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| REGISTRATION REPORT  Part B  Section 5  Analytical Methods  Detailed summary of the risk assessment |
| Product code: SHA 148000 A  Product name: METROPOLITAN  Chemical active substance:  Metazachlor, 500 g/L |
| Central Zone  Zonal Rapporteur Member State: POLAND |
| CORE ASSESSMENT  (Authorization) |
| Applicant: XXXX  Submission date: October 2022  Evaluation date: July 2023  MS Finalisation date: dd/mm/yyyy |

Version history

|  |  |
| --- | --- |
| When | What |
| July 2023 | Initial RR |
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# Analytical methods

This application is to request authorization of Metropolitan, suspension concentrate (SC) formulation containing 500 g/L of Metazachlor, for use as an herbicide in winter and spring oilseed rape. The application follows the data requirements for the active substance laid down in Regulation (EC) No. 283/2013 and the data requirements for the plant protection product laid down in Regulation (EC) No. 284/2013.

## Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps are: none

* ~~data gap 1~~
* ~~data gap 2~~
* ~~data gap 3~~

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Acc. to EFSA Scientific Report (2008) 145, 1-132 Conclusion on the peer review of metazachlor adequate methods are available to monitor all compounds given in the respective residue definitions in food/feed of plant and animal origin.

A GC-MS enforcement analytical method is available to monitor residues of metabolites 479-M04, 479-M08 and 479-M16 in plant matrices, determined as 2,6 dimethyl aniline, with LOQ of 0.05 mg/kg for rape seed, wheat grain, wheat straw, cabbage and carrot.

HPLC-MS/MS methods are also available allowing determination of the metabolites 479-M04, 479-M08 and 479-M16 without hydrolysis to the common moiety as in the enforcement method, serving also as a confirmatory methods, with LOQ of 0.01 mg/kg for wheat grain, wheat straw, lettuce, cauliflower, lemon and oilseed rape.

The applicability of the multi-residue method DFG S19 was tested with metazachlor only, in conclusion it cannot be used for monitoring the compounds in the residue definition.

An HPLC-MS/MS method is available to monitor residues of metazachlor, determined as 2,6 dimethylaniline, in food/feed of animal origin (milk, liver) with LOQs of 0.01 mg/kg.

Adequate methods are available (HPLC-MS/MS) to monitor metazachlor and metabolites 479M-04 and 479M-08 in soil, with LOQ of 0.01 mg/kg, and in water (drinking water, surface water) with LOQ of 0.05 µg/L. Subject to the final agreement on the hazard classification of metazachlor however, monitoring methods for metabolites 479M09, 479M11 and 479M12 would also be required.

Residues of metazachlor in air can be determined with GC-MS method with LOQ of 0.5 µg/m³

Analytical methods for the determination of residues in body fluids and tissues are not required as metazachlor is not classified as toxic or highly toxic.

In the context of the authorisation request noticed data gaps are: none

| Commodity/crop | Supported/ Not supported |
| --- | --- |
| OSR | Supported |
| Brassica | Supported |

## Methods used for the generation of pre-authorization data (KCP 5.1)

### Analysis of the plant protection product (KCP 5.1.1)

#### Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | The method is validated and accepter for analysing active substance in the PPP. |

The following analytical method, and its validation, for the determination of metazachlor in the plant protection product Metazachlor 50% SC, has not previously been reviewed and is provided in support of this assessment.

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1 |
| Report | Physical chemical studies of Metazachlor 50% SC. Azeema, G.B. 2022. Report No 10874/2022. |
| Guideline(s): | SANCO/3030/99 rev.5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

*Test item:* Metazachlor 50% SC

*Analytical standard:* Metazachlor, analytical standard

*Blank formulation:* Blank formulation of Metazachlor 50 SC.

Preparation of the standard, test item and blank formulation solutions were carried out in acetonitrile.

The analysis was carried out by HPLC-DAD detector with external standards.

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substance metazachlor in plant protection product Metazachlor 50 SC

|  | Metazachlor |
| --- | --- |
| Author(s), year | Azeema, G.B., 2022 |
| Principle of method | HPLC-DAD with external standard |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r)  n = 6 | 40.1 – 60.5 mg/L (±20% range of the declared content)  y = 1637.6-213.6, R² = 1.0 |
| Precision – Repeatability Mean  n = 5  (%RSD) | RSD = 0.03 % < 1.519% (Horwits RSDr)  Horrat value <1 |
| Accuracy  n = 10 (2 levels by quintuplicate)  (% Recovery) | Average recovery 100.36 %.  Recovery range at two spiking levels on blank formulation: 100.5– 100.2 % |
| Interference/ Specificity | No interference >3%. A comparison of the chromatograms of metazachlor reference material, test item sample, blank formulation, show that the active ingredient peaks are well separated and there is no evidence of interferences with the test item peaks. |
| Comment |  |

Conclusion

According to the SANCO/3030/99 rev.5 guidance document, the analytical method for the determination of metazachlor in the test item Metazachlor 50% SC was validated.

#### Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

|  |  |
| --- | --- |
| Comments of zRMS: | Accepted |

The following analytical method, and its validation, for the determination of toluene in the plant protection product Metazachlor 50% SC, has not previously been reviewed and is provided in support of this assessment.

|  |  |
| --- | --- |
| Reference: | KCP 5.1.1 |
| Report | Physical chemical studies of Metazachlor 50% SC. Azeema, G.B. 2022. Report No 10874/2022. |
| Guideline(s): | SANCO/3030/99 rev.5 |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

*Test item:* Metazachlor 50% SC

*Analytical standard:* toluene, analytical standard

Preparation of the standard, test item solutions were carried out in acetone.

The analysis was carried out by GC-FID detector with external standards.

Validation - Results and discussions

Table 5.2‑1: Methods suitable for the determination of active substance metazachlor in plant protection product Metazachlor 50 SC

|  | Toluene |
| --- | --- |
| Author(s), year | Azeema, G.B., 2022 |
| Principle of method | GC-FID with external standard |
| Linearity  (linear between  mg/L / % range of the declared content)  (correlation coefficient, expressed as r)  n = 6 | 0.135-0.204 mg/L  y = 17036-31.071, R² = 0.999 |
| Precision – Repeatability Mean  n = 5  (%RSD) | at 0.025 g/L (toluene’s content)  RSD = 0.9874 % < 6.479% (Horwits RSDr)  Horrat value <1 |
| Accuracy  n = 10 (2 levels by quintuplicate)  (% Recovery) | Average recovery 100.04 %.  Recovery range at two spiking levels on blank formulation: 100.3– 99.8 % |
| LOQ | 0.151 mg/L |
| Interference/ Specificity | No interference >3%. A comparison of the chromatograms of toluene reference material, test item sample, show that the substance peaks are well separated and there is no evidence of interferences with the test item peaks. |
| Comment |  |

Conclusion

According to the SANCO/3030/99 rev.5 guidance document, the analytical method for the determination of toluene in the test item Metazachlor 50% SC was validated.

#### Description of analytical methods for the determination of formulants (KCP 5.1.1)

Non relevant.

#### Applicability of existing CIPAC methods (KCP 5.1.1)

There is currently no CIPAC method.

### Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of metazachlor for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/additional studies refer to Appendix 2.

Table 5.2‑3: Validated methods for the generation of pre-authorization data

| Component of residue definition: Metazachlor | | | | | |
| --- | --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method | Author(s), year / missing / EU agreed | Annex point of  pre-registration study that method validation supports |
| Soil, water  (Ecotoxicology) | Primary | 0.5 μg/L | LC-MS | Kolek L., 2021a  See Appendix 2 | KCP 5.1.2/01 (Acute immobilisation study in *Daphnia magna*) |
| Primary | 0.5 μg/L | LC-MS | Nowrotek M., 2021  See Appendix 2 | KCP 5.1.2/02 (Growth Inhibition test in *Lemna gibba*) |
| Primary | 0.5 μg/L | LC-MS | Kolek L., 2021b  See Appendix 2 | KCP 5.1.2/03 (Growth Inhibition test in *Freshwater Alga and Cyanobacteria*) |
| Primary | 0.05 mg/kg | LC-MS | Swoboda T., 2021  See Appendix 2 | KCP 5.1.2/04 (Earthworm Reproduction Test (*Eisenia andrei*) |
| Primary | 20 mg/kg | LC-MS | Parma P., 2021  See Appendix 2 | KCP 5.1.2/05 (Chronic oral toxicity test on Honeybees (*Apis* *mellifera* L.) |

## Methods for post-authorization control and monitoring purposes (KCP 5.2)

### Analysis of the plant protection product (KCP 5.2)

These methods are already submitted in accordance with the requirements set out in point 5.2.1.

### Description of analytical methods for the determination of residues of metazachlor (KCP 5.2)

#### Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3‑1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

| Matrix | Residue definition | MRL / limit | Reference for MRL/level Remarks |
| --- | --- | --- | --- |
| Plant, high water content | Sum of metabolites M479H004, M479H008 and M479H016 expressed as metazachlor | LOQ = 0.05 mg/kg  LOQ = 0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Plant, high acid content | LOQ = 0.05 mg/kg  LOQ = 0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Plant, high protein/high starch content (dry commodities) | LOQ = 0.05 mg/kg  LOQ = 0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Plant, high oil content | LOQ = 0.05 mg/kg  LOQ = 0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Muscle | Metazachlor including all degradation products which can be determined as 2,6-dimethylaniline | LOQ = 0.05 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Milk | LOQ = 0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Eggs | LOQ = 0.05 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Fat | LOQ = 0.05 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Liver, kidney | LOQ = 0.05 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Soil  (Ecotoxicology) | Metazachlor, | LOQ =0.01 mg/kg | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Drinking water | metazachlor | LOQ = 0.1 µg/L | general limit for drinking water |
| Surface water  (Ecotoxicology) | metazachlor | LOQ = 0.05 µg/L  EC50 2.3 µg/L | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Air | metazachlor | LOQ = 0.001 μg/l  LOQ = 0.5 μg/m3 | EFSA Conclusion, Metazachlor (2008) 145, 1-132 |
| Tissue (meat or liver) | metazachlor | not required | not classified as T |
| Body fluids | not required | not classified as T |

#### Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in plant matrices is given in the following tables.

Table 5.3‑2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

**Enforcement methods**

| Component of residue definition:  Sum of metabolites M479H004, M479H008 and M479H016 expressed as metazachlor | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing / EU agreed |
| High oil content | Primary | * 1. mg/kg   0.05 mg/kg | LC-MS/MS  GC/MS | Bross, M. and Mckenroth, C., 2003; Hopf, B. and Mackenroth, C., 2007 / DAR 2005, Addendum 2007.  Wittig, 2000; Chambers J.G. and Taylor N.W., 2002 / DAR 2005 |
| ILV | * 1. mg/kg   0.05 mg/kg | LC-MS/MS  GC/MS | Edwards 2003; Class, T., 2006 / DAR 2005, Addendum 2007.  Witte, 2004; Wasser, C., 2000 / DAR 2005 |
| Confirmatory | 0.01 mg/kg  0.05 mg/kg | LC-MS/MS  GC/MS | Bross, M. and Mckenroth, C., 2003; Hopf, B. and Mackenroth, C., 2007 / DAR 2005, Addendum 2007.  Wittig, 2000; Chambers J.G. and Taylor N.W., 2002 / DAR 2005 |
| High water content | Primary | 0.01 mg/kg | LC-MS/MS | Bross, M. and Mckenroth, C., 2003; Hopf, B. and Mackenroth, C., 2007 / DAR 2005, Addendum 2007. |
| ILV | 0.01 mg/kg | LC-MS/MS | Edwards 2003; Class, T., 2006 / DAR 2005, Addendum 2007. |
| Confirmatory | 0.01 mg/kg | LC-MS/MS | Bross, M. and Mckenroth, C., 2003; Hopf, B. and Mackenroth, C., 2007 / DAR 2005, Addendum 2007. |

Table 5.3‑3: Statement on extraction efficiency

|  | Method for products of plant origin |
| --- | --- |
| Required, available from: | Metazachlor DAR (June 2005), Volume 3, Annex B-7: Residue Data. Satisfactory extraction efficiency was found. |

#### Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in animal matrices is given in the following tables.

Table 5.3‑4: Validated methods for food and feed of animal origin (if appropriate)

| Component of residue definition: Metazachlor including all degradation products which can be determined as 2,6-dimethylaniline | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Milk | Primary | 0.01 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| ILV | 0.01 mg/kg | LC-MS/MS | Rawle N.W., 2003a / EFSA Conclusion, Metazachlor (2008) |
| Confirmation | 0.01 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| Eggs | Primary | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| ILV | 0.05 mg/kg | LC-MS/MS | Rawle N.W., 2003a / EFSA Conclusion, Metazachlor (2008) |
| Confirmation | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| Muscle | Primary | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| ILV | 0.05 mg/kg | LC-MS/MS | Rawle N.W., 2003a / EFSA Conclusion, Metazachlor (2008) |
| Confirmation | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| Fat | Primary | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| ILV | 0.05 mg/kg | LC-MS/MS | Rawle N.W., 2003a / EFSA Conclusion, Metazachlor (2008) |
| Confirmation | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| Kidney, liver | Primary | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |
| ILV | 0.05 mg/kg | LC-MS/MS | Rawle N.W., 2003a / EFSA Conclusion, Metazachlor (2008) |
| Confirmation | 0.05 mg/kg | LC-MS/MS | Tilting N., 2003c / EFSA Conclusion, Metazachlor (2008) |

There are no special comments or remarkable points concerning the analytical methods the determination of residues in animal matrices.

Table 5.3‑5: Statement on extraction efficiency

|  | Method for products of animal origin |
| --- | --- |
| Required, available from: | Metazachlor DAR (June 2005), Volume 3, Annex B-7: Residue Data. Satisfactory extraction efficiency was found. |

#### Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in soil is given in the following table. Method have been previously reviewed and deemed acceptable (Metazachlor DAR, June 2005 and EFSA Conclusion, Metazachlor (2008)).

Table 5.3‑6: Validated methods for soil (if appropriate)

| Component of residue definition: metazachlor | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (*i.e.* GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.01 mg/kg | LC-MS-MS | Grote, C., 2003b /EFSA Conclusion, Metazachlor (2008) |
| Confirmatory | 0.01 mg/kg | LC-MS-MS | Grote, C., 2003b /EFSA Conclusion, Metazachlor (2008) |

There are no special comments or remarkable points concerning the analytical methods for soil.

#### Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in surface and drinking water is given in the following tables.

Table 5.3‑7: Validated methods for water (if appropriate)

| Component of residue definition: metazachlor | | | | |
| --- | --- | --- | --- | --- |
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | 0.05 μg/L | LC-MS/MS | Grote, C., 2003a /EFSA Conclusion, Metazachlor (2008) |
| Confirmatory | 0.05 μg/L | LC-MS/MS | Grote, C., 2003a /EFSA Conclusion, Metazachlor (2008) |
| Surface water | Primary | 0.05 μg/L | LC-MS/MS | Grote, C., 2003a /EFSA Conclusion, Metazachlor (2008) |
| Confirmatory | 0.05 μg/L | LC-MS/MS | Grote, C., 2003a /EFSA Conclusion, Metazachlor (2008) |

There are no special comments or remarkable points concerning the analytical methods for water.

#### Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of metazachlor in air is given in the following tables.

Table 5.3‑8: Validated methods for air (if appropriate)

| Component of residue definition: metazachlor | | | |
| --- | --- | --- | --- |
| Method type | Method LOQ | Principle of method  (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | 0.001 μg/l | GC-ECD | Zangmeister, W., 2000 /EFSA Conclusion, Metazachlor (2008) |
| Primary | 0.5 μg/m3 | GC-MSD | Wittig, 2000 /EFSA Conclusion, Metazachlor (2008) |
| Confirmatory | 0.5 μg/m3 | GC-MSD | Wittig, 2000 /EFSA Conclusion, Metazachlor (2008) |

There are no special comments or remarkable points concerning the analytical methods for air.

#### Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Method is not required as metazachlor is not classified as toxic or acutely toxic.

#### Other studies/ information

None.

# 

1. Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| KCP 5.1.1 | Azeeema, G.B. | 2022 | Physical chemical studies of Metazachlor 50% SC.  BRF Report No 10874/2022  GLP  Unpublished | N | Sharda Cropchem Ltd |
| KCP 5.1.2/01 | Kolek L. | 2021a | Daphnia sp., Acute Immobilisation Test’’  Report No.: EMI/4/8/2019  Ecomelius Institute Sp. z o. o.  GLP  Unpublished | N | Sharda Cropchem Ltd |
| KCP 5.1.2/02 | Nowrotek M. | 2021 | Lemna sp. Growth Inhibition Test  Report No.: EMI/4/29/2019  Ecomelius Institute Sp. z o. o.  GLP  Unpublished | N | Sharda Cropchem Ltd |
| KCP 5.1.2/03 | Kolek L. | 2021b | Freshwater Alga and Cyanobacteria, Growth Inhibition Test  Report No.: EMI/4/103/2020  Ecomelius Institute Sp. z o. o.  GLP  Unpublished | N | Sharda Cropchem Ltd |
| KCP 5.1.2/04 | Swoboda T. | 2021 | Earthworm Reproduction Test (Eisenia andrei)  Report No.: EMI/4/36/2019  Ecomelius Institute Sp. z o. o.  GLP  Unpublished | N | Sharda Cropchem Ltd |
| KCP 5.1.2/05 | Parma P. | 2021 | Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test  Report No.: EMI/4/67/2020  Ecomelius Institute Sp. z o. o.  GLP  Unpublished | N | Sharda Cropchem Ltd |

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
| 5.3 | Bross M.,  Mackenroth C. | 2003 | Validation of the analytical method 522/0:  Determination of the BAS 479 H  (Metazachlor) metabolite 479M16 in  plant matrices by LC/LC/MS/MS  BASF AG, Agrarzentrum Limburgerhof;  Limburgerhof; Germany Fed.Rep.  2003/1000956  Yes  unpublished | N | BASF |
| 5.3 | Wasser, C. | 2000 | Analysis of Metazachlor residues in  tomatoes, lemons, wheat grains and rape  seeds - Validation of BASF analytical  method No. 147  Anadiag SA; Haguenau; France  2000/1000244  Yes  unpublished | N | BASF |
| 5.3 | Edwards, J. | 2003 | Validation of BASF method number 522/0  for the analysis of BAS 479 H metabolite  479M16 in various crop matrices to an  LOQ of 0.01 mg/kg  BASF Agro Research Gosport; Gosport;  United Kingdom  2003/1012653  Yes  unpublished | N | BASF |
| 5.3 | Witte, A. | 2004 | Independent laboratory validation of an analytical  method for the determination of Metazachlor in  rape seed  GAB&IFU, Niefern-Öschelbronn, Germany  20041092/01-RVRA  Yes  Unpublished | N | Adama |
| 5.3 | Class, T. | 2006 | Independent laboratory validation of BASF method No. L0074/01 for the determination of BH 479-4 and BH 479-8 (metabolites of Metazachlor) in plant matrices  2006/1040649  PTRL Europe GmbH, Ulm, Germany Fed.Rep.  yes  Unpublished | N | BASF |
| 5.3 | Hopf B.,  Mackenroth C. | 2007 | Validation of the analytical method L0074/01: Method for the determination of BH 479-4 and BH 479-8 (metabolites of BAS 479 H) in plant matrices  2006/1038859  BASF AG, Limburgerhof, Germany Fed.Rep.  yes  Unpublished | N | BASF |
| 5.3 | Taylor N.W.,  Chambers J.G. | 2002 | Validation of an analytical method for the  determination of BAS 479 H residues in  vegetables  Synergy Laboratories Ltd.; Thaxted Essex  CM6 2PY; United Kingdom  2003/1001417  Yes  unpublished | N | BASF |
| 5.3 | Wittig, A. | 2000 | Validation of an analytical method to monitor  Metazachlor residues in rape seed  UCL GmbH, Köln, Germany  PR00/012  Yes  Unpublished | N | Adama |
| 5.3 | Tilting N. | 2003c | Validation of analytical method No 516/0  for the determination of BAS 479 H  (Metazachlor) in products of animal  origin  BASF AG, Agrarzentrum Limburgerhof;  Limburgerhof; Germany Fed.Rep.  2003/1001496  Yes  unpublished | N | BASF |
| 5.3 | Rawle N.W. | 2003b | Independent laboratory validation of a  method for the determination of residues  of BAS 479 H (Metazachlor) in matrices  of animal origin  CEM Analytical Services Ltd.; Berkshire  SL5 8JB; United Kingdom  2003/1001497  Yes  unpublished | N | BASF |
| 5.3 | Grote, C. | 2003b | Validation of analytical method No. 508/0:  LC/MS determination of BAS 479 H  (Metazachlor, 114 252) and its  metabolites BH 479-4 (211 193) and BH  479-8 (291 634) in soil  BASF AG, Agrarzentrum Limburgerhof;  Limburgerhof; Germany Fed.Rep.  2003/1005472  Yes  unpublished | N | BASF |
| 5.3 | Grote, C. | 2003a | Validation of analytical method No. 519/0:  LC/MS determination of BAS 479 H  (Metazachlor, 114 252) and its  metabolites BH 479-4 (211 193) and BH  479-8 (291 634) in tap, surface and  leachate water  BASF AG, Agrarzentrum Limburgerhof;  Limburgerhof; Germany Fed.Rep.  2003/1005471  Yes  unpublished | N | BASF |
| 5.3 | Zangmeister W. | 2000 | Validation of analytical method 372.  Determination of Metazachlor (BAS 479  H) (Reg.No. 114252) in air by GC  BASF AG, Agrarzentrum Limburgerhof;  Limburgerhof; Germany Fed.Rep.  2000/1000145  Yes  unpublished | N | BASF |
| 5.3 | Wittig, A. | 2000 | Validation of an analytical method for the  determination of residues of Metazachlor in air -  monitoring method  UCL GmbH, Köln, Germany  PR00/013  Yes  Unpublished | N | Adama |

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |
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List of data relied on not submitted by the applicant but necessary for evaluation

| Data point | Author(s) | Year | Title Company Report No.  Source (where different from company) GLP or GEP status Published or not | Vertebrate study  Y/N | Owner |
| --- | --- | --- | --- | --- | --- |
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|  |  |  |  |  |  |

1. Detailed evaluation of submitted analytical methods
   1. Analytical methods for the metazachlor

Summarised below are the analytical methods used in support of the ecotoxicology pre-registration studies.

* + 1. Methods used for the generation of pre-authorization data (KCP 5.1)

**A.2.1.1.1 Analytical method 1 (Daphnia sp., Acute Immobilisation Test)**

**A.2.1.1.1.1 Method validation**

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted.  The validation parameters were in the required range. |

|  |  |
| --- | --- |
| Reference: | 5.1.2/01 (Cross reference: KCP 10.2.1/01) |
| Report | Daphnia sp., Acute Immobilisation Test,  Kolek L., 2021a, Report No.: EMI/4/8/2019 |
| Guideline(s): | Yes (OECD 202, (2004)) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

A 10 mL sample was taken from each replicate of test concentrations and control. Samples were diluted if needed. Then they were centrifuged 3000 rcf, 10 min and supernatant were collected, and analysed by UHPLC-MS/MS using Column Zorbax SB-C18 RRHT 2,1×50 mm, 1,8 μm and isocratic elution with a mobile phase of (a) water + ammonium formate (0.1%): 55% (b) acetonitrile + formic acid (0.05%): 45%.

Results and discussions

Table A 1: Recovery results from method validation of metazachlor using the analytical method in culture medium - reconstituted water according to OECD 202

| Matrix | Analyte | Fortification level (μg/L) (*n* = 5) | Mean  recovery (%) | RSD (%) |
| --- | --- | --- | --- | --- |
| Culture medium | metazachlor | 0.5 | 81.88 | 6.190 |
| 5.0 | 92.86 | 1.298 |

Table A 2: Characteristics for the analytical method used for validation of metazachlor residues in culture medium - reconstituted water according to OECD 202

|  | Metazachlor |
| --- | --- |
| Specificity | The analysis showed that no signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions.  Two ion transitions were recorded:  Target: 278.1 → 134; Qualifier: 278.1 → 210  Specificity was verified using the ion transition ratio of 63.8% ± 30% (relative). Specificity of the method was confirmed. |
| Calibration (type, number of data points) | calibration line equation: Signal = 4101 C + 521 |
| Calibration range | Linearity range: 0.5 – 150 μg/L  Coefficient of determination R2: 0.9996 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | LOD: 0.15 μg/L  LOQ: 0.50 μg/L (Mean recovery: 81.88 %, % RSD: 6.19) |

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,   
accuracy and LOQaccording to guideline SANTE/2020/12830, Rev.1.

**A.2.1.1.2 Analytical method 2 (Lemna sp. Growth Inhibition Test)**

**A.2.1.1.1.2 Method validation**

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted. |

|  |  |
| --- | --- |
| Reference: | 5.1.2/02 (Cross reference: KCP 10.2.1/02) |
| Report | Lemna sp. Growth Inhibition Test,  Nowrotek M.., 2021, Report No.: EMI/4/29/2019 |
| Guideline(s): | Yes (OECD 221, (2006)) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

To prepare the stock solution, the appropriate amount of the test item, i.e. 311.6 mg, was weighed into a glass beaker and quantitatively transferred into volumetric flask by multiple washing with 20X AAP medium and filled up to appropriate volume (500 mL) of the 20X AAP medium. All test concentrations of test item were prepared by dilution of stock solution with concentration 623.2 mg/L. After splitting the concentrations of the test item into the crystallizers, to each crystallizer 3 plants per 3 fronds were added.

From each test item concentration, and the control, 20 mL were taken as a sample for chemical determinations. Analysis was done by UHPLC-MS/MS using Column Zorbax SB-C18 RRHT 2.1×50 mm, 1.8 μm and isocratic elution with a mobile phase of (a) water + ammonium formate (0.1%): 55% (b) acetonitrile + formic acid (0.05%): 45%.

Results and discussions

Table A 3: Recovery results from method validation of metazachlor using the analytical method in culture medium - 20X AAP medium recommended by the OECD Guideline No. 221

| Matrix | Analyte | Fortification level (μg/L) (*n* = 5) | Mean  recovery (%) | RSD (%) |
| --- | --- | --- | --- | --- |
| 20X AAP medium | metazachlor | 0.5 | 81.88 | 6.190 |
| 5.0 | 92.86 | 1.298 |

Table A 4: Characteristics for the analytical method used for validation of metazachlor residues in culture medium - 20X AAP medium recommended by the OECD Guideline No. 221

|  | Metazachlor |
| --- | --- |
| Specificity | The analysis showed that no signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions.  Two ion transitions were recorded:  Target: 278.1 → 134; Qualifier: 278.1 → 210  Specificity was verified using the ion transition ratio of 63.8% ± 30% (relative). Specificity of the method was confirmed. |
| Calibration (type, number of data points) | calibration line equation: Signal = 4101 C + 521 |
| Calibration range | Linearity range: 0.5 – 150 μg/L  Coefficient of determination R2: 0.9996 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | LOD: 0.15 μg/L  LOQ: 0.50 μg/L (Mean recovery: 81.88 %, % RSD: 6.19) |

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,   
accuracy and LOQaccording toguideline SANTE/2020/12830, Rev.1.

**A.2.1.1.3 Analytical method 3 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test)**

**A.2.1.1.1.3 Method validation**

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted. |

|  |  |
| --- | --- |
| Reference: | 5.1.2/03 (Cross reference: KCP 10.2.1/03) |
| Report | Freshwater Alga and Cyanobacteria, Growth Inhibition Test,  Kolek L., 2021b, Report No.: EMI/4/103/2020 |
| Guideline(s): | Yes (OECD 201, (2011)) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

A 1 mL sample was taken from each replicate of test concentrations and control. Samples were mixed from replicates of the same concentration of test item and control. Then they were centrifuged (3000 rpm, 10 min). Supernatant was collected and analysed by UHPLC-MS/MS using Column Zorbax SB-C18 RRHT 2.1×50 mm, 1.8 μm and isocratic elution with a mobile phase of (a) water + ammonium formate (0.1%): 55% (b) acetonitrile + formic acid (0.05%): 45%.

Results and discussions

Table A 5: Recovery results from method validation of metazachlor using the analytical method in AAP medium recommended by OECD Guideline No. 201

| Matrix | Analyte | Fortification level (μg/L) (*n* = 5) | Mean  recovery (%) | RSD (%) |
| --- | --- | --- | --- | --- |
| AAP medium | metazachlor | 0.5 | 81.88 | 6.190 |
| 5.0 | 92.86 | 1.298 |

Table A 6: Characteristics for the analytical method used for validation of metazachlor residues in AAP medium recommended by OECD Guideline No. 201

|  | Metazachlor |
| --- | --- |
| Specificity | The analysis showed that no signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions.  Two ion transitions were recorded:  Target: 278.1 → 134; Qualifier: 278.1 → 210  Specificity was verified using the ion transition ratio of 63.8% ± 30% (relative). Specificity of the method was confirmed. |
| Calibration (type, number of data points) | calibration line equation: Signal = 4101 C + 521 |
| Calibration range | Linearity range: 0.5 – 150 μg/L  Coefficient of determination R2: 0.9996 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | LOD: 0.15 μg/L  LOQ: 0.50 μg/L (Mean recovery: 81.88 %, % RSD: 6.19) |

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,   
accuracy and LOQaccording to guideline SANTE/2020/12830, Rev.1.

**A.2.1.1.4 Analytical method 4 (Earthworm Reproduction Test (*Eisenia andrei*)**

**A.2.1.1.1.4 Method validation**

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted. |

|  |  |
| --- | --- |
| Reference: | KCP 5.1.2/04 (Cross reference KCP 10.4.1.1) |
| Report | Earthworm Reproduction Test (*Eisenia andrei*),  Swoboda T., 2021, Report no: EMI/4/36/2019 |
| Guideline(s): | Yes (OECD 222 (2016)) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

A 4 mL of cold (< 4 ⁰C) ultrapure water and 8 mL of acetonitrile were added to 1.25 ± 0.5 g of a sample. The sample was shaken (5 min, >800 rpm), mixed with 2.6 g ± 0.1 g of salt mixture, shaken again (15 min, >800 rpm), and centrifuged (5 min, 6500 rcf). The supernatant was collected and stored in a refrigerator (<-18 ⁰C) during the night. 0.45 mL of the sample and 0.55 ml of ultrapure water were added to a vial and mixed. Samples containing the expected conc. of metazachlor greater than 2.6 mg/kg were diluted with acetonitrile before the addition of ultrapure water and then analysed with LC-MS/MS. LC-MS/MS equipped with column Zorbax SB-C18 RRHT 2.1X 50 mm, 1.8 µm with isocratic elution and with a mobile phase of (a) water + ammonium formate (0.1%): 55% (b) acetonitrile + formic acid (0.05%): 45%.

Results and discussions

Table A 7: Recovery results from method validation of metazachlor using the analytical method

| Matrix | Analyte | Fortification level (µg/L) (*n* = 5) | Mean  recovery (%) | RSD (%) |
| --- | --- | --- | --- | --- |
| Artificial soil substrate | metazachlor | 2.8 | 93.72 | 8.272 |
| 28 | 105.76 | 1.77 |
| 70 | 96.4 | 0.568 |

Table A 8: Characteristics for the analytical method used for validation of metazachlor residues in artificial soil substrate

|  | Metazachlor |
| --- | --- |
| Specificity | The analysis showed that signal of the detected substance was overlapping with the matrix signal of the control samples under the experimental conditions but mean matrix blank response (2123, n=2) is equal to 18% of mean LOQ response which is acceptable.  Two ion transitions were recorded:  Target: 278.1 → 134; Qualifier: 278.1 → 210  Specificity was verified using the ion transition ratio of 63.8% ± 30% (relative). Specificity of the method was confirmed |
| Calibration (type, number of data points) | calibration line equation: signal= 4094 C + 809 |
| Calibration range | Linearity range: 0.5 – 150 μg/L  Coefficient of determination R2: 0.9997 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | LOD: 0.015 mg/kg  LOQ: 0.05 mg/kg (mean recovery: 93.72%, %RSD: 8.27) |

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,   
accuracy and LOQ according to guideline SANTE/2020/12830, Rev.1.

**A.2.1.1.5 Analytical method 5 (Honeybees (*Apis mellifera* *L*.), Chronic Oral Toxicity Test)**

**A.2.1.1.1.5 Method validation**

|  |  |
| --- | --- |
| Comments of zRMS: | The method has been accepted. |

|  |  |
| --- | --- |
| Reference: | 5.1.2/05 (Cross reference: KCP 10.3.1.2) |
| Report | Honeybees (*Apis mellifera L*.), Chronic Oral Toxicity Test,  Parma P., 2021, Report No.: EMI/4/67/2020 |
| Guideline(s): | Yes (OECD 245, (2017)) |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | Yes |

Materials and methods

A 3.9 mL of ultrapure water and 0.1 mL of sample were added to 8 mL of acetonitrile. The sample was shaken (5 min, >800 rpm), mixed with 2.6 g ± 0.1 g of salt mixture, shaken again (15 min, >800 rpm), and centrifuged (5 min, 6500 rcf). The supernatant was collected and stored in a refrigerator (<-18 ⁰C) during the night. 0.45 mL of the sample and 0.55 ml of ultrapure water were added to a vial and mixed. Samples were diluted with acetonitrile before the addition of ultrapure water and then analysed with LC-MS/MS. LC-MS/MS equipped with column Zorbax SB-C18 RRHT 2.1X 50 mm, 1.8 µm with isocratic elution and with a mobile phase of (a) water + ammonium formate (0.1%): 55% (b) acetonitrile + formic acid (0.05%): 45%.

Results and discussions

Table A 9: Recovery results from method validation of metazachlor using the analytical method in culture medium - reconstituted water according to OECD 202

| Matrix | Analyte | Fortification level (μg/L) (*n* = 5) | Mean  recovery (%) | RSD (%) |
| --- | --- | --- | --- | --- |
| 50% sachharose solution | metazachlor | 3 | 88.52 | 7.62 |
| 30 | 87.52 | 10.93 |

Table A 10: Characteristics for the analytical method used for validation of metazachlor residues in 50% saccharose solution

|  | Metazachlor |
| --- | --- |
| Specificity | The signal of matrix blank sample did not exceed 30% of the LOQ.  Two ion transitions were recorded:  Target: 278.1 → 134; Qualifier: 278.1 → 210  Specificity was verified using the ion transition ratio of 63.8% ± 30% (relative). Specificity of the method was confirmed. |
| Calibration (type, number of data points) | calibration line equation: Signal = 8764.3 C + 433.8 |
| Calibration range | Linearity range: 0.9 – 100 μg/L  Coefficient of determination R2: 0.9995 |
| Assessment of matrix effects is presented | yes |
| Limit of determination/quantification | LOD: 200 mg/kg  LOQ: 20 mg/kg (Mean recovery: 88.52 %, % RSD: 7.625) |

Conclusion

The analytical procedure has been successfully validated in terms of specificity, linearity, precision,   
accuracy and LOQaccording to SANCO/3029/99 rev.4.

* + 1. Methods for post-authorization control and monitoring purposes (KCP 5.2)
       1. Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

* + - 1. Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Air (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted

* + - 1. Other Studies/ Information

No new or additional studies have been submitted.