In special cases, the CEF Panel may require OM data, e.g. when larger amounts of oligomers are suspected (see item 5.3). For the determination of OM, refer to the JRC 'Technical guidelines for compliance testing of plastic food contact materials in the framework of Regulation (EU) No 10/2011'.

Ref:

5.2.1. Test sample: Set out what food contact material sample was subjected to testing, e.g. composition, shape (bottles, film, cups, tins, etc.), thickness and dimensions. For selection of test samples, etc., see 5.1.2. Where relevant use the same grade of test material in specific and overall migration testing. However there may be reasons to take different grades of material. Where the overall migration of one grade gives highest results while from another grade the specific migration is the highest, then different test samples could be used.

Ref:

5.2.2. Treatment of sample prior to testing: Set out to what treatment food contact material was subjected prior to testing.

5.2.3. Food simulant(s): Set out food simulant(s) used in testing.
Commission Regulation (EU) No 10/2011, as amended, should be followed for the selection of food simulants. The JRC 'Technical guidelines for compliance testing of plastic food contact materials in the framework of Regulation (EU) No 10/2011', should be also taken into account. The necessity of the use of substitute test medium should be explained, preferably supported by some analytical data.

5.2.4. Contact mode: Set out whether the sample was tested on one or on two sides. Set out in which way contact with the simulants was achieved, like cell, pouch, total immersion etc. If tested on two sides, set out whether one or both sides of the test specimen are used in the calculation of the contact area.

5.2.5. Contact time and temperature: Set out duration of test and test temperature in °C. Time temperature combinations should be determined in accordance with Annex V to Regulation (EU) No 10/2011.
In case of short contact times ($\leq$ 2 h) at high temperature ($\geq$ 100°C), describe in acceptable manner or demonstrate maintenance of the temperature over the test period.

5.2.6. Surface to volume ratio: Set out area of test sample in dm^2/L simulant.
Conventionally, the ratio is 6 dm^2/kg simulant.
The actual ratio in the migration tests may deviate.

5.2.7. Test method: Set out analytical methods used. Reference to valid CEN methods (see item 5.2) should be given, where relevant. Any deviation from those methods should be reported. If other methods are used to determine the overall migration, then a detailed description of the analytical method should be provided.
Please note that Article 20 (2)(c) of Regulation (EC) No 1935/2004 applies, i.e. information relating to the analytical methods shall not be considered confidential.

5.2.8. Other information: Set out any other information that may be relevant for the evaluation.

5.2.9. Results: Give all individual migration data obtained, if relevant, blanks inclusive. The data should preferably be presented in a table,

which should contain sufficient details to follow the way the final results are obtained, e.g.:

- test conditions of time and temperature (in °C)
- simulant
- contact area (dm^2)
- volume of food simulant used in the test (mL)
- migration in the food simulant expressed in mg/dm^2
- migration in the food simulant using the conventional factor of 6 dm^2/kg or any other relevant ratio.

Ref:

5.3. Quantification and identification of: (a) migrating oligomers and (b) reaction products derived from monomers and starting substances and additives:

Answer 'determined', or 'not determined'. Where it is not determined, a justification should be given.
Experimental data show that in polymers the migration of oligomers ($M_w < 1{,}000$) or reaction products occurs and in some cases high levels were found. Therefore, there is a need for information on:

(a) the migration of oligomers from polymers produced from monomers or which are produced by means of polymerisation aids that influence the molecular structure or molecular weight of the polymer.
(b) the migration of reaction products from polymers produced from monomers or additives.

In the first instance, there is a need for information on the identity and level of substances that migrate as a consequence of the use of a new monomer or additive (see also 2.2).
Tests with olive oil may not be suitable for identification purposes. Substitute simulants or alternative test media may be more convenient for identification purposes.
In principle, the identity of the migratable substances may be required, however, in some cases, a simple characterisation by identification of the functional groups may be sufficient.

Ref:

5.3.1. Test sample:

The test sample composition and its thickness should always represent the worst case. In general, the highest concentration of the substance, and the largest thickness, should be used. If the substance is intended to be used in a range of materials of different polymers or grades, then each type of material should be tested. However, if it is properly argued, only tests with the material representing the worst case may be acceptable.

Ref:

5.3.1.1. Chemical composition:

Set out chemical composition of the test sample. nformation should be provided on the initial concentration of the substance(s), and also on the total composition, as this may influence the final migration of the substance(s).

5.3.1.2. Physical composition:

Set out physical composition of test sample, such as homogeneous material, multilayer material, etc
In the case of a multilayer material, it should be indicated in which layer the substance(s) is present. If this is not the direct food contact side, then relevant information should also be given on the top layers.

5.3.1.3.	**Density, melt flow index of polymer:**	Set out density and melt flow index (if relevant) of the polymer containing the substance(s). This information is required for mathematical modelling. The density of the barrier layers should also be given in multilayer constructions.
5.3.1.4.	**Dimensions of test sample:**	Set out dimensions of test sample. Test sample is the sample manufactured or used for the study. Provide information on shape, e.g.: bottle, film, sheet, etc., and thickness. For laminates, the total thickness and the thickness of each relevant layer should be indicated. For articles with non-homogeneous thickness, the thickness at various places should be given. The dimensions of an article should be set out (height, length, width and/or diameter).
5.3.1.5.	**Dimensions of test specimen:**	Describe briefly the part or section of the test sample from which the test specimen was taken. Particular attention should be paid to this step in case of variable thickness materials (e.g. bottle). Set out spatial dimensions of test specimen (length, height, width, diameter). Calculate the total area of the test specimen. In case of two-sided contact (see 6.3.1.4), also calculate the total area of both sides. If the test specimen does not come into contact completely with the simulant, then calculate the actual contact area. In case of extraction, the weight of the test sample may suffice.
5.3.2.	**Treatment of test sample prior to testing:**	Set out to what treatment the food contact material was subjected prior to testing, e.g. cleaning, washing etc. Treatment of a test sample should be representative of use in practice.
5.3.3.	**Test food(s)/food simulant(s)/extraction solvent(s):**	Set out foodstuff(s) or food simulant(s) or extraction solvent (s) used in migration testing. For quantitative determinations, the use of food simulants selected according to Commission Regulation (EU) No 10/2011 as amended should be followed. Identification or characterisation of migratable substances may be possible in aqueous food simulants. In general, use of olive oil may not be feasible for various reasons. The use of volatile simulants or extraction solvents may be required to allow identification or characterisation of the migratable substances.
5.3.4.	**Contact mode:**	Set out whether the sample was tested on one or on two sides. Set out in which way contact with the simulants was achieved, e.g. cell, pouch, total immersion, etc. If tested on two sides, set out whether one or both sides of the test specimen are used in the calculation of the contact area. Set out conditions of extraction, if relevant.
5.3.5.	**Contact time and temperature:**	Set out test duration and temperature.
5.3.6.	**Surface to volume ratio in migration tests:**	Give the actual contact area and the volume of simulant used in the migration experiment. Calculate their ratio expressed as dm^2/kg food simulant.

In principle, the ratio should be equivalent to the ratio occurring in real use. If this ratio is not known, then the conventionally 6 dm^2/kg simulant may be used. For analytical reasons, it may be necessary to deviate from that ratio, which in principle is acceptable. However it should be carefully considered whether or not using a higher ratio of area to volume, could influence the final migration due to saturation of the simulant, which may occur with substances poorly soluble in the simulant used.
In extraction experiments, this most likely will not occur.

5.3.7. Analytical method:

Set out the principle of analytical method(s) used, and submit a full copy of the method in the technical dossier. Follow the description of item 5.1.8.
Identification or characterisation of migratable substances usually require application of various sophisticated and complementary techniques. The analytical methods applied should be described in the technical dossier in such detail to allow appropriate evaluation of the results. This requires information on chromatographic, mass spectrometric systems, or other means of isolation or detection. Chromatograms, spectra, etc should be provided with a proper legend. Information or conclusions to be deduced from such documents should be accompanied by an explanatory text.
Details should be given on the quantitative gravimetric analysis method. When using quantitative chromatographic methods, all details of the method that may be relevant for the evaluation of the results should be provided, e.g. actual data concerning the calibration procedure, typical chromatograms or spectra, calibration curves, correlation coefficients, etc.
Please note that Article 20 (2)(c) of Regulation (EC) No 1935/2004 applies, i.e. information relating to the analytical methods shall not be considered confidential.

Ref:

5.3.8. Detection/ determination limit:

Give detection and/or determination limit of the method, and set out the way the detection limit was established for quantitative determinations. Where relevant, visual information such as typical chromatograms, calibration curve, blank values should be provided.
An indication on the detection limit should also be provided in quantitative analyses.

Ref:

5.3.9. Recovery:

Set out percentage recovery of substance as determined in recovery experiments under time-temperature conditions of migration test.
Recovery experiments as required in specific migration testing may or may not be possible, as no reference substances may be available. If there are proper arguments, then the recovery tests are not required.

Ref:

5.3.10. Other information:

Set out any other information that may be relevant for evaluation.

Ref:

5.3.11. Results: Describe the migratable substance(s) that have been characterised or identified and give their migration levels (expressed in mg/6 dm^2). The presentation of the results of the characterised or identified migratable substance(s) may not be a straightforward issue. Any conclusions drawn from the investigations will need to be justified with some clear reasoning and explanation.

Ref:

6. DATA ON RESIDUAL CONTENT OF SUBSTANCE IN THE FOOD CONTACT MATERIAL

6.1. Actual content: Answer 'actual content determined' or 'actual content not determined'. The need for the determination of the actual or residual content of the substance in the test material depends on the type of substance and the data provided in the specific migration determination. For guidance, the following examples are given:

– monomer (case 1)
Full data on specific migration are provided. Determination of residual content is not required.
– monomer (case 2)
Specific migration is not determined, but calculation of migration based on residual content and assuming 100% migration is provided. Determination of residual content is required. Full details concerning the method and results shall be provided.
– monomer (case 3)
Worst case migration is based on the amount of monomer initially added to the polymerisation process, while assuming 100% migration.
Determination of residual content is not required. However, a properly described method for the determination of the residual content shall be provided for enforcement purposes.
– additive
Migration of additive is determined by specific and/or overall migration. The presence of the additive at the intended level in the actual test material used in migration experiments (see Section 5) should be demonstrated by means of analytical data. In general, it is sufficient to demonstrate by analytical experiments the presence of the additive at the intended level. In this situation, validation of the analytical method and extensive description of the analytical method is of less importance. Nevertheless, sufficient information should be provided to make the data provided transparent and acceptable.
– monomer or additive
Determination of the specific migration of monomer or additive is not possible because of, e.g. instability of the substance in food simulants, or because a QM limit is more appropriate. The determination of the actual content should be described in full detail according to standard format. In addition, the method should be validated, and, where relevant, visual information (e.g. chromatograms) should be added.

Ref:

6.2. Substance: Set out substance.

6.3. Test sample: Where relevant, the test sample shall be equivalent to the test sample used in the migration experiments. In other situations,

		the sample shall represent a worst case situation. If the test sample is intended to represent a range of materials of different brands or grades, then it should be assured that a material is selected that will represent the worst case situation. If the substance is used in different kinds of polymers then, in principle, each type of polymer should be examined for the residual content of the substance. However, if it is properly argued only determination of the residual content in a polymer representing the worst case can be acceptable. Criteria of selection will depend on the substance and the manufacturing process.
		Ref:
6.3.1.	**Chemical composition:**	Set out chemical composition of the test sample. Information should particularly be provided on the initial concentration of the substance, but information on the total composition is also required as the composition of the test specimen may influence the applicability of the analytical method and/or the residual content.
6.3.2.	**Physical composition:**	Set out physical composition of test sample, such as homogeneous material, multilayer material. In case of multi-layer material, it should be indicated in which layer the substance is present. If this is not the direct food contact side, then also relevant information on the top layers shall be given.
6.3.3.	**Density, melt flow index of polymer:**	Set out density and melt flow index (if relevant) of the polymer containing the substance. This information is required for mathematic modelling. In multilayer constructions, also the density of the barrier layers shall be given.
6.3.4.	**Dimensions of test sample:**	Set out dimensions of test sample. The test sample is the sample manufactured for the purpose of the determination of the residual or actual content of substance. Provide information on shape, e.g. bottle, film, sheet, etc., and thickness. For laminates, the total thickness and the thickness of each relevant layer should be indicated. For articles with in-homogeneous thickness, the thickness at various places should be given. The dimensions of an article should be set out (height, length, width, diameter).
6.3.5.	**Dimensions of test specimen:**	Set out dimensions or weight of test specimen. The test specimen is the actual part of material submitted to the residual content determination. Set out actual dimensions (height, length, width, diameter) or weight of the test specimen. If a subsample is taken from in-homogeneous materials (e.g. bottle), then set out which part was taken.
6.4.	**Treatment of sample:**	Set out treatment of the test sample, if not included in the test method.
6.5.	**Test method:**	If relevant, follow the description of item 5.1.8. The technical dossier shall contain the following information: actual data concerning the preparation of calibration solutions, typical chromatograms, calibration curves, correlation coefficients and all relevant data needed for a proper evaluation of the method as well as the data related to the residual content. Useful guidance can be found in the JRC 'Guidelines for

		performance criteria and validation procedures of analytical methods used in controls of food contact materials' and in the JRC 'Technical guidelines for compliance testing of plastic food contact materials in the framework of Regulation (EU) No 10/2011'. The method of determination may be used by enforcement laboratories in order to enforce restriction set for the substance. Therefore, the method should use generally available equipment. Use of very sophisticated methods should be justified. Where relevant, visual information such as typical chromatograms, calibration lines, etc. should be included. Please note that Article 20 (2)(c) of Regulation (EC) No 1935/2004 applies, i.e. information relating to the analytical methods shall not be considered confidential. *Ref:*
6.5.1.	**Detection/ determination limit:**	Give detection and/or determination limit of method, and set out the way the detection limit was established. Detection limits are particularly important when a substance is not detectable or at the level of the detection limit. Where relevant visual information such as typical chromatograms, calibration curve, blank values should be provided. *Ref:*
6.5.2.	**Precision of test method:**	Give repeatability (r) of method at residual content level. For example, repeatability of the method can be obtained from the standard deviation of the triplicate determination or from recovery experiments. *Ref:*
6.5.3.	**Recovery:**	Set out percentage recovery of substance as determined in recovery experiments. To obtain data on the suitability of the analytical method, recovery experiments (triplicate) shall be performed by standard addition of the substance to the polymer sample at a level of interest or at the level of the actual content. Also, the use of similar test material not containing the substance may be allowed. The spiked samples shall be treated in the same way as the test samples itself. Where relevant, visual information should be provided. If low recovery values are obtained, reasons for this should be provided. *Ref:*
6.5.4.	**Other information:**	Give any other relevant information. *Ref:*
6.6.	**Results:**	Give individual test results, including blank and recovery data. Preferably the data should be presented in a table, which should contain sufficient details to follow the way the final results are obtained. *Ref:*
6.7.	**Calculated migration (worst case):**	Set out calculation of migration of substance assuming total migration. In case, worst case calculation is acceptable an analytical method for analysis has to be provided. See also the 'Technical guidelines for compliance testing of plastic food contact materials in the framework of Regulation (EU) No 10/2011'. *Ref:*

6.8.	**Residual content versus specific migration:**	Give the relationship between residual content and specific migration, if determined. *Ref:*

7. MICROBIOLOGICAL PROPERTIES OF SUBSTANCE

This section focuses on the use of antimicrobial substances incorporated into food contact materials. Biocidal products are defined in Commission Regulation (EU) No 528/2012[11] concerning the making available on the market and use of biocidal products, as amended, as 'any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action, any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action'.
According to art. 3 (1)(g) of the same Regulation, a harmful organism is understood to be 'an organism, including pathogenic agents, which has an unwanted presence or a detrimental effect on humans, their activities or the products they use or produce, on animals or the environment'.

This section provides information to applicants regarding documentation to be supplied in order to permit the assessment of the public health implications, i.e. safety and efficacy including the microbiological effects of the use of an antimicrobial substance incorporated into food contact materials. Deviations are allowed provided that an appropriate justification is given.
It is not possible to give more specific guidance as to the methods to be used, as no validated methodology has been agreed at international level. Furthermore, different approaches may have to be followed for different substances depending on their intended use.
It should be noted that any effect of the biocidal active substance incorporated into the food contact material on the microbial flora of the food is strongly dependent on the contact time of the food contact material with the food (dose–time relation). This should be taken into account when assessing the effect of the **antimicrobial substance** on the microbial flora.
The evaluation of the microbiological data may lead to a restriction of use or of migration. If there is also another restriction based on toxicology, the lower should apply.
Substances with antimicrobial properties, which are intended to be incorporated into food contact materials will be evaluated on a case by case basis. Applicants shall provide all data required in items 1–7 of this Note for Guidance. Toxicological data shall be provided for new substances or substances not evaluated before by the EFSA CEF panel. Active ingredients evaluated before will NOT need new toxicological data, provided the carrier system is inert and/or already approved and does not actively contribute to the antimicrobial properties of the food contact material. A typical example is the use of silver based antimicrobial agents where different supports for the silver ions may be used.
It should be emphasised that the use of the **antimicrobial substance** should not replace the need for good hygiene practices.

7.1.	**Is the substance used as an antimicrobial agent?**	**Answer 'yes' or 'no'.** If 'no' go to Section 8, if 'yes' go to 7.2.
7.2.	**What is the intended microbiological function?**	Set out the technological function of the biocide If the **antimicrobial substance** is used: a) as a 'protection agent' during production process or storage of products to be used in the manufacture of the final article, go to 7.2.1

[11] Commission Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products, OJ L 167, 27.6.2012, p. 1–123, repealing Commission Directive 98/8/EC of 16 February 1998 concerning the placing of biocidal products on the market.

b) to reduce the microbiological contamination on the surface of the finished food contact material (FCM) and thereby improve hygiene in food preparation areas, go to 7.2.2

Ref:

7.2.1. Protection agent during production process or storage of products:

An **antimicrobial** substance may be added to protect from microbial spoilage the products to be used in the manufacture of the final article during production process or storage, e.g. an aqueous emulsion or process water containing these products.
In this case, it should be argued from Minimum Inhibitory Concentration (MIC) values, migration data and/or concentrations in the final product that there could be no antimicrobial activity on the surface of the finished article. Alternatively it could be demonstrated using an appropriate method e.g. JIS Z 2801[12] (adapted to use a wider range of microorganisms). If this is demonstrated, go to Section 8.

7.2.2. Means of reducing microbial contamination on the surface of a FCM

An antimicrobial substance may be added to a FCM to reduce the numbers of microorganisms on its surface and in turn to reduce the possibility of cross contamination.
In this case, all information requested below should be provided.

7.2.2.1. Intended applications of use

Describe as far as possible the intended applications.
Information should be provided on whether it is intended to be used for industrial food processing applications, consumer use (including catering) or both.
Information should also be provided, whether each application is intended for 'repeated use' or 'single use'.

7.2.2.2. Other information

Give any information on the intended use other than those mentioned under 7.2.2.1 and in Section 3 if it may be useful for the risk assessment of the biocide.

7.3. Spectrum of microbiological activity:

Provide data on the spectrum of activity against various food-associated microorganisms, including pathogens. Any insensitive genera or species known or identified should be included.

Ref:

7.4. Level of activity:

Provide information on MICs of the pure biocidal substance or preferably its active component, e.g. silver ions, for the microorganisms likely to be exposed to the substance. The concentration of the microorganisms and the nature of the test medium in which they are exposed to the antimicrobial substance should be described
Include any dose–time–response information if available, e.g. varying doses of antimicrobial substance for a constant time or a single concentration of antimicrobial substance for varying times. Describe the nature of the test medium in which the microorganisms are exposed to the biocide.
Document the possibility of resistance arising to the antimicrobial substance in the sensitive population or cross-resistance to other antimicrobials developing.

Ref:

[12] Japanese Industrial Standard/Antimicrobial products – Test for antimicrobial activity and efficacy (Japanese Standards Association – 4-1-24, Akasaka, Minato-ku, Tokyo, 107-8440 JAPAN)

7.5.	**Possible consequences of the use of the antimicrobial substance:**	Describe any possible encouragement to favour selective overgrowth of the flora on the surface of the food contact material containing the biocidal substance(s) by organisms that are insensitive to the biocidal substance(s). *Ref:*
7.6.	**Efficacy:**	Efficacy strongly depends on migration of the antimicrobial substance to the surface of the material, and therefore on the type of polymer and on its antimicrobial substance content. On the other hand, migration should not be so high that it causes preservative effect on food (see item 7.8). Consequently, efficacy testing should be performed with polymers mentioned in 3.1, especially using that giving the highest and that giving the lowest migration (e.g. LDPE and PET respectively). The concentration of the antimicrobial substance in these test materials should not exceed that indicated in 3.4 and 5.1.2.1. Provide data to demonstrate the efficacy under the intended conditions of use describing the testing methodology that demonstrates this efficacy. When the biocide is to be used at low temperatures, e.g. in chill rooms, refrigerators, efficacy should be demonstrated at these temperatures. However, when this is technically impossible, e.g. in large scale industrial applications, provide data obtained from experiments that simulate the intended conditions of use. An alternative approach may rely for instance on comparison of predicted migration values with MICs, taking into account intrinsic and extrinsic conditions. The model should be properly validated. *Ref:*
7.7.	**Efficacy upon repeated use:**	Information should be provided to describe the behaviour of the biocidal surface after, for example, repeated cleaning procedures. Preferably, demonstration of efficacy under in-use conditions could be done using microbiological tests or by establishing the concentration of the active substance. *Ref:*
7.8.	**Demonstration of the lack of antimicrobial activity against microbes in/on the food:**	Describe the evidence for absence of any effect on the microbiological flora in/on the food including comparison with data obtained from use of the same/comparable FCM not containing the biocidal substance(s).This should cover the worst case, which could include: • the most sensitive microorganism(s), • the highest release level of the biocidal substance(s) or FCM with the highest concentration applied for, • foodstuffs spiked with the biocidal substance(s) at concentrations exceeding the observed or calculated migration levels. This consideration includes: • comparison of the observed or calculated migration levels with MIC values, • information on interaction of the biocidal substance(s) with food constituents which may lead to the inactivation of the biocide. *Ref:*

7.9.	**Other information:**	Set out any other information that may be relevant for evaluation. *Ref:*
7.10.	**Information on claim or disclaimer in accordance with the requirement of the relevant Regulation:**	The claim should be consistent with the data described above on efficacy and activity.
7.11.	**Information on authorisation as biocidal product in the frame of Commission Regulation (EU) No 528/2012:**	Supply information if the substance is listed in Annex I of Commission Regulation (EC) No 528/2012 or if it is a constituent of biocidal products authorised under Article 55(2) of Commission Regulation (EC) No 528/2012 or if it is a constituent of biocidal products allowed under the transitional measures or subject to the 10-year work programme provided for in Article 89 of Commission Regulation (EC) No 528/2012.

8. TOXICOLOGICAL DATA

A complete report of the toxicity studies performed should be provided. The studies should be performed following prevailing EU or OECD guidelines or other internationally agreed methods and should be in compliance with Good Laboratory Practice, as better specified below.

The substances tested should be the commercial substances for which the authorisation is requested. Especially, the percentage of purity and the identity of impurities should be the same as those of the substances to be used in practice. In any case, the substances used in any toxicological experiment should be described properly and their samples tested must be traceable. In the absence of specifications on the identity (see Section 1) of the substances tested, a justification should be provided.

The general principle for developing the toxicological data as reported in the 2001 'SCF Guidelines on Food Contact Materials' should apply, i.e. the greater the exposure through migration, the more toxicological information will be required:

(a) In case of high migration (i.e. 5–60 mg/kg per food), a full data set is needed to establish the safety.
(b) In case of migration between 0.05 and 5 mg/kg food, a reduced data set may suffice.
(c) In case of low migration (i.e. < 0.05 mg/kg food), only a limited data set is needed.

In determining the appropriate extent of the data set required, the migration values should not be regarded as absolute limits but as indicative values.

a) Full data set comprises:

- at least two *in vitro* genotoxicity tests, in line with the testing strategies of the EFSA Scientific Committee recommendations on genotoxicity testing strategies[6]:
  - i) A bacterial reverse mutation test
  - ii) An *in vitro* mammalian cell micronucleus test
- A 90-day oral toxicity study
- Studies on absorption, distribution, metabolism and excretion
- Studies on reproduction and developmental toxicity
- Studies on long-term toxicity/carcinogenicity

Under certain circumstances the extensive set of tests as described above may not be required and only the tests indicated below may have to be provided.
Reduced data sets (b) and (c) comprise:

b) In cases where migration is in the range from 0.05 to 5 mg/kg of food / food simulant, the following data are needed:

- at least two genotoxicity tests as indicated above

- A 90-day oral toxicity study
- Data to demonstrate the absence of potential for accumulation in man

c) In cases where migration is below 0.05 mg/kg of food/food simulant the following data are needed:

- at least two genotoxicity tests as indicated above

8.1. Genotoxicity

In line with the EFSA Scientific Committee recommendations on genotoxicity testing strategies,[6] the two *in vitro* genotoxicity assays described in items 8.1.1 and 8.1.2 should be performed. The combination of these two tests fulfils the basic requirement to cover the three genetic endpoints with the minimum number of tests: the bacterial reverse mutation assay covers gene mutations and the *in vitro* micronucleus test covers both structural and numerical chromosome aberrations.
In case of positive results obtained from the *in vitro* genotoxicity tests, further *in vivo* genotoxicity tests may be required.
Consistent with the recommendations of the EFSA Scientific Committee on genotoxicity testing strategies, the *in vivo* tests described in items 8.1.3, 8.1.4 and 8.1.5 would be suitable for following up substances that test positive in the *in vitro* basic battery. These EFSA Scientific Committee recommendations should be consulted for further details of the testing strategy.

8.1.1. Bacterial reverse mutation assay: According to the OECD Guideline 471.[13]
Ref:

8.1.2. *In vitro* mammalian cell micronucleus test: According to the OECD Guideline 487.[14]
Ref:

8.1.3. *In vivo* micronucleus test: According to the OECD Guideline 474.[15]
The *in vivo* micronucleus test covers the endpoints of structural and numerical chromosomal aberrations and is an appropriate follow-up for *in vitro* clastogens and aneugens.
Ref:

8.1.4. *In vivo* Comet assay: According to the OECD Guideline 489.[16]
The *in vivo* Comet assay evaluating DNA primary lesions is an indicator test sensitive to substances that cause gene mutations and/or structural chromosomal aberrations *in vitro*. The assay can be applied to any tissues from which single cell suspensions can be prepared. It is also suitable for the detection of DNA damage at the first site of contact.
Ref:

[13] OECD (1997), Test No. 471: Bacterial Reverse Mutation Test, OECD Publishing, Paris. https://doi.org/10.1787/9789264071247-en
[14] OECD (2010), Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Publishing, Paris. https://doi.org/10.1787/9789264091016-en
[15] OECD (2014), Test No. 474: Mammalian Erythrocyte Micronucleus Test, OECD Publishing, Paris. https://doi.org/10.1787/9789264224292-en
[16] OECD (2014), Test No. 489: In Vivo Mammalian Alkaline Comet Assay, OECD Publishing, Paris. https://doi.org/10.1787/9789264224179-en

8.1.5.	**Transgenic rodent gene mutation assays:**	According to the OECD Guideline 488.[17] Transgenic rodent assays can detect point mutations and small deletions and are without tissue restrictions. The combination of tests assessing different endpoints in different tissues in the same animal, or the incorporation of such testing within other repeated-dose toxicity studies that will be conducted anyway, should be considered. *Ref:*
8.1.6.	**Other information:**	Include any other information that may be relevant for evaluation of the genotoxicity of the substance (e.g. chemical reactivity of the substance, structural alerts and 'read-across' from structurally related substances, data on bioavailability, metabolism, toxicokinetics, target organ specificity, any relevant published data on the genotoxicity of the substance).
8.2.	**General toxicity**	
8.2.1.	**Repeated dose 90-day oral toxicity study:**	According to the OECD guideline 408.[18] *Ref:*
8.2.2.	**Combined Chronic Toxicity/ Carcinogenicity:**	According to the OECD guideline 453.[19] *Ref:*
8.2.3.	**Reproduction/ teratogenicity:**	According to the EC Methods B.34–B.35 and the OECD guidelines 421 and 422.[20,21] *Ref:*
8.2.4.	**Other information:**	Set out any other information that may be relevant for evaluation, e.g. acute or subacute (28 days) toxicity,[22] dermal and inhalation effects should be provided when available. *Ref:*
8.3.	**Metabolism**	
8.3.1.	**Absorption, distribution, biotransformation and excretion:**	Give any relevant information when available *Ref:*

[17] OECD (2011), Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays, OECD Publishing, Paris. https://doi.org/10.1787/9789264122819-en

[18] OECD (1998), Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents, OECD Publishing, Paris. https://doi.org/10.1787/9789264070707-en

[19] OECD (2009), Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, OECD Publishing, Paris. https://doi.org/10.1787/9789264071223-en

[20] OECD (1996), Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, OECD Publishing, Paris. https://doi.org/10.1787/9789264070981-en

[21] The following OECD guidelines have also been developed to assess additional reproduction toxicity endpoints:
- OECD (2001), Test No. 414: Prenatal Development Toxicity Study, OECD Publishing, Paris. https://doi.org/10.1787/9789264070820-en
- OECD (2001), Test No. 416: Two-Generation Reproduction Toxicity, OECD Publishing, Paris. https://doi.org/10.1787/9789264070868-en
- OECD (2011), Test No. 443: Extended One-Generation Reproductive Toxicity Study, OECD Publishing, Paris. https://doi.org/10.1787/9789264122550-en

[22] OECD (2008), Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Publishing, Paris. https://doi.org/10.1787/9789264070684-en

8.3.2.	**Accumulation in man:**	To assess the potential for this consider the approaches listed in Annex 3. As detailed guidelines for methodology are absent the relevant sections of existing EU guidelines on veterinary drugs, additives in animal nutrition and human drugs may be consulted. Also IPCS (EHC 70[23] & EHC 57[24]) as well as the FDA Red Book II[25] may provide guidance. *Ref:*
8.3.3.	**Other information:**	Set out any other information that may be relevant for evaluation. *Ref:*
8.4.	**Miscellaneous**	
8.4.1.	**Effects on immune system:**	Give relevant information, if any. *Ref:*
8.4.2.	**Neurotoxicity:**	Phosphoric and phosphorous acid esters should be tested for neurotoxicity, if migration exceeds 0.05 mg/kg food/food simulants. According to OECD guideline 424.[26,27] *Ref:*
8.4.3.	**Other information:**	Set out any other information that may be relevant for evaluation. *Ref:*

[23] IPCS, Environmental Health Criteria 70, Principles for the safety assessment of food additives and contaminants in food, 1987.
[24] IPCS, Environmental Health Criteria 57, Principles of toxicokinetic studies, 1986.
[25] FDA, Redbook II, Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients, 2007.
[26] OECD (1997), Test No. 424: Neurotoxicity Study in Rodents, OECD Publishing, Paris. https://doi.org/10.1787/9789264071025-en
[27] The following OECD guidelines have also been developed to assess additional neurotoxicity endpoints:
- OECD (1995), Test No. 418: Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure, OECD Publishing, Paris. DOI: https://doi.org/10.1787/9789264070905-en
- OECD (1995), Test No. 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study, OECD Publishing, Paris. https://doi.org/10.1787/9789264070929-en
- OECD (2007), Test No. 426: Developmental Neurotoxicity Study, OECD Publishing, Paris. https://doi.org/10.1787/9789264067394-en

Annex 1

MEASUREMENT OF HYDROLYSIS OF PLASTICS MONOMERS AND ADDITIVES IN DIGESTIVE FLUID SIMULANTS

Contents

INTRODUCTION

For the protection of human health, plastic food contact materials shall be in compliance with Commission Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food, as amended, with regard to composition and migration of constituents to foodstuffs coming into contact with these materials.

Constituents that may migrate to foodstuffs comprise residual monomers and other starting substances, residual process chemicals and additives as well as breakdown products and impurities of these substances.

Certain constituents may hydrolyse when ingested. The method described in this Guideline allows determination of the extent of hydrolysis, especially of esters, in order to assess whether the constituents break down into innocuous substances.

1. SCOPE

The method can be used to measure the extent of hydrolysis of monomers and additives *in vitro*, using standard digestive fluid simulants for saliva, gastric juice and intestinal fluid.

The method does not describe the analytical procedures required for the determination of the parent constituent and its hydrolysis products in the simulants.

2. PRINCIPLE

The test substance (monomer or additive) is dissolved in an appropriate solvent. An aliquot of the solution is transferred to the digestive fluid simulant, which is maintained at 37°C with continual agitation. After a specified time period, the concentrations of both parent constituent and hydrolysis products are determined in the simulant, whereupon percentage hydrolysis is calculated.

3. REAGENTS

NOTE: All reagents should be of recognised analytical quality unless otherwise specified.

3.1. Chemicals

3.1.1. Water, distilled or deionised
3.1.2. Sodium bicarbonate ($NaHCO_3$)
3.1.3. Sodium chloride (NaCl)
3.1.4. Sodium taurocholate?
3.1.5. Potassium carbonate (K_2CO_3)

3.1.6. Sodium hydroxide standard solution, 0.2 M
3.1.7. Hydrochloric acid standard solutions, 2 M and 0.1 M
3.1.8. Potassium dihydrogen orthophosphate (KH_2PO_4)
3.1.9. Porcine pancreatin extract, activity equivalent to 8x SUP specification
3.1.10. Dispersing solvents, one of:

- acetonitrile
- *N*,*N*-dimethylacetamide
- 1,4-dioxane
- ethanol
- methanol
- propan-2-ol
- tetrahydrofuran
- water

3.2. Digestive fluid simulants

3.2.1. Saliva simulant

Dissolve 4.2 g of sodium bicarbonate ($NaHCO_3$), 0.5 g of sodium chloride (NaCl) and 0.2 g of potassium carbonate (K_2C0_3) in 1 L of water. The pH of the solution should be approximately 9.

3.2.2. Gastric-juice simulant

Dilute 0.1 M hydrochloric acid standard solution to a concentration of 0.07 M. The pH of the solution should be 1.2 $\pm$ 0.1.

3.2.3. Intestinal-fluid simulant

NOTE: Care should be taken to ensure that the simulant is prepared in the order given.

Dissolve 6.8 g of potassium dihydrogen orthophosphate (KH_2PO_4) in 250 mL of water, transfer to a 1 L volumetric flask and add 190 mL of 0.2 M sodium hydroxide (NaOH). Add 400 mL of water and shake briefly to mix. Weigh 10.0 g of pancreatin extract into a 250 mL beaker. Add a little water, and stir to make a stiff, homogenous paste. Gradually dilute the paste with small portions of water, stirring well after each dilution, to give approximately 150 mL of a lump-free solution. Transfer the solution to the volumetric flask, rinsing the beaker and funnel with water. Add 0.5 g of sodium taurocholate, gently shake the flask and make the volume up to the neck of the flask. Adjust the pH of the solution to 7.5 $\pm$ 0.1 with 0.2 M sodium hydroxide (NaOH). Make the volume up to the mark with water and shake thoroughly to mix.

4. APPARATUS

NOTE: An instrument or item of apparatus is listed only where it is special, or made to a particular specification, usual laboratory equipment being assumed to be available.

4.1. Glass vials, 100 mL or 125 mL, with crimp-on type PTFE/silicone rubber septa.
4.2. Crimping and decapping pliers.
4.3. Device for mechanical agitation of the simulant, e.g. a flask shaker, or a magnetic stirrer bar for use with a stirrer plate, situated in a cabinet or water bath controlled to a temperature of 37 $\pm$ 1°C.

5. SAMPLES

NOTE: The test substance should be of similar purity as the substance used in food contact materials.

5.1. Preparation of stock solutions

Weigh out the required weight of the test substance to the nearest 0.1 mg into a 10 mL volumetric flask and dissolve in a suitable dispersing solvent such as one listed in section 3.1.10. Make the volume up to the mark, and shake the flask thoroughly to mix.

NOTE: The solvent selected must completely dissolve the test substance and must not chemically react with it.

The final concentration of solvent (other than water) in the digestive fluid simulant should not exceed 0.1% (v/v).

The concentration of the test substance in the digestive fluid simulant should be selected such as to enable determination of the substance down to 5% of the amount added to the simulant. Anyhow, that concentration should not be lower than the maximum likely human intake predicted from migration studies.

6. PROCEDURE

6.1. Hydrolysis equation

Set out the hydrolysis equation, using the following model expression:
PC $\Longrightarrow$ HP-1 + HP-2 (+ HP-3 +..... HP-N), in which:
PC = parent constituent
HP = hydrolysis product

6.2. Selection of simulants

Select simulants to be used in the test such that the analytical effort is kept to the minimum, e.g. a test with intestinal fluid simulant is often sufficient to demonstrate hydrolysis of esters. So, if the test substance is an ester, a test with intestinal fluid simulant should be carried out first. If complete hydrolysis is demonstrated, it is not necessary to perform tests with other simulants.

6.3. Performance of hydrolysis test

Transfer for each test 100 mL of the digestive fluid simulant to a glass vial using a measuring cylinder. Crimp-seal the vial with a PTFE-silicone rubber septum. Commence shaking the vial or stirring its contents and equilibrate the simulant at 37 $\pm$ 1°C.

NOTE: As for analytico-technical reasons each substance in the hydrolysis equation selected for determination has to be assessed in a separate hydrolysis test and each of the determinations has to be carried out in triplicate, the number of glass vials needed for the test amounts to thrice the number of combinations of substances (be it parent constituent or hydrolysis product) to be determined, specified time period and simulant.

Subsequently add a suitable aliquot of the stock solution (25–100 µL) to the simulant, using a 100 µL syringe. Inject the solution through the septum, below the surface of the simulant, and continue agitation or stirring for the duration of the test. Take the duration of the test from the following table:

- saliva simulant 0.5 h
- gastric-juice simulant 1, 2 and 4 h
- intestinal-fluid simulant 1, 2 and 4 h

NOTE: If gastric-juice simulant or intestinal-fluid simulant is used for the test, a test for 1 h should be performed first. If complete hydrolysis is demonstrated, it is not necessary to perform tests for 2 and 4 h.

6.4. Analysis of hydrolysates

After termination of the hydrolysis test, determine the hydrolysis products in the hydrolysate. Use an appropriate analytical method and calculate percentage hydrolysis from the results.

NOTE It is insufficient to only measure disappearance of the parent constituent. A case-by-case selection should be made about which hydrolysis products need be measured in order to permit a judgement about mass balance.

Suitability of the analytical methods should be demonstrated by performing tests with standard addition of the hydrolysis product(s) of interest set out in the CEN standard format.

7. TEST REPORT

The test report should conform to the CEN standard format.

Annex 2

POLYMERIC ADDITIVES

Components with a molecular mass above 1,000 Dalton (Da) are very unlikely to be absorbed by the gastro-intestinal tract and thus are not considered to present a toxicological risk. The value of 1,000 Da was chosen because it takes into account the effect of the shape of the molecule, which has an important influence on the likelihood of absorption of substances in the molecular mass range 600–1,000 Da. Below 600 Da, most substances are absorbed and the rate of absorption is determined by factors other than size and shape of the molecule.

Since only the fraction of the polymeric additive with molecular mass below 1,000 Da is regarded as toxicologically relevant a distinction has been made between polymeric additives with a weight averaged molecular mass (Mw) below 1,000 Da and those with Mw above 1,000 Da. For polymeric additives with Mw above 1,000 Da, the fraction with molecular mass below 1,000 Da will vary and a case-by-case consideration of the specification will determine whether further data are required.

The following data should be supplied:

I) Data according to the 'Explanatory Guidance to the SCF Guidelines for Food Contact Materials' on:

- paragraph 1.4 'Identity'
- paragraph 2 'Properties'
- paragraph 3 'Use'
- paragraph 4 'Authorisation'

II) Genotoxicity data on the monomer(s) according to the 'Explanatory Guidance to the SCF Guidelines for Food Contact Materials'.

IIIa) For those additives with Mw less than 1,000 Da: migration and toxicity data on the polymeric additive itself, according to 'SCF Guidelines' with the exception that mutagenicity studies on the polymeric additive itself are not required.

IIIb) For those additives with Mw above 1,000 Da: data, including migration and toxicity, may be required on the polymeric additive itself once the CEF Panel has examined the specification; especially for those additives containing a significant fraction with molecular mass below 1,000 Da.

In deciding whether further data are needed, the CEF Panel will take into account both the size of the fraction with molecular weights below 1,000 Da and the proportion of the additive in the plastic.

N.B. As regards the migration, the level of the migrated fraction with molecular mass less than 1,000 Da should preferably be supplied. However, if the applicant(s) is (are) unable to determine this or decides (decide) not to determine the migrated fraction with molecular mass less than 1,000 Da, the total migration of the polymeric additive will be attributed to the fraction with molecular mass less than 1,000 Da.

These guidelines apply to polymeric additives in general. The CEF Panel will however consider any scientific arguments put forward by applicants for deviation from the guidelines. For example, in cases of additives made using hydrogenation, or additives in which residual monomers have been removed from the final product, not all the data mentioned in the guidelines may be required.

If relevant toxicological data are available, they may be submitted because they may support evaluation.

Annex 3

ACCUMULATION IN MAN

This Annex focuses on accumulation in man and not on bioaccumulation in general. Many experts are familiar with the term 'bioaccumulation' as it relates to the fate of a chemical in the environment. It covers e.g. the behaviour in aquatic organisms and potential for accumulation through the food web.

In the case of food contact materials, the interest centres on the potential for direct accumulation in mammalian tissues and not on biomagnification through the food chain. However, normally a log $k_{o/w}$ value below 3 would be considered sufficient evidence for the lack of accumulative potential in the mammalian body, unless special considerations, e.g. chemical structure, give cause for concern. On the other hand, a log $k_{o/w}$ of 3 and higher will not by itself be proof of accumulation as a substance may not be absorbed or be metabolised to substances with no accumulation potential. In these circumstances, other evidence for the absence of accumulative potential is needed.

It is not possible to give definitive guidance as to the methods to be used, as different approaches must be followed for different substances according to their chemical structures and physical properties. If it can be shown by appropriate kinetic studies (absorption distribution, metabolism, excretion (ADME)) after oral exposure that the biological half-life excludes accumulation, this would be considered sufficient evidence. Furthermore, the use of appropriately radioactively labelled substances and autoradiography can demonstrate the existence/absence of an accumulative potential of a substance.

Guidelines describing in detail the procedures for such studies do not appear to exist, but some relevant information may be found in existing EU guidelines on veterinary drugs, additives in animal nutrition, and human drugs. Also, IPCS (EHC70 and EHC57) as well as the FDA Red Book II could be useful sources on possible methodology.

In principle, accumulation is undesirable but not automatically associated with any toxic effects. In cases where accumulation potential has been demonstrated or its lack not demonstrated, it remains the responsibility of the applicant to provide evidence that any accumulation found will not be associated with toxic effects even after long-term exposure.

Applicability and transitional period

This Note for Guidance for Food Contact Materials shall apply and may be referred to by applicants as of its publication in the EFSA Journal.

The requirements on genotoxicity testing, as described in item 8.1 of this document, which are in line with the 'EFSA Scientific Committee opinion on genotoxicity testing strategies', shall apply to new performed genotoxicity studies as of one year from the publication of this document on the EFSA Journal. During this transitional period, applicants may submit their genotoxicity testing based on the testing strategy as described in the 2001 'SCF guidelines for Food Contact Materials' or as described in the EFSA Scientific Committee opinion on genotoxicity testing strategies.