2,5 ml 1M hydrochloric acid in 100 ml water and add 200 mg Alizarin Red to this solution).
- 2.1.2.1.3. Mounting media
 - Lye (NaOH 2,5 % w/v or KOH 2,5 % w/v).
 - Glycerol (undiluted, viscosity: 1 490 cP) or a mounting medium with equivalent properties for nonpermanent slide preparation.
- 2.1.2.1.4. Mounting media with staining properties
 - Lugol solution (dissolve 2 g potassium iodide in 100 ml water and add 1 g iodine while frequently shaking).
 - Cystine reagent (2 g lead acetate, 10 g NaOH/100 ml water).
 - Fehling's reagent (prepared before use from equal parts (1/1) of two-stock solutions A and B: solution A (dissolve 6,9 g copper (II) sulphate pentahydrate in 100 ml water); solution B (dissolve 34,6 g potassium sodium tartrate tetrahydrate and 12 g NaOH in 100 ml water).
 - Tetramethylbenzidine/Hydrogen peroxide (dissolve 1 g 3,3',5,5' tetramethylbenzidine (TMB) in 100 ml glacial acetic acid and 150 ml water. Before use, mix 4 parts of this TMB solution with 1 part 3 % hydrogen peroxide).
- 2.1.2.1.5. Rinsing agents
 - Ethanol \geq 96 % (technical grade).
 - Acetone (technical grade).
- 2.1.2.1.6. Bleaching reagent
 - Commercial sodium hypochlorite solution (9 14 % active chlorine).
- 2.1.2.2. Equipment
 - Analytical balance with an accuracy of 0,001 g.
 - Grinding equipment: knife or rotor mill. If a rotor mill is used, mill sieves ≤ 0,5 mm shall be prohibited.
 - Sieves with square meshes of 0,25 mm and 1 mm width. With the exception of sample pre-sieving, the diameter of the sieves shall not exceed 10 cm to avoid loss of materials. Calibration of sieves is not required.
 - Conical glass separation funnel with a content of 250 ml with Teflon or ground glass stopcock at the base of the cone. Stopcock opening diameter shall be \geq 4mm. Alternatively, for single TCE sedimentation only, a conical bottomed settling beaker may be used provided the laboratory has demonstrated that detection levels are equivalent to that obtained using the conical glass separation funnel.



Separation funnel

- Stereomicroscope covering at least a 6,5x to 40x final magnification range.
- Compound microscope covering at least a 100x to 400x final magnification range with transmitted light bright field. Polarised light, differential interferential contrast can additionally be used.
- Standard laboratory glassware.
- Equipment for slide preparation: classical microscope slides, hollow slides, coverslips (20x20 mm), tweezers, fine spatula.
- Laboratory oven.
- Centrifuge.
- Filter paper: qualitative cellulose filter (pore size 4-11 μm).

2.1.3. Sampling and sample preparation

2.1.3.1. Sampling

A representative sample, taken in accordance with Annex I shall be used.

2.1.3.1.1. Sample drying

Samples with a moisture content > 14 % shall be dried prior to handling in accordance with Annex III.

2.1.3.1.2. Sample pre-sieving

In order to collect information on possible environmental contamination of the feed, it is recommended to pre-sieve at 1 mm pelleted feeds and kernels and to subsequently prepare, analyse, and report separately on the two resulting fractions, which must be considered as distinct samples.

2.1.3.2. Precautions to be taken

In order to avoid laboratory cross-contamination, all reusable equipment shall be carefully cleaned before use. Separation funnel pieces shall be disassembled before cleaning. Separation funnel pieces and glassware shall be pre-washed manually and then washed in a washing machine. Sieves shall be cleaned by using a brush with stiff synthetic hairs. A final cleaning of sieves with acetone and compressed air is recommended after sieving of fatty material like fishmeal.

2.1.3.3. Preparation of samples consisting of fat or oil

The following protocol shall be followed for the preparation of samples consisting of fat:

- If the fat is solid, it shall be warmed in an oven until it is liquid.
- By using a pipette, 40 ml of fat shall be transferred from the bottom of the sample to a centrifugation tube.
- The sample shall be centrifuged during 10 min at 4 000 r.p.m.
- If the fat is solid after centrifugation, it shall be warmed in an oven until it is liquid.
- The centrifugation shall be repeated during 5 min at 4 000 r.p.m.
- By using a small spoon or a spatula, one half of the decanted impurities shall be transferred to
 microscopic slides for examination. Glycerol is recommended as mounting medium.
- The remaining impurities shall be used for preparing the sediment as described in point 2.1.3.4.3, first indent.

The same protocol, with the exception of the first and fourth indents, shall be applied for the preparation of samples consisting of oil.

- 2.1.3.4. Preparation of samples other than fat or oil
- 2.1.3.4.1. Sub-sampling and grinding: at least 50 g of the sample shall be sub-sampled for analysis and subsequently ground.

- 2.1.3.4.2. Preparation of raw material: a portion of at least 5 g of the ground sub-sample shall be prepared. It shall be sieved at 0,25 mm and the two resulting fractions shall be examined.
- 2.1.3.4.3. Single TCE sedimentation for the detection of constituents of animal origin other than terrestrial invertebrates.
 - Extraction and preparation of the sediment:

A portion of 10 g (accurate to 0,01 g) of the ground sub-sample shall be transferred into the separation funnel or conical bottomed settling beaker and 50 ml of TCE shall be added. The portion transferred into the funnel shall be limited to 3 g in case of fishmeal or other pure animal products, mineral ingredients or premixtures which generate more than 10 % of sediment. The mixture shall be vigorously shaken for at least 30 s and 50 ml more of TCE shall be added cautiously while washing down the inside surface of the funnel to remove any adhering particles. The resulting mixture shall be left to stand for at least 5 min before the sediment is separated off by opening the stopcock.

If a conical bottomed settling beaker is used then the mixture shall be vigorously stirred for at least 15 s and any particles adhering to the side of the beaker shall be carefully washed down the inside surface with at least 10 ml of clean TCE. The mixture shall be left to stand for 3 min and then stirred again for 15 s and any particles adhering to the side of the beaker shall be carefully washed down the inside surface with at least 10 ml of clean TCE. The resulting mixture shall be left to stand for at least 5 min and then the liquid fraction is removed and discarded by careful decanting, taking care not to lose any of the sediment.

The sediment shall be collected on a filter paper placed into a funnel to allow the separation of the remaining TCE while avoiding fat deposition into the sediment. The sediment shall be dried. It is recommended to subsequently weigh the sediment (accurate to 0,001 g) to control the sedimentation step. Lastly, the sediment shall be sieved at 0,25 mm and the two resulting fractions shall be examined, unless sieving is not deemed necessary.

— Extraction and preparation of the flotate:

After recovery of the sediment with the method described above, two phases shall remain in the separation funnel: a liquid one consisting of TCE and a solid one made of floating material. This solid phase is the flotate and shall be recovered by pouring off completely TCE from the funnel by opening the stopcock. By inverting the separation funnel, the flotate shall be transferred into a large petri dish and air dried in a fume hood. It shall be sieved at 0,25 mm and the two resulting fractions shall be examined.

— Use of staining reagents:

In order to facilitate the correct identification of the constituents of animal origin, the operator may use staining reagents during the sample preparation in accordance with guidelines issued by the EURL-AP and published on its website.

In case Alizarin Red solution is used to colour the sediment, the following protocol shall apply:

- The dried sediment shall be transferred into a glass test tube and rinsed twice with approximately 5 ml of ethanol (each time a vortex of 30 s shall be used, the solvent shall be let settle about 1 min 30 s and poured off).
- The sediment shall be bleached by adding at least 1 ml sodium hypochlorite solution. The reaction shall be allowed to continue for 10 min. The tube shall be filled with water, the sediment shall be let settle 2-3 min, and the water and the suspended particles shall be poured off gently.
- The sediment shall be rinsed twice more with about 10 ml of water (a vortex shall be used for 30 s, let settle, and pour off the water each time).
- 2 to 10 drops of the Alizarin Red solution shall be added and the mixture shall be vortexed. The reaction shall be let occur for 30 s and the coloured sediment shall be rinsed twice with approximately 5 ml ethanol followed by one rinse with acetone (each time a vortex of 30 s shall be used, the solvent shall be let settle about 1 min and poured off).
- The coloured sediment shall be dried.

2.1.3.4.4. Double PE/TCE sedimentation for the detection of terrestrial invertebrate constituents.

All steps shall be realised in a conical glass separation funnel of 250 ml as described in point 2.1.2.2, fourth indent.

- A portion of 10 g (accurate to 0,01 g) of the ground sub-sample shall be transferred into the separation funnel and submitted first to a single TCE sedimentation as described in point 2.1.3.4.3 including the recovery of the sediment on a filter paper placed on a funnel. This sediment may be used as the one obtained from point 2.1.3.4.3.
- The small volume of TCE drained together with the sediment shall be transferred into a graduated cylinder. By opening the stopcock of the separation funnel the graduated cylinder has to be filled further until obtaining 30 ml of TCE. Once this volume is achieved, the stopcock shall be closed.
- This collected volume of TCE shall be substituted by adding a volume of 30 ml of petroleum ether boiling point 40– 60 °C into the separation funnel. The content of the separation funnel shall be mixed thoroughly to obtain a 30 % PE/70 % TCE mixture (with a density of approximately 1,26 g.cm⁻³). Allow the material to settle down for 10 min. Two new fractions will segregate: a second sediment and a final flotate (< 1,26 g.cm⁻³). The second sediment is to recover in a petri dish (or a filter paper placed on a funnel) by opening the stopcock until only a few solvent mixture and the final flotate remain in the separation funnel. The remaining liquid and the final flotate shall be collected separately on a filter paper placed on a funnel. The wall of the separation funnel shall be rinsed with a flush of PE to collect all material from the final flotate. The final flotate shall be allowed to dry. The final flotate shall be sieved at 0,25 mm and the two resulting fractions shall be examined for the detection of terrestrial invertebrate constituents, unless sieving is not deemed necessary.
- 2.1.4. Microscopic examination
- 2.1.4.1. Slide preparation

Microscopic slides shall be prepared from the sediment and, depending on operator's choice, from either the flotate or the raw material. When appropriate, for the detection of terrestrial invertebrate constituents only, slides shall also be prepared from the final flotate obtained as described in point 2.1.3.4.4. The two resulting fractions (the fine and the coarse one) shall be prepared. Test portions of fractions spread on slides shall be representative of the whole fraction.

A sufficient number of slides shall be prepared in order to ensure that a complete examination protocol as laid down in point 2.1.4.2 can be carried-out.

Microscopic slides shall be mounted with the adequate mounting medium in accordance with the SOP established by the EURL-AP and published on its website. The slides shall be covered with coverslips.

2.1.4.2. Observation flowchart for the detection of animal particles in compound feed, feed material and premixtures.

The prepared microscopic slides shall be observed in accordance with the observation flowcharts in Diagrams 1 and 2.

The microscopic observations shall be conducted using the compound microscope on the sediment and, depending on the operator's choice, either on the flotate or on the raw material. Additionally, for the detection of terrestrial invertebrate constituents, observations shall also be conducted on the final flotate obtained as described in point 2.1.3.4.4 in accordance with Diagram 3. The stereomicroscope may be used in addition to the compound microscope for the coarse fractions. Each slide shall be screened entirely at various magnifications. Precise explanations on how to use the flowcharts are detailed by a SOP established by the EURL-AP and published on its website.

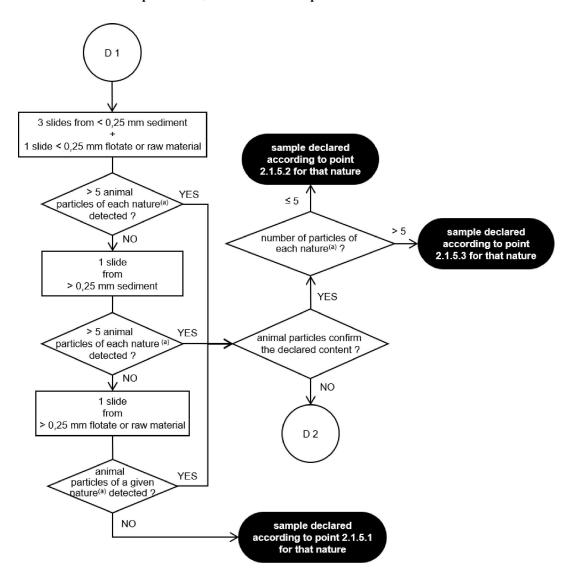
The minimum numbers of slides to be observed at each step of the observation flowcharts shall be strictly respected unless the entire fraction material does not permit to reach the stipulated slide number, for instance when no sediment is obtained. No more than 6 slides per determination shall be used for recording of the number of particles.

When additional slides are prepared using a more specific mounting medium with staining properties, as described in point 2.1.2.1.4, on the flotate or the raw material to further characterise structures (e.g. feathers, hairs, muscle or blood particles), which have been detected on slides prepared by other mounting media, as described in point 2.1.2.1.3, the number of particles shall be counted based on a number of slides per determination not exceeding 6, including the additional slides with a more specific mounting medium. The additional slides prepared from the final flotate obtained, as described in point 2.1.3.4.4, for the detection of terrestrial invertebrate constituents shall not be considered for the identification of other natures (terrestrial vertebrates and fish).

In order to facilitate the identification of the particles' nature and origin, the operator may use support tools like decision support systems, image libraries and reference samples.

Diagram 1

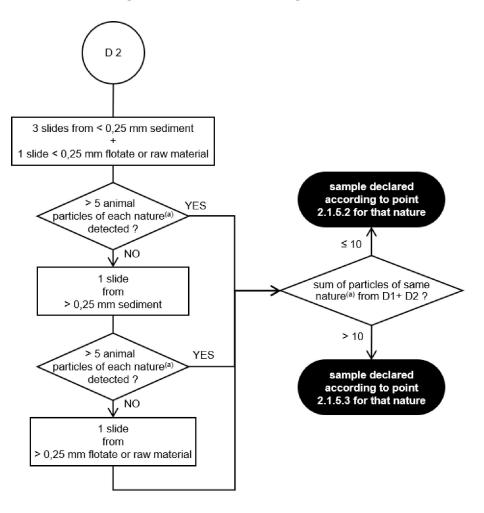
Observation flowchart after single TCE sedimentation for the detection of animal particles other than from terrestrial invertebrates in compound feed, feed materials and premixtures for the first determination



("D1" and "D2" refer to the first and second determinations; (a): terrestrial vertebrates, fish)

Diagram 2

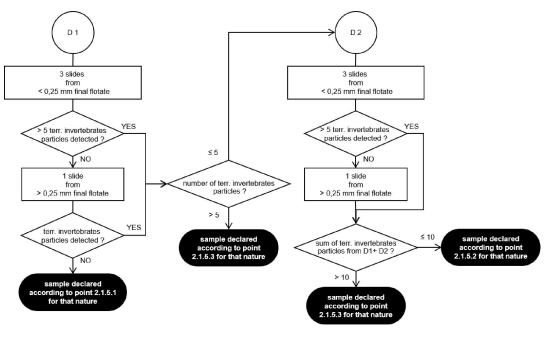
Observation flowchart after single TCE sedimentation for the detection of animal particles other than from terrestrial invertebrates in compound feed, feed materials and premixtures for the second determination



("D1" and "D2" refer to the first and second determinations; (a): terrestrial vertebrates, fish)

Diagram 3

Observation flowchart after double PE/TCE sedimentation for the detection of terrestrial invertebrates' constituents in compound feed, feed materials and premixtures



("D1" and "D2" refer to the first and second determinations)

2.1.4.3. Number of determinations

Determinations shall be performed on different sub-samples of 50 g each.

If, following the first determination carried out in accordance with the observation flowchart in Diagram 1, or Diagram 3 when appropriate, no animal particles are detected, no additional determination shall be necessary and the result of the analysis shall be reported using the wording set out in point 2.1.5.1.

If, following the first determination carried out in accordance with the observation flowchart in Diagram 1, one or more animal particles of a given nature (i.e. terrestrial vertebrates or fish) are detected and the nature of the particles found confirms the declared content of the sample, no second determination shall be necessary. If the number of the animal particles of a given nature detected during this first determination is higher than 5, the result of the analysis shall be reported per animal nature using the wording set out in point 2.1.5.3. Otherwise, the result of the analysis shall be reported per animal nature using the wording set out in point 2.1.5.2.

If, following the first determination carried out in accordance with the observation flowchart in Diagram 3, more than 5 particles of terrestrial invertebrates are detected, no second determination shall be necessary and the result of the analysis shall be reported using the wording set out in point 2.1.5.3 for this nature.

In all other cases, including when no declaration of content has been provided to the laboratory, a second determination shall be carried out from a new sub-sample. If, following the second determination carried out in accordance with the observation flowchart in Diagram 2, or Diagram 3 when appropriate, the sum of the animal particles of a given nature detected over the two determinations is higher than 10, the result of the analysis shall be reported per animal nature using the wording set out in point 2.1.5.3. Otherwise, the result of the analysis shall be reported per animal nature using the wording set out in point 2.1.5.2.

2.1.5. Expression of the results

When reporting the results, the laboratory shall indicate on which type of material the analysis has been carried out (sediment, flotate, final flotate or raw material). The reporting shall clearly indicate how many determinations have been carried out and if sieving of the fractions prior to slide preparation, in accordance with point 2.1.3.4.3, first indent, third paragraph, or point 2.1.3.4.4, third indent, was not performed.

The laboratory report shall at least contain information on the presence of constituents derived from terrestrial vertebrates and from fish.

The different situations shall be reported in the following ways.

- 2.1.5.1. No animal particle of a given nature is detected:
 - "As far as was discernible using a light microscope, no particle derived from terrestrial vertebrates was
 detected in the submitted sample."
 - "As far as was discernible using a light microscope, no particle derived from fish was detected in the submitted sample."
 - "As far as was discernible using a light microscope, no particle derived from terrestrial invertebrates was
 detected in the submitted sample."
- 2.1.5.2. Between 1 and 5 animal particles of a given nature are detected when only one determination has been performed, or between 1 and 10 particles of a given nature are detected in case of two determinations (the number of detected particles is below the decision limit established in the SOP of the EURL-AP and published on its website):

When only one determination has been performed:

- "As far as was discernible using a light microscope, no more than 5 particles derived from terrestrial vertebrates were detected in the submitted sample. The particles were identified as ... [bone, cartilage, muscle, hair, horn, other (please specify as appropriate)]. This low level presence is below the decision limit established for this microscopic method."
- "As far as was discernible using a light microscope, no more than 5 particles derived from fish were detected in the submitted sample. The particles were identified as ... [fishbone, fish scale, cartilage, muscle, otolith, gill, other (please specify as appropriate)]. This low level presence is below the decision limit established for this microscopic method."

When two determinations have been performed:

- "As far as was discernible using a light microscope, no more than 10 particles derived from terrestrial vertebrates were detected over the two determinations in the submitted sample. The particles were identified as ... [bone, cartilage, muscle, hair, horn, other (please specify as appropriate)]. This low level presence is below the decision limit established for this microscopic method."
- "As far as was discernible using a light microscope, no more than 10 particles derived from fish were detected over the two determinations in the submitted sample. The particles were identified as ... [fishbone, fish scale, cartilage, muscle, otolith, gill, other (please specify as appropriate)]. This low level presence is below the decision limit established for this microscopic method."
- "As far as was discernible using a light microscope, no more than 10 particles derived from terrestrial invertebrates were detected over the two determinations in the submitted sample. The particles were identified as ... [cuticle fragments, mouthparts, muscles, tracheal structures, other (please specify as appropriate)]. This low level presence is below the decision limit established for this microscopic method."

Additionally:

— In case of sample pre-sieving, the laboratory report shall mention in which fraction (sieved fraction, pelleted fraction or kernels) the animal particles have been detected insofar as the detection of animal particles only in the sieved fraction may be the sign of an environmental contamination.

- When only animal particles which cannot be categorised as either terrestrial vertebrates or fish are detected (e.g. muscle fibres), the report shall mention that only such animal particles were detected and that it cannot be excluded that they originate from terrestrial vertebrates.
- 2.1.5.3. More than 5 animal particles of a given nature are detected when only one determination has been performed, or more than 10 particles of a given nature are detected in case of two determinations:

When only one determination has been performed:

- "As far as was discernible using a light microscope, more than 5 particles derived from terrestrial vertebrates were detected in the submitted sample. The particles were identified as ... [bone, cartilage, muscle, hair, horn, other (please specify as appropriate)]."
- "As far as was discernible using a light microscope, more than 5 particles derived from fish were detected in the submitted sample. The particles were identified as ... [fishbone, fish scale, cartilage, muscle, otolith, gill, other (please specify as appropriate)]."
- "As far as was discernible using a light microscope, more than 5 particles derived from terrestrial invertebrates were detected in the submitted sample. The particles were identified as ... [cuticle fragments, mouthparts, muscles, tracheal structures, other (please specify as appropriate)]."

When two determinations have been performed:

- "As far as was discernible using a light microscope, more than 10 particles derived from terrestrial vertebrates were detected over the two determinations in the submitted sample. The particles were identified as ... [bone, cartilage, muscle, hair, horn, other (please specify as appropriate)]."
- "As far as was discernible using a light microscope, more than 10 particles derived from fish were detected over the two determinations in the submitted sample. The particles were identified as ... [fishbone, fish scale, cartilage, muscle, otolith, gill, other (please specify as appropriate)]."
- "As far as was discernible using a light microscope, more than 10 particles derived from terrestrial invertebrates were detected over the two determinations in the submitted sample. The particles were identified as ... [cuticle fragments, mouthparts, muscles, tracheal structures, other (please specify as appropriate)]."

Additionally:

- In case of sample pre-sieving, the laboratory report shall mention in which fraction (sieved fraction, pelleted fraction or kernels) the animal particles have been detected insofar as the detection of animal particles only in the sieved fraction may be the sign of an environmental contamination.
- When only animal particles which cannot be categorised as either terrestrial vertebrates or fish are detected (e.g. muscle fibres), the report shall mention that only such animal particles were detected and that it cannot be excluded that they originate from terrestrial vertebrates.'