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9 **Guidance document on pesticide residue**
10 **analytical methods**

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17 [Revision 8 is the version of this guidance document that is currently valid. It is, however, under
18 continuous review and will be updated when necessary. The document is aimed at
19 manufacturers seeking pesticides authorisations and parties applying for setting or modification
20 of an MRL. It gives requirements for methods that would be used in post-registration
21 monitoring and control by the competent authorities in Member States in the event that
22 authorisations are granted. For authorities involved in post-registration control and monitoring,
23 the document may be considered as being complementary to the documents: Method Validation
24 and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed (for the valid
25 revision visit http://ec.europa.eu/food/plant/protection/resources/publications_en.htm) and the
26 OECD document “Guidance Document on pesticide residue analytical methods”, 2007.
27 (ENV/JM/ ENV/JM/MONO(2007)17).

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96 **1 Preamble**

97 This document provides guidance to applicants, Member States and EFSA on the data
98 requirements and assessment for residue analytical methods for post-registration control and
99 monitoring purposes. It is not intended for biological agents such as bacteria or viruses. It
100 recommends possible interpretations of the provisions of section 3.5.2 of Annex II of
101 Regulation (EC) No 1107/2009 [1] and of the provisions of section 4, part A of Annex II and
102 section 5, part A of Annex III of Council Directive 91/414/EEC [2]. It also applies to
103 applications for setting or modification of an MRL within the scope of Regulation (EC) No
104 396/2005 [3]. It has been elaborated in consideration of the ‘Guidance Document on pesticide
105 residue analytical methods’ of the OECD [4] and SANCO/10684/2009 “Method validation
106 and quality control procedures for pesticide residue analysis in food and feed” [5].

107 This document has been conceived as an opinion of the Commission Services and elaborated
108 in co-operation with the Member States. It does not, however, intend to produce legally
109 binding effects and by its nature does not prejudice any measure taken by a Member State nor
110 any case law developed with regard to this provision. This document also does not preclude
111 the possibility that the European Court of Justice may give one or another provision direct
112 effect in Member States.

113 This guidance document must be amended at the latest if new data requirements as referred to
114 in Article 8 (1)(b) and 8 (1)(c) of Regulation (EC) No 1107/2009 will have been established
115 in accordance with the regulatory procedure with scrutiny referred to in Article 79 (4).

116 **2 General**

117 **2.1 Good Laboratory Practice**

118 According to Guidance Document 7109/VI/94-Rev. 6.c1 (Applicability of Good Laboratory
119 Practice to Data Requirements according to Annexes II, Part A, and III, Part A, of Council
120 Directive 91/414/EEC) [6] the development and validation of an analytical method for
121 monitoring purposes and post-registration control is not subject to GLP. However, where the
122 method is used to generate data for registration purposes, for example residue data, these
123 studies must be conducted to GLP.

124 **2.2 Selection of analytes for which methods are required**

125 The definition of the residues relevant for monitoring in feed and food as well as in
126 environmental matrices and air is not the subject matter of this document. Criteria for the
127 selection of analytes in case that no legally binding definition is available are given in the
128 respective sections 3 - 8. In addition, sections 5.2, 6.2, 7.2 and 8.2 clarify under which
129 circumstances analytical methods for residues may not be necessary.

130 **2.3 Description of an analytical method and its validation results**

131 Full descriptions of validated methods shall be provided. The submitted studies must include
132 the following points:

- 133 • Itemisation of the fortified compounds and the analytes, which are quantified
- 134 • Description of the analytical method
- 135 • Validation data as described in more detail below
- 136 • Description of calibration including calibration data
- 137 • Recovery and Repeatability
- 138 • Data proving the selectivity of the method
- 139 • Confirmatory data, if not presented in a separate study
- 140 • References (if needed)

141

142 The following information should be offered in the description of the analytical method:

- 143 • An introduction, including the scope of the method
- 144 • Outline/summary of method, including validated matrices, limit of quantification (LOQ),
145 range of recoveries, fortification levels and number of fortifications per level

- 146 • Apparatus and reagents
- 147 • instrument parameters used as example if appropriate
- 148 • Description of the analytical method, including extraction, clean-up, derivatisation (if
- 149 appropriate), chromatographic conditions (if appropriate) and quantification technique
- 150 • Hazards or precautions required
- 151 • Time required for one sample set
- 152 • Schematic diagram of the analytical method
- 153 • Stages where an interruption of the method is possible
- 154 • Result tables (if results are not presented in separate studies)
- 155 • Procedure for the calculation of results from raw data
- 156 • Extraction efficiency of solvents used
- 157 • Important points and special remarks (e.g. volatility of analyte or its stability with regard
- 158 to pH)
- 159 • Information on stability of fortified/incurred samples, extracts and standard solutions (If
- 160 the recoveries in the fortified samples are within the acceptable range of 70-120 %, stability is sufficiently proven.)
- 161
- 162 Sometimes it may be necessary for other information to be presented, particularly where
- 163 special methods are considered.

164 **2.4 Hazardous reagents**

165 Hazardous reagents (carcinogens category I and II [7]) shall not be used. Among these
166 compounds are diazomethane, chromium (VI) salts, chloroform and benzene.

167 **2.5 Acceptable analytical techniques considered commonly available**

168 Analytical methods shall use instrumentation regarded as "commonly available":

- 169 • GC detectors: FPD, NPD, ECD, FID, MS, MSⁿ (incl. Ion Traps and MS/MS), HRMS
- 170 • GC columns: capillary columns
- 171 • HPLC detectors: MS, MS/MS, HRMS, FLD, UV, DAD
- 172 • HPLC columns: reversed phase, ion-exchange, normal phase
- 173 • AAS, ICP-MS, ICP-OES

174 Other techniques can be powerful tools in residue analysis, therefore the acceptance of
175 additional techniques as part of enforcement methods should be discussed at appropriate
176 intervals. Whilst it is recognised that analytical methodology is constantly developing, some
177 time elapses before new techniques become generally accepted and available.

178 **2.6 Multi-residue methods**

179 Multi-residue methods that cover a large number of analytes and that are based on GC-MS
180 and/or HPLC-MS/MS are routinely used in enforcement laboratories for the analysis of plant
181 matrices. Therefore, validated residue methods submitted for food of plants, plant products
182 and foodstuff of plant origin (Section 3) should be multi-residue methods published by an
183 international official standardisation body such as the European Committee for
184 Standardisation (CEN) (e.g. [8 - 12]) or the AOAC International (e.g. [13]). Single residue
185 methods should only be provided if data show and are reported that multi-residue methods
186 involving GC as well as HPLC techniques cannot be used.

187 If validation data for the residue analytical method of an analyte in at least one of the
188 commodities of the respective matrix group have been provided by an international official
189 standardisation body and if these data have been generated in more than one laboratory with
190 the required LOQ and acceptable recovery and RSD data (see Section 2.9.2), no additional
191 validation by an independent laboratory is required.

192 **2.7 Single methods and common moiety methods**

193 Where a pesticide residue cannot be determined using a multi-residue method, one or where
194 appropriate more alternative method(s) must be proposed. The method(s) should be suitable
195 for the determination of all compounds included in the residue definition. If this is not
196 possible and an excessive number of methods for individual compounds would be needed, a
197 common moiety method may be acceptable, provided that it is in compliance with the residue
198 definition. However, common moiety methods shall be avoided whenever possible.

199 **2.8 Single methods using derivatisation**

200 For the analysis of some compounds by GC, such as those of high polarity or with poor
201 chromatographic properties, or for the detection of some compounds in HPLC, derivatisation
202 may be required. These derivatives may be prepared prior to chromatographic analysis or as
203 part of the chromatographic procedure, either pre- or post-column. Where a derivatisation
204 method is used, this must be justified.

205 If the derivatisation is not part of the chromatographic procedure, the derivative must be
206 sufficiently stable and should be formed with high reproducibility and without influence of
207 matrix components on yield. The efficiency and precision of the derivatisation step should be
208 demonstrated with analyte in sample matrix against pure derivative. The storage stability of

209 the derivative should be checked and reported. For details concerning calibration refer to
210 Section 2.9.1.

211 The analytical method is considered to remain specific to the analyte of interest if the
212 derivatised species is specific to that analyte. However, where – in case of pre-column
213 derivatisation – the derivative formed is a common derivative of two or more active
214 substances or their metabolites or is classed as another active substance, the method should be
215 considered non-specific and may be deemed unacceptable.

216 **2.9 Method validation**

217 Validation data must be submitted for all analytes included in the residue definition for all
218 representative sample matrices to be analysed at adequate concentration levels.

219 Basic validation data are:

- 220 • Calibration data
- 221 • Concentration of analyte(s) found in blank samples
- 222 • Concentration level(s) of fortification experiments
- 223 • Concentration and recovery of analyte(s) found in fortified samples
- 224 • Number of fortification experiments for each matrix/level combination
- 225 • Mean recovery for each matrix/level combination
- 226 • Relative standard deviation (RSD) of recovery, separate for each matrix/level combination
- 227 • Limit of quantification (LOQ), corresponding to the lowest validated level
- 228 • Representative clearly labelled chromatograms
- 229 • Data on matrix effects, e.g. on the response of the analyte in matrix as compared to pure
230 standards

231 .Further data may be required in certain cases, depending on the analytical method used, and
232 the residue definition to be covered.

233 **2.9.1 Calibration**

234 The calibration of the detection system shall be adequately demonstrated at a minimum of 3
235 concentration levels in duplicate or (preferably) 5 concentration levels with single
236 determination. Calibration should be generated using standards prepared in blank matrix
237 extracts (matrix matched standards) for all sample materials included in the corresponding
238 validation study (Sections 3 - 8). Only, if experiments clearly demonstrate that matrix effects
239 are not significant (i.e. < 20 %), calibration with standards in solvent may be used. Calibration
240 with standards in solvent is also acceptable for methods to detect residues in air (Section 7).

241 In case that aqueous samples are analysed by direct injection HPLC-MS/MS calibration shall
242 be performed with standards in aqueous solution.

243 The analytical calibration must extend to at least the range which is suitable for the
244 determination of recoveries and for assessment of the level of interferences in control
245 samples. For that purpose a concentration range shall be covered from 30 % of the LOQ to
246 20 % above the highest level (Section 2.9.2).

247 All individual calibration data shall be presented together with the equation of the calibration.
248 Concentration data should refer to both, the mass fraction in the original sample (e.g. mg/kg)
249 and to the concentration in the extract (e.g. µg/L). A calibration plot should be submitted, in
250 which the calibration points are clearly visible. A plot showing the response factor¹ versus the
251 concentration for all calibration points is preferred over a plot of the signal versus the
252 concentration.

253 Linear calibrations are preferred if shown to be acceptable over an appropriate concentration
254 range. Other continuous, monotonically increasing functions (e.g. exponential/power,
255 logarithmic) may be applied where this can be fully justified based on the detection system
256 used.

257 When quantification is based on the determination of a derivative, the calibration shall be
258 conducted using standard solutions of the pure derivative generated by weighing, unless the
259 derivatisation step is an integral part of the detection system. If the derivative is not available
260 as a reference standard, it should be generated within the analytical set by using the same
261 derivatisation procedure as that applied for the samples. Under these circumstances, a full
262 justification should be given.

263 **2.9.2 Recovery and Repeatability**

264 Recovery and precision data must be reported for the following fortification levels, except for
265 body fluids and body tissues (Section 8):

- 266 • LOQ 5 samples
- 267 • 10 times LOQ, or MRL (set or proposed) or other relevant level ($\geq 5 \times \text{LOQ}$)
268 5 samples

269 Additionally, for unfortified samples residue levels must be reported:

- 270 • blank matrix 2 samples

271 According to the residue definition the LOQ of chiral analytes usually applies to the sum of
272 the two enantiomers. In this case it is not necessary to determine the enantiomers separately.

¹ The response factor is calculated by dividing the signal area by the respective analyte concentration.

273 Enantioselective methods would only be required if a single enantiomer is included in the
274 residue definition.

275 In cases of complex residue definitions (e.g. a residue definition which contains more than
276 one compound) the validation results shall be reported for the single parts of the full residue
277 definition, unless the single elements cannot be analysed separately.

278 The mean recovery at each fortification level and for each sample matrix should be in the
279 range of 70 % - 120 %. In certain justified cases mean recoveries outside of this range will be
280 accepted.

281 For plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff recovery
282 may deviate from this rule as specified in Table 1.²

283 **Table 1: Mean recovery and precision criteria for plant matrices and animal matrices [4]**

Concentration level	Range of mean recovery (%)	Precision, RSD (%)
> 1 µg/kg ≤ 0.01 mg/kg	60 - 120	30
> 0.01 mg/kg ≤ 0.1 mg/kg	70 - 120	20
> 0.1 mg/kg ≤ 1.0 mg/kg	70 - 110	15
> 1 mg/kg	70 - 110	10

284

285 If blank values are unavoidable, recoveries shall be corrected and reported together with the
286 uncorrected recoveries.

287 The precision of a method shall be reported as the relative standard deviation (RSD) of
288 recovery at each fortification level. For plants, plant products, foodstuff (of plant and animal
289 origin) and feeding stuff the RSD should comply with the values specified in Table 1. In other
290 cases the RSD should be ≤ 20 % per level. In certain justified cases, e.g. determination of
291 residues in soil lower than 0.01 mg/kg, higher variability may be accepted.

292 When outliers have been identified using appropriate statistical methods (e.g. Grubbs or
293 Dixons test), they may be excluded. Their number must not exceed 1/5 of the results at each
294 fortification level. The exclusion should be justified and the statistical significance must be

² According to Annex IIA 4.2 of Directive 91/414/EEC the mean recovery should normally be 70 % - 110 % and the RSD should preferably be ≤ 20 %.

295 clearly indicated. In that case all individual recovery data (including those excluded) shall be
296 reported.

297 **2.9.3 Selectivity**

298 Representative clearly labelled chromatograms of standard(s) at the lowest calibrated level,
299 matrix blanks and samples fortified at the lowest fortification level for each analyte/matrix
300 combination must be provided to prove selectivity of the method. Labelling should include
301 sample description, chromatographic scale and identification of all relevant components in the
302 chromatogram.

303 When mass spectrometry is used for detection, a mass spectrum (in case of MS/MS: product
304 ion spectrum) should be provided to justify the selection of ions used for determination.

305 Blank values (non-fortified samples) must be determined from the matrices used in
306 fortification experiments and should not be higher than 30 % of the LOQ. If this is exceeded,
307 detailed justification should be provided.

308 **2.10 Confirmation**

309 Confirmatory methods are required to demonstrate the selectivity of the primary method for
310 all representative sample matrices (Sections 3 – 8). It has to be confirmed that the primary
311 method detects the right analyte (analyte identity) and that the analyte signal of the primary
312 method is quantitatively correct and not affected by any other compound.

313 **2.10.1 Confirmation simultaneous to primary detection**

314 A confirmation simultaneous to the primary detection using one fragment ion in GC-MS and
315 HPLC-MS or one transition in HPLC-MS/MS may be accomplished by one of the following
316 approaches:

- 317 • In GC-MS, HPLC-MS, by monitoring at least 2 additional fragment ions (preferably
318 $m/z > 100$) for low resolution system and at least 1 additional fragment ion for high
319 resolution/accurate mass system
- 320 • In GC-MSⁿ (incl. Ion Traps and MS/MS), HPLC-MS/MS, by monitoring at least 1
321 additional SRM transition

322 The following validation data are required for the additional fragment ions (MS and HRMS)
323 or the additional SRM transition (MSⁿ and MS/MS): calibration data (Section 2.9.1), recovery
324 and precision data according to Section 2.9.2 for samples fortified at the respective LOQ ($n =$
325 5) and for 2 blank samples.

326 For all mass spectrometric techniques a mass spectrum (in case of single MS) or a product ion
327 spectrum (in case of MSⁿ) should be provided to justify the selection of the additional ions.

328 **2.10.2 Confirmation by an independent analytical technique**

329 Confirmation can also be achieved by an independent analytical method. The following are
330 considered sufficiently independent confirmatory techniques:

- 331 • chromatographic principle different from the original method
- 332 • e.g. HPLC instead of GC
- 333 • different stationary phase and/or mobile phase with significantly different selectivity
- 334 • the following are not considered significantly different:
 - 335 • in GC: stationary phases of 100 % dimethylsiloxane and of 95 % dimethylsiloxane
336 + 5 % phenylpolysiloxane
 - 337 • in HPLC: C18- and C8-phases
- 338 • alternative detector
- 339 • e.g. GC-MS vs. GC-ECD, HPLC-MS vs. HPLC-UV/DAD
- 340 • derivatisation, if it was not the first choice method
- 341 • high resolution/accurate mass MS
- 342 • in mass spectrometry an ionisation technique that leads to primary ions with different m/z
343 ratio than the primary method (e.g. ESI negative ions vs. positive ions)

344 It is preferred that confirmation data are generated with the same samples and extracts used
345 for validation of the primary method.

346 The following validation data are required: calibration data (Section 2.9.1), recovery and
347 precision data (Section 2.9.2) for samples fortified at the respective LOQ ($n \geq 3$) and of a
348 blank sample and proof of selectivity (Section 2.9.3).

349 **2.11 Independent laboratory validation (ILV)**

350 A validation of the primary method in an independent laboratory (ILV) must be submitted for
351 methods used for the determination of residues in plants, plant products, foodstuff (of plant
352 and animal origin) and in feeding stuff. The ILV shall confirm the LOQ of the primary
353 method, but at least the lowest action level (MRL).

354 The extent of independent validation required is given in detail in sections 3 and 4.

355 In order to ensure independence, the laboratory chosen to conduct the ILV trials must not
356 have been involved in the method development and in its subsequent use. In case of multi-

357 residue methods it would be accepted if the ILV is performed in a laboratory that has already
358 experience with the respective method.

359 The laboratory may be in the applicant's organisation, but should not be in the same location.
360 In the exceptional case that the lab chosen to conduct the ILV is in the same location,
361 evidence must be provided that different personnel, as well as different instrumentation and
362 stocks of chemicals etc have been used.

363 Any additions or modifications to the original method must be reported and justified. If the
364 chosen laboratory requires communication with the developers of the method to carry out the
365 analysis, this should be reported.

366 **2.12 Availability of standards**

367 All analytical standard materials used in an analytical method must be commonly available.
368 This applies to metabolites, derivatives (if preparation of derivatives is not a part of the
369 method description), stable isotope labelled compounds or other internal standards.

370 If a standard is not commercially available the standard should be made generally available by
371 the applicant and contact details be provided.

372 **2.13 Extraction Efficiency**

373 The extraction procedures used in residue analytical methods for the determination of residues
374 in plants, plant products, foodstuff (of plant and animal origin) and in feeding stuff should be
375 verified for all matrix groups for which residues \geq LOQ are expected, using samples with
376 incurred residues from radio-labelled analytes.

377 Data or suitable samples may be available from pre-registration metabolism studies or
378 rotational crop studies or from feeding studies. In cases where such samples are no longer
379 available to validate an extraction procedure, it is possible to "bridge" between two solvent
380 systems (details in [4]). The same applies if new matrices are to be included.

381 **3 Analytical methods for residues in plants, plant products, foodstuff (of**
 382 **plant origin), feedingstuff (of plant origin)**
 383 **(Annex IIA Point 4.2.1 of Directive 91/414/EEC; Annex Point IIA, Point**
 384 **4.3 of OECD)**

385 **3.1 Purpose**

- 386 • Analysis of plants and plant products, and of foodstuff and feeding stuff of plant origin for
 387 compliance with MRL [3].

388 **3.2 Selection of analytes**

389 The selection of analytes for which methods for food and feed are required depends upon the
 390 definition of the residue for which a maximum residue level (MRL) is set or is applied for
 391 according to Regulation (EC) No 396/2005.

392 **3.3 Commodities and Matrix Groups**

393 Methods validated according to Section 2.9 and 2.10 must be submitted for representative
 394 commodities (also called “matrices” by analytical chemists) of all four matrix groups in
 395 Table 2.

396 **Table 2: Matrix groups and typical commodities**

Matrix group	Examples for commodities
dry commodities (high protein/high starch content)	barley, rice, rye, wheat, dry legume vegetables
commodities with high water content	apples, bananas, cabbage, cherries, lettuce, peaches, peppers, tomatoes
commodities with high oil content	avocados, linseed, nuts, olives, rape seed
commodities with high acid content	grapefruits, grapes, lemons, oranges

397 Important Note: This list of commodities is not a comprehensive list of commodities/matrices.

398 Applicants may consult regulatory authorities for advice on the use of other commodities.

399 If samples with high water content are extracted at a controlled pH a particular method or
 400 validation for commodities with high acid content is not required.

401 Where a previously validated method has been adopted to a new matrix group, validation data
 402 must be submitted for representative matrices of this group.

403 If a method is required for a commodity which is difficult to analyse (see Table 3 for
 404 examples), full validation data for that specific commodity shall be presented to prove the
 405 suitability of the method.

406 **Table 3: Examples of matrices which are difficult to analyse**

Matrix group	Examples for Commodities
no group	coffee beans, cocoa beans, herbal infusions, hops, spices, tea, tobacco

407

408 The decision on whether a commodity must be considered “difficult to analyse” may depend
 409 upon the sample preparation and the analytical method selected for analysis. For example
 410 matrices like brassica or onion may be considered ”difficult to analyse” if detection
 411 techniques like ECD, NPD or UV are used.

412 **3.4 Limit of quantification**

413 Generally, an LOQ of 0.01 mg/kg should be met. Only in justified cases it may be sufficient
 414 that the LOQ complies with the lowest MRL in the respective matrix group [3]. For
 415 commodities which are difficult to analyse the LOQ must meet 50 % of the MRL of that
 416 commodity unless the MRL is set at the LOQ.

417 **3.5 Independent laboratory validation (ILV)**

418 An ILV must be conducted with samples of representative commodities of all matrix groups
 419 for which a primary method is required, with the same number of samples and fortification
 420 levels. If the primary method is identical for all matrix groups, it is sufficient to perform the
 421 ILV for commodities of two of these groups, one of them with high water content.

422 If a validated primary method is required for commodities difficult to analyse (Section 3.3) an
 423 ILV must be performed.

424 No ILV may be required if a multi-residue method published by an international official
 425 standardisation body is sufficiently validated in more than one laboratory (see Section 2.6 for
 426 details).

427 **4 Analytical methods for residues in foodstuff (of animal origin)**
428 **(Annex IIA Point 4.2.1 of Directive 91/414/EEC; Annex Point IIA, Point**
429 **4.3 of OECD)**

430 **4.1 Purpose**

- 431 • Analysis of foodstuff and feeding stuffs of animal origin for compliance with MRL [3].

432 **4.2 Selection of analytes**

433 The selection of analytes for which methods for foodstuff of animal origin are required
434 depends upon the definition of the residue for which a maximum residue level (MRL) is set or
435 is applied for according to Regulation (EC) No 396/2005.

436 **4.3 Commodities**

437 A residue analytical method for foodstuff of animal origin shall be provided for the following
438 animal matrices, if an MRL is established or is likely to be proposed:

- 439 • Milk
440 • Eggs
441 • Meat (e.g. bovine or poultry)
442 • Fat
443 • Liver/kidney

444 Methods must be validated according to Section 2.9 and 2.10.

445 **4.4 Limit of quantification**

446 Generally, an LOQ of 0.01 mg/kg should be met. Only in justified cases it may be sufficient
447 that the LOQ complies with the lowest MRL in the respective matrix [3].

448 **4.5 Independent laboratory validation (ILV)**

449 An ILV must be conducted with samples of representative commodities of all matrices for
450 which a primary method is required, with the same number of samples and fortification levels.
451 If a primary method is identical for all matrices listed under Section 4.3, it may be sufficient
452 to perform the ILV with at least two of these matrices.

453 **5 Analytical methods for residues in soil**
 454 **(Annex IIA, Point 4.2.2 of Directive 91/414/EEC; Annex Point IIA, Point**
 455 **4.4 of OECD)**

456 **5.1 Purpose**

457 Monitoring, enforcement of restrictions, post-registration control, emergency measures in the
 458 case of an accident, surveillance of buffer zones to surface waters.

459 **5.2 Selection of analytes**

460 The residue definition for monitoring purposes in soil is based on the assessment of fate and
 461 ecotoxicology and may include the active substance and/or relevant metabolites.

462 EFSA Conclusions provide recommendations for the analytes “relevant for monitoring” in
 463 soil for active substances which were already peer reviewed. However such a definition may
 464 be subject to national legal provisions.

- 465 • Analytical methods for residues in soil may not be necessary, if more than 90 % of the
 466 start concentration of the active substance and its relevant metabolites are degraded within
 467 3 days ($DT_{90} < 3 \text{ d}$).
- 468 • Methods for naturally occurring non-toxic substances are usually not required.

469 **5.3 Samples**

470 Methods must be validated according to Section 2.9 and 2.10 with representative soil of crop
 471 growing. Characteristics of the soil sample (e.g. soil type, pH and organic matter/carbon
 472 content) should be provided in the method description to support its selection.

473 **5.4 Limit of quantification**

474 Usually, the limit of quantification for residues in soil should be 0.05 mg/kg.

475 If the toxic concentration (LC_{50}) for the most sensitive non-target organism is lower than
 476 0.05 mg/kg (= 75 g/ha)³ the LOQ must comply with this LC_{50} value. For phytotoxic
 477 herbicides the LOQ should also comply with the EC_{10} -value of the most sensitive crop.

³ Expected concentrations in soil can be calculated from the application rate of an active substance (in [g/ha]) using the following equation:

$$c = \frac{\text{application rate}}{\text{soil depth} \cdot \text{soil density}}$$

with soil depth : 10[cm]; soil density : 1.5[g/cm³]:

$$c = \text{application rate} \cdot \frac{1}{1500} \left[\frac{\text{mg}}{\text{kg}} \right]$$

478 Methods for highly phytotoxic compounds possibly demand highly sophisticated equipment
479 to meet the required LOQ. Therefore techniques that are not considered as commonly
480 available can be accepted, if justified.

481 **6 Analytical methods for residues in water**
482 **(Annex IIA, Point 4.2.3 of Directive 91/414/EEC; Annex Point IIA;**
483 **Point 4.5 of OECD)**

484 **6.1 Purpose**

485 Enforcement of the drinking water limit [14] or the groundwater limit [15] of 0.1 µg/L, post-
486 registration control, emergency measures in the case of an accident.

487 **6.2 Selection of analytes**

488 The residue definition for monitoring purposes in drinking water and surface water is based
489 on the assessment of fate and ecotoxicology and may include the active substance and/or
490 relevant metabolites.

491 EFSA Conclusions provide recommendations for the analytes relevant for monitoring in
492 drinking water/groundwater and surface water for active substances which were already peer
493 reviewed. However such a definition may be subject to national legal provisions.

- 494 • Analytical methods for residues in water may not be necessary, if more than 90 % of the
495 start concentration of the active substance and its relevant metabolites are degraded within
496 3 days ($DT_{90} < 3$ d).
- 497 • Methods for naturally occurring non-toxic substances are usually not required.

498 **6.3 Samples**

499 Methods must be validated according to Section 2.9 and 2.10 for the following matrices:

- 500 • Drinking water or groundwater
- 501 • Surface water (freshwater, e.g. from rivers or ponds)

502 In the method description the sampling site should be provided. For the surface water used in
503 method validation quality data shall be provided to demonstrate that the sample is a typical
504 surface water in terms of its inorganic load (e.g. conductivity, hardness, pH) and its organic
505 load (e.g. dissolved organic carbon content (DOC)).

506 Provided that a method has been successfully validated for surface water at the LOQ required
507 for drinking water, no further validation in drinking water is required.

508 **6.4 Limit of quantification**

509 For drinking water or groundwater the limit of quantification must meet 0.1 µg/L [14]. For
510 surface water the LOQ must comply with the lowest effect concentration [16] mentioned in
511 Table 4.

512 **Table 4: Effect concentrations relevant for setting of LOQs in surface water**

	Acute test	Long-term test
Fish	LC ₅₀	NOEC
<i>Daphnia</i>	EC ₅₀	NOEC
<i>Chironomus</i> sp	EC ₅₀	NOEC
Algae	EC ₅₀	
Higher aquatic plants	EC ₅₀	

513

514 **6.5 Direct injection**

515 In case that HPLC-MS/MS is used the direct injection of water samples is desirable, provided
516 that it complies with the LOQ. In that case recovery data cannot be calculated. Thus,
517 calibration and precision data have to be presented, only.

518 **7 Analytical methods for residues in air**
519 **(Annex IIA, Point 4.2.4 of Directive 91/414/EEC; Annex Point IIA;**
520 **Point 4.7 of OECD)**

521 **7.1 Purpose**

522 Monitoring of the exposure of operators, workers or bystanders and working place.

523 **7.2 Selection of analytes**

524 For air analyte selection is governed by the safety of operators, workers and/or bystanders as
525 the primary criterion, and comprises the active substance in most cases.

526 EFSA Conclusions provide recommendations for the analytes relevant for monitoring for
527 active substances which were already peer reviewed.

- 528 • Methods may not be necessary if the application technique makes an exposure unlikely.
529 However, consideration should be given to spray drift and particle associated substances
530 which can cause relevant exposures. Therefore, in such cases an analytical method is also
531 required for substances with a low vapour pressure ($< 10^{-5}$ Pa).
- 532 • Methods are usually not required for naturally occurring non-toxic substances and
533 substances which are not classified as T+, T, Xi, Xn and are not labelled with the
534 following symbols according to GHS:



538

539 **7.3 Samples**

540 Methods shall be validated according to Section 2.9 with air at 35 °C and at least 80 %
541 relative humidity (RH). In justified cases (e.g. heat sensitive analyte) and if it is shown that a
542 method does not work at 35 °C and 80 % RH, other conditions are applicable (e.g. ambient
543 temperature and normal humidity).

544 **7.4 Limit of quantification**

545 If a limit was established according to Council Directive 98/24/EC [17], the LOQ should
546 comply with this value. If no limit is set the LOQ should comply with the concentration c
547 calculated from the $AOEL_{inhalative}$ (in [mg/kg bw d]) according to the following equation:

$$c = AOEL_{inhalative} \cdot \frac{\text{safety factor} \cdot \text{body weight}}{\text{air intake}}$$

548 with safety factor : 0.1; body weight : 60 [kg]; air intake : 20 [m³/day]

$$c = AOEL_{inhalative} \cdot 300 \left[\frac{\mu\text{g}}{\text{m}^3} \right]$$

549 Instead of the $AOEL_{inhalative}$ the $AOEL_{systemic}$ can be used for calculation. In case that no
550 AOEL is available, the ADI-value can be employed instead.

551 In case that inhalation toxicity studies show that an active substance induces local effects on
552 the respiratory tract rather than systemic effects, the LOQ should comply with the $AEC_{inhalation}$
553 [18].

554 **7.5 Sorbent characteristics**

555 The sorbent must be suitable for enrichment of particle associated and gaseous residues. It is
556 sufficient to quote literature proving that the sorbent also adsorbs particle associated residues.
557 For polymer based sorbents such a proof is not required.

558 **7.6 Further validation data**

559 The retention capacity of the sorbent material must be proven. This may be carried out by
560 determining the recovery of the analyte, added onto the sorbent in a small volume of solvent,
561 at defined air temperature and relative humidity, after passage of a defined volume of air
562 (> 100 L) for at least 6 hours. The capacity is considered sufficient if no significant
563 breakthrough occurs.

564 It is desirable to submit data on the extractability of the analyte from the sorbent and on the
565 storage stability of the analyte loaded onto the sorbent.

566 **7.7 Confirmatory methods**

567 No confirmatory methods are required for the determination of residues in air if sufficient
568 confirmatory methods are available for the determination in soil or water.

569 **8 Analytical methods for residues in body fluids and tissues**
570 **(Annex IIA, Point 4.2.5 of Directive 91/414/EEC; Annex Point IIA Point**
571 **4.8 of OECD)**

572 **8.1 Purpose**

573 Detection of intoxications in humans and animals.

574 **8.2 Selection of analytes**

575 EFSA Conclusions provide recommendations for the analytes relevant for monitoring in body
576 fluids and tissues for active substances which were already peer reviewed. This may include
577 analytes defined as “relevant for monitoring” and classified as toxic or very toxic (T, T+) or
578 are classified according to GHS as follows: Acute toxicity (cat. 1 - 3), CMR (cat. 1) or STOT
579 (cat. 1). This may also include analytes that exhibit high acute toxicity in humans or animals
580 equivalent to those classifications.

581 **8.3 Samples**

582 Methods must be validated according to Section 2.9 and 2.10 with the following matrix
583 groups:

- 584 • Body fluids (either blood, serum, plasma or urine)
- 585 • Body tissues (either meat, liver or kidney)

586 Methods for body fluids and tissues should be validated with the matrix which is most
587 suitable to prove intoxication. If a primary method for food of animal origin (Section 4) with
588 sufficient sensitivity covers the respective tissue no additional method or validation study for
589 body tissue is required.

590 **8.4 Sample set**

- 591 • LOQ 5 samples
- 592 • control 2 samples

593 No validation data for an elevated concentration level (10 x LOQ) are required for body fluids
594 and tissues.

595 **8.5 Limit of quantification**

596 The LOQ shall meet 0.05 mg/L for body fluids and 0.1 mg/kg for body tissues.

597 **9 Summary - List of methods required**

598 Table 5 gives an overview on the methods that may be required. It can be used by the
 599 applicant to check prior to submission of the application whether all required studies are
 600 addressed.

601 **Table 5: Completeness check of analytical methods for monitoring purposes and post-**
 602 **registration control**

Matrix group / crop group	Residue definition for monitoring	LOQ	Methods		
			Primary method	Confirmatory method	Independent lab validation
Dry commodities (high protein/high starch content)					
Commodities with high water content					
Commodities with high oil content					
Commodities with high acid content					
Commodities which are difficult to analyse					
Milk					
Eggs					
Meat					
Fat					
Kidney/liver					
Soil					Not necessary
Drinking water					Not necessary
Surface water					Not necessary
Air					Not necessary
Body fluids					Not necessary
Body tissues					Not necessary

604 **10 Abbreviations**

AAS	atomic absorption spectroscopy
ADI	acceptable daily intake
AOEL _{inhalative}	acceptable operator exposure level for exposure by inhalation; according to [19]
AEC _{inhalation}	adverse effect concentration for exposure by inhalation [18]
AOEL _{systemic}	acceptable operator exposure level concerning systemic effects [19]
CEN	European Committee for Standardisation
DAD	diode array detector
DT ₉₀	time required for 90 % degradation
EC ₁₀	concentration showing 10 % effect
EC ₅₀	concentration showing 50 % effect
ECD	electron capture detector
EFSA	European Food Safety Authority
ESI	electrospray ionisation
FID	flame ionisation detector
FLD	fluorescence detector
FPD	flame photometric detector
GC	gas chromatography
HPLC	high-performance liquid-chromatography
HRMS	high resolution mass spectrometry
ICP	inductively coupled plasma
ILV	independent laboratory validation
LC ₅₀	concentration showing 50 % lethal effect
LOQ	limit of quantification (here: lowest successfully validated level)
MRL	maximum residue level
MS	mass spectrometry
MS ⁿ	multiple-stage mass spectrometry (with $n \geq 2$)
NOEC	no observed effect concentration
NPD	nitrogen phosphorus detector
OECD	Organisation of Economic Cooperation and Development
OES	optical emission spectroscopy

RSD	relative standard deviation (coefficient of variation) (here: within laboratory)
SRM	selected reaction monitoring
UV	ultraviolet (detector)

605 11 References

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