



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE SUBGROUP MEETING ON ANALYTICAL METHODS OF THE EFSA WORKING GROUP ON COMPARATIVE *IN VITRO* METABOLISM STUDIES

**Held on 16 December 2020 (teleconference)**

**(Agreed on 4 January 2021)**

#### Participants

■ **Working Group Members:**

Olavi Pelkonen and Serge Rudaz

■ **Hearing Experts:**

Not applicable

■ **European Commission and/or Member States representatives:**

Not applicable

■ **EFSA:**

Pesticide Peer Review (PREV) Unit: Juan Parra Morte (Chair) and Mathilde Colas.

## 1. Welcome and apologies for absence

The Chair welcomed the WG members.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence<sup>1</sup> and the Decision of the Executive Director on Competing Interest Management<sup>2</sup>, EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process, and no interests were declared orally by the members at the beginning of this meeting.

## 4. Scientific topic(s) for discussion

The chapter on chemical analysis of the draft Scientific Opinion was discussed.

### **Analytical workflow for unique human metabolite**

The experts discussed the confidence in defining a unique human metabolite. The different steps of the workflow were agreed to enable the detection of unique human metabolites (e.g. incubation of the radiolabelled compound, the comparison of the relative peak area to the sum of radioactivity recovered, etc.).

### **Analytical workflow for disproportionate metabolites**

The experts also drafted the analytical workflow to cover disproportionate metabolites. Specific topics were highlighted, such as the use of biological replicates with the related statistical method and the recommended criteria for considering the ratio of 4 in the chemical analysis chapter.

### **Other points of discussion**

The use of radiolabelled and non-radiolabelled material to perform comparative in vitro metabolism studies was questioned. In addition, the concept of stereoisomers was introduced as well as the detection of potential reactive intermediate.

## 5. Any Other Business

Not applicable.

## 6. Next meeting(s)

Next WG meeting is planned on the 10<sup>th</sup> and 11<sup>th</sup> of February 2020 by teleconference.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/competing\\_interest\\_management\\_17.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf)



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 6<sup>TH</sup> MEETING OF THE EFSA WORKING GROUP ON COMPARATIVE *IN VITRO* METABOLISM STUDIES

**Held on 23 and 24 November 2020 (via teleconference)**

**(Agreed on 4 December 2020)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Emanuela Testai, Olavi Pelkonen and Serge Rudaz

■ **Hearing Experts:**

Gerrit Wolterink

■ **European Commission and/or Member States representatives:**

Sandra Coecke (Joint Research Center)

■ **EFSA:**

Pesticides Peer Review (PREV) Unit: Juan Parra Morte (Chair) and Mathilde Colas  
Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne

## 1. Welcome and apologies for absence

The Chair welcomed the WG members and the hearing expert, Gerrit who was invited to give his advice on how metabolites and degradation products of pesticides should be assessed regarding human health.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

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## 4. Scientific topic(s) for discussion

### **Status of the comparative *in vitro* metabolism project: adoption of a new charter and terms of reference**

The status of the project was presented by Juan. The amended charter considering extension of the deadline, the second problem formulation of the charter (i.e. toxicological relevance of human metabolites) as well as the stronger involvement of the PPR Panel in the project was presented. The next steps of the project were presented to the experts, including working group meetings and the public consultation.

### **Definition of the terms "unique human metabolite" and "disproportionate metabolite"**

The terms "unique human metabolite" and "disproportionate metabolite" were defined and agreed by the Working Group members.

### **Discussion on the second problem formulation**

The second problem formulation regarding the toxicological relevance of disproportionate and unique human metabolites was approached. In line with the toxicological data package available or recommended in other guidelines (e.g. EFSA guidance on the Residue definition), the relevance assessment of human metabolites was examined under different scenarios. The hazard and risk assessment of disproportionate and unique human metabolite were considered. Theoretical case studies and examples of calculations were presented by the experts.

### **Presentation of the main changes and remaining comments**

The main changes from the last meeting were presented to the experts. Comments provided by the Working Group members were addressed along the draft Scientific Opinion.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

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## **5. Any Other Business**

A sub-group (Olavi, Serge and EFSA) dedicated on the analytical part will be organised in December. Hearing experts from the industry and academia will be invited to address specific questions during the next working group meeting on the second problem formulation.

## **6. Next meeting(s)**

Next meeting is planned for December 2020 by teleconference.



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 5<sup>TH</sup> MEETING OF THE EFSA WORKING GROUP ON THE GUIDANCE ON THE *IN VITRO* COMPARATIVE METABOLISM STUDIES

**Held on 8 and 9 July 2020 (Teleconference)**

**(Agreed on 22 July 2020)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Emanuela Testai, Olavi Pelkonen and Serge Rudaz.

■ **Hearing Experts:**

Not applicable.

■ **European Commission and/or Member States representatives:**

Sandra Coecke (Joint Research Center).

■ **EFSA:**

Pesticide Peer Review (PREV) Unit: Alfonso Lostia (Chair), Juan Parra Morte (Co-Chair) and Mathilde Colas.

Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne

## 1. Welcome and apologies for absence

The Chair welcomed the participants.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

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## 4. Scientific topic(s) for discussion

### Discussion on experimental layout

The interpretation part on disproportionate metabolites was discussed with the support of an example of calculation. Case studies were presented by one expert to contribute to the definition of criteria to be recommended for the study design (i.e. concentrations, time points).

### Discussion on the remaining comments of the draft guidance

Comments provided by the experts were addressed along the draft guidance on the study design and experimental strategy, *in vitro* system, test item and reference compounds, *in vitro* method, chemical analysis, and interpretation of the results.

### Hearing experts

The experts agreed to invite hearing experts for the next meeting to discuss the study design for long-term incubation using plated hepatocytes (e.g. pooling, positive controls, sampling points, etc).

## 5. Any Other Business

### Use of teams

The experts were trained on the use of Teams. From now on the experts will work on Teams.

## 6. Next meeting(s)

Next meeting is planned on the 12th and 13th of October 2020 by teleconference.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/competing\\_interest\\_management\\_17.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf)



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 4<sup>TH</sup> MEETING OF THE EFSA WORKING GROUP ON THE GUIDANCE ON THE *IN VITRO* COMPARATIVE METABOLISM STUDIES

**Held on 12 June 2020 (Teleconference)**

**(Agreed on 2 July 2020)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Emanuela Testai, Olavi Pelkonen and Serge Rudaz.

■ **Hearing Experts:**

Not applicable

■ **European Commission and/or Member States representatives:**

Sandra Coecke (Joint Research Center).

■ **EFSA:**

Pesticides Peer Review (PREV) Unit: Alfonso Lostia (Co-Chair), Juan Parra Morte (Chair) and Mathilde Colas.

Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne



## 1. Welcome and apologies for absence

The Chair welcomed the participants.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence<sup>1</sup> and the Decision of the Executive Director on Competing Interest Management<sup>2</sup>, EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process, and no interests were declared orally by the members at the beginning of this meeting.

## 4. Scientific topic(s) for discussion

### **Discussion on remaining comments of the draft guidance**

Some of the remaining comments on the draft guidance were addressed directly by all the WG members. The general editorials changes were accepted and the relevant references were included in the draft guidance. The discussion focused in particular on the interpretation part, the study design and the strategy recommended. Therefore, a more advanced version of the guidance has been designed.

## 5. Any Other Business

Not applicable

## 6. Next meeting(s)

Next meeting is planned on July 2020 (8-9<sup>th</sup>) by teleconference.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/competing\\_interest\\_management\\_17.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf)



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 3<sup>RD</sup> MEETING OF THE EFSA WORKING GROUP ON THE GUIDANCE ON THE *IN VITRO* COMPARATIVE METABOLISM STUDIES

**Held on 04-05 May 2020 (Teleconference)**

**(Agreed on 20 May 2020)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Emanuela Testai, Olavi Pelkonen and Serge Rudaz.

■ **Hearing Experts:**

Ugo Zanelli (Monday 04<sup>th</sup>, 14.00-16.00) and Paul Whalley (Tuesday 05<sup>th</sup>, 9.00-11.00).

■ **European Commission and/or Member States representatives:**

Sandra Coecke (Joint Research Center).

■ **EFSA:**

Pesticide Peer Review (PREV) Unit: Alfonso Lostia (Co-Chair), Juan Parra Morte (Chair) and Mathilde Colas.

Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne

## 1. Welcome and apologies for absence

This meeting, originally scheduled as a physical meeting, was converted into a teleconference to avoid traveling to EFSA in line with the measures established to reduce the risk of coronavirus infection.

The Chair welcomed the participants.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

In accordance with EFSA's Policy on Independence<sup>1</sup> and the Decision of the Executive Director on Competing Interest Management<sup>2</sup>, EFSA screened the Annual Declarations of Interest filled out by the Working Group members invited to the present meeting. No Conflicts of Interest related to the issues discussed in this meeting have been identified during the screening process, and no interests were declared orally by the members at the beginning of this meeting.

## 4. Scientific topic(s) for discussion

### **Discussions with hearing experts.**

The hearing experts Ugo Zanelli and Paul Whalley were asked to present their views on several issues related to *in vitro* metabolism assessment. Ugo Zanelli presented his view on the study design (positive control, concentration, time points, species), long-term incubation, preliminary clearance experiment, the use of radiolabel and non-radiolabel material and structural determination. Paul Whalley presented his views on rationale for testing one concentration and one time point, the definition of unique and disproportionate metabolite and their follow-up.

The WG members thanked the hearing experts and discussed some points on study design, methodologies for specific cases (slow metabolism, disproportionate metabolites), clearance measurements and analytical methods.

### **Discussions on experimental strategy, the interpretation part and review of main comments on the draft guidance**

During the meeting, the interpretation part was addressed in priority. Jochem Louisse prepared and presented his synthesis on the specific case of disproportionate metabolites and discussions were undertaken with the WG members.

Additionally, discussions were focused on the study design such as the number of time-points and concentrations required, and recommendations for species in short-term incubation. The section on analytical methods described by Serge Rudaz was also reviewed by all experts.

Main comments on the draft guidance were reviewed and agreed by all the WG members. Tasks were allocated for the next WG meeting to the experts.

## 5. Any Other Business

The potential extension of the WG's project was introduced to the experts and will be further defined by EFSA during the next following meeting.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/competing\\_interest\\_management\\_17.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf)

## **6. Next meeting(s)**

Next meetings are planned on July 2020 (8-10<sup>th</sup>) by teleconference. An additional meeting in mid-June may take place.



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 2<sup>ND</sup> MEETING OF THE EFSA WORKING GROUP ON THE GUIDANCE ON THE *IN VITRO* COMPARATIVE METABOLISM STUDIES

**Held on 18-20 February 2020, Parma (Italy)**

**(Agreed on 11 March 2020)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Olavi Pelkonen, Serge Rudaz.

■ **Hearing Experts:**

Not applicable.

■ **European Commission and/or Member States representatives:**

Sandra Coecke (Joint Research Center).

■ **EFSA:**

Pesticides Peer Review (PREV) Unit: Alfonso Lostia (Co-Chair), Juan Parra Morte (Chair) and Mathilde Colas.

Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne (attending 20<sup>th</sup> February).

## 1. Welcome and apologies for absence

The Chair welcomed the participants. Apologies were received from Ms Emanuela Testai.

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

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## 4. Scientific topic(s) for discussion

### **Interpretation chapter with support of theoretical case-studies**

During the 2<sup>nd</sup> meeting of the EFSA WG, discussions were mainly focused on the interpretation chapter. Case-studies were presented by the WG members to support the discussion. The main topics addressed were the strategy for long-term exposure, the definition of slow-metabolised chemicals and unique human metabolite and metabolic competence. Additionally, recommendations about the criteria for selecting the most appropriate test system (s), test item and reference compounds were set by the EFSA WG. The case of disproportionate metabolites was also addressed. Based on the discussion, a new structure of the guidance was proposed.

### **Analytical methods chapter**

Most relevant methods of analysis to be recommended for *in vitro* comparative metabolism studies were commented by the WG members. The main points of discussion were about identification methods, the case of isomers, tentative analysis to qualify *in vitro* rat compared to *in vivo* rat and radioactive techniques.

## 5. Any Other Business

### **Hearing experts**

The experts agreed to invite hearing experts for the next WG meeting to support the discussion on the experimental layout and concentrations. Specific questions were prepared by the WG.

## 6. Next meeting(s)

Next meeting is planned on May 2020.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

<sup>2</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/competing\\_interest\\_management\\_17.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/competing_interest_management_17.pdf)



## PESTICIDE PEER REVIEW UNIT

### MINUTES OF THE 1<sup>ST</sup> MEETING OF THE EFSA WORKING GROUP ON THE GUIDANCE ON THE *IN VITRO* COMPARATIVE METABOLISM STUDIES

**Held on 20-22 November 2019, Parma (Italy)**

**(Agreed on 11 December 2019)**

#### Participants

■ **Working Group Members:**

Ursula Gundert-Remy, Jochem Louisse, Olavi Pelkonen, Serge Rudaz and Emanuela Testai.

■ **Hearing Experts:**

Not applicable.

■ **European Commission and/or Member States representatives:**

Not Applicable.

■ **EFSA:**

Pesticide Peer Review (PREV) Unit: Alfonso Lostia (Chair) and Mathilde Colas.

Scientific Committee and Emerging Risk (SCER) Unit: Jean Lou Dorne.

## 1. Welcome and apologies for absence

The Chair welcomed the participants. Apologies were received from Ms Sandra Coecke (European Commission, DG Joint Research Center) and Mr Juan Manuel Parra Morte (EFSA, PREV Unit).

## 2. Adoption of agenda

The agenda was adopted without changes.

## 3. Declarations of Interest of Working Groups members

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## 4. Scientific topic(s) for discussion

### Introduction and presentation of the project

EFSA introduced the Peer review process and presented the three Work Packages (WP) of the *in vitro* comparative metabolism project: Workshop (WP1), Guidance (WP2) and Public Consultation (WP3). The chair presented the outcome of the Workshop on *in vitro* metabolism held in November 2018 in Parma (EFSA, 2018). The planning for Guidance finalisation and Public Consultation was shared with the experts (i.e. First Quarter 2020).

### Case-studies

The WG members presented and discussed examples from the peer review process as case-studies. Recommendations about the biological test systems, test item, reference control and the study design were set by the WG.

### Structure and chapters of the EFSA draft guidance

The structure of the guidance (table of contents) was determined by the WG and each chapter was allocated to WG members for the draft.

## 5. Any Other Business

### Promotion of the project

The project will be presented in the future externally during conferences or through publications in a scientific journal.

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<sup>1</sup> [http://www.efsa.europa.eu/sites/default/files/corporate\\_publications/files/policy\\_independence.pdf](http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/policy_independence.pdf)

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## **Endorsement of the EFSA Guidance by the PPR Panel**

The WG agreed with the endorsement by the PPR Panel. Olavi kindly agreed to regularly inform the PPR Panel on the guidance development.

## **6. Next meeting(s)**

Next meeting is planned on February 2020.

Next discussions will focus on the “interpretation” chapter.