Metiram Negligible Exposure Dossier – Non-target Organisms

Authors:

[redacted]

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Written on behalf of:

BASF SE
Agricultural Solutions
Global Product Safety and Registration (APD)
67117 Limburgerhof, Germany

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1 Background

EFSA has concluded that the scientific criteria for the determination of endocrine disrupting (ED) properties are met for metiram. As such, information is requested to demonstrate that metiram may be used such that exposure is negligible, and/or documentary evidence for the application of the derogation under Art.4(7)2 of Regulation (EC) No 1107/2009 (EFSA Request, 22nd February 2019).

Commission Regulation (EC) No. 1107/2009 (Annex II, 3.8.2) states:

"An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible."

The following assessment addresses two key points, I) the application of the ED criteria tor metiram and II) demonstration of negligible exposure.

The GAP considered is for use on potato only, at up to three applications of 1.26 kg a.s./ha, at BBCH 21 to 89.

2 Metiram ED assessment

An extensive ED assessment has been conducted by BASF SE (BASF DocID 2018/1181256; October 2018), in line with the ECHA/EFSA Guidance (2018). This was submitted as part of the EU review in November 2018.

BASF SE's assessment demonstrates that, from the available data with **mammals** there is no indication of estrogenic, androgenic or steroidogenic (EAS) activity of metiram, which is consistent with the absence of possibly mediated adverse effects by these modalities on any reproductive organ or sexual development. While there is some evidence for thyroid (T) toxicity after exposure to metiram in different mammalian species the adverse effects related to the thyroid organ are not considered population relevant for wild mammals since none of the potentially endocrine sensitive parameters related to fertility, reproduction and development of offspring were adversely affected. Impact on endpoints potentially sensitive to endocrine modalities as detected in developmental toxicity studies are secondarily related to maternal toxicity and thus cannot be linked to any endocrine activity.

Further evidence for the absence population relevant effects of metiram is obtained from the field effect study conducted in Central Europe with a small mammalian representative species, the common vole. Under environmental relevant exposure conditions there were no impact on population dynamics and reproduction over several reproductive cycles.

From the minutes of the expert meeting on ED (EFSA Request, 22nd February 2019, Annex B), the experts agreed that effects observed in mammals cannot be considered population relevant. Therefore, it was concluded by the experts that **metiram does not fulfil the ED criteria in mammals related to non-target organisms.**

BASF SE's assessment demonstrates that, for **birds** the available reproduction studies with metiram provide evidence for adverse population-relevant effects, which are consistently found in studies with quails and mallard ducks. The effect pattern shows impacts on reproductive parameters, which in accordance with the ED guidance (ECHA/EFSA, 2018), are not diagnostic for an endocrine mode of action and thus cannot be linked to a specific endocrine mechanism. Endpoints, which might be influenced by the thyroid, like growth and body weight of chicks, remained unaffected in the studies with metiram. Indeed, from gross-pathological investigations in the avian studies with metiram no changes in endocrine organs were evident.

Thus, from the avian studies with metiram there is no evidence for endocrine activity or endocrine mediated adverse effects. From the minutes of the expert meeting on ED (EFSA Request, 22nd February 2019, Annex B), the experts concluded that the available studies on birds did not allow to draw a firm conclusion on ED.

BASF SE considers that the **fish** studies show little evidence that metiram fulfils the criteria for endocrine disruption. However, the EATS parameters are not sufficiently investigated according the EFSA guidance on ED assessments (ECHA/EFSA, 2018) and relevant data are missing to judge on mechanisms and adversity, which are key in the definition of an ED. Therefore, further aquatic studies with metiram on fish and amphibians have been proposed by the applicant. A staggered testing strategy is planned and can be performed upon request by EFSA.

To minimise animal testing and in the light of the known information from the available data, the proposed staggered testing strategy is as follows:

- To get detailed information about EAS parameters a fish sexual development test (FSDT; OECD TG 234) is proposed. In addition to cover the T parameter, inclusion of thyroid endpoints in this test is proposed. This would make the testing more efficient and reduces vertebrate animal testing because parallel amphibian testing could be avoided if reliable thyroid endpoints are included in the fish test.
- As all parameters should have been investigated with the fish study, no further animal test is considered necessary. However, to cover effects on amphibians, a *Xenopus* embryonic thyroid signalling assay (XETA) is proposed. This screening assay is not a vertebrate test and would complement the fish test.
- If there is a positive signal in the XETA and also indications from the fish study with regard to thyroid effects, a full amphibian metamorphosis assay (AMA) will be conducted as a follow up.

It should be noted that, no information from the peer-review meeting is given on the experts conclusion on the ED assessment of the available fish studies or the proposed testing strategy covering amphibian studies. Although, there is a comment from Germany that they agree the data are not sufficient to evaluate potential ED properties of metiram to fish and that further studies would be needed.

3 Comments on the ED Assessment of metiram related to non-target organism

During the peer review meeting further studies related to other non-target organism were considered and evaluated as follows.

Four different studies were performed for amphibians, but only with the metabolite ETU (common metabolite of metiram and mancozeb). […]

In all the studies with amphibians, changes in thyroid histopathology, when investigated, were observed. Those changes were considered consistent with the adverse effects observed in mammals. Since in amphibians the effects on thyroid histopathology were accompanied with delay in development, some experts considered the observed effects adverse and relevant at a population level. However, it was clarified that in line with the guidance and OECD GD 150 effects level 3 studies are not sufficiently robust for the definition of adversity. Furthermore, the available studies were performed with the metabolite and not with the active substance and it was clarified that the ED criteria do not apply to metabolites formed in the environment.
Since ETU is formed in the animal metabolism (rat and hen), the RMS proposed that the same can be expected in amphibians and, therefore, similar adverse effects than the ones observed in the studies done with ETU can be expected after exposure to the a.s.

The suitability of this extrapolation was questioned since there were some concerns in assuming that the metabolism is comparable in birds/mammals and amphibians. It was questioned whether the ETU level potentially formed during metabolism in amphibians would be sufficient to trigger similar adverse effects than the ones observed in the available studies.

Indeed four studies on amphibian metamorphosis (AMA) are available, but only with the metabolite ETU (common metabolite of metiram and mancozeb). These studies were not included in BASF's ED assessment in line with 1107/2009 and the ECHA/EFSA Guidance, as this addressed the ED properties of the active substance only (2018; BASF DocID 2018/1181256). The studies are presented and discussed in the RAR for metiram (November 2018) and they were also discussed at the expert meeting on ED (EFSA Request, 22nd February 2019, Annex B). From the evaluation of the applicant there is some uncertainty over the reliability of the literature studies on amphibians conducted with ETU (see also Appendix for more details). As noted in the experts meeting there is also uncertainty over the interpretation of the results, and they are not sufficiently robust for the definition of adversity.

Further, the relevance of these studies on the metabolite ETU to the assessment of metiram in relation to ED is also questionable.

As agreed in the expert meeting, and consistent with the criteria given in Commission Regulation (EC) No. 1107/2009, the ED criteria do not apply to metabolites formed in the environment. Since ETU is formed in the animal metabolism (rat and hen), the minutes of the expert meeting state that, the RMS proposed that the same can be expected in amphibians and, therefore, similar adverse effects than the ones observed in the studies done with ETU can be expected after exposure to the active substance. The suitability of this extrapolation was questioned in the expert meeting since there were some concerns in assuming that the metabolism is comparable in birds/mammals and amphibians. It was also questioned whether the ETU level potentially formed during metabolism in amphibians would be sufficient to trigger similar adverse effects than the ones observed in the available studies. The testing strategy proposed by BASF would address these questions.

From the peer-review meeting following points were discussed further:

Although it was acknowledged in the meeting that the available evidence would suggest that it is likely that metiram is an ED for non-target organisms, in order to address the uncertainties discussed above, EFSA and some experts considered that in line with the guidance further data (i.e. LAGDA) with the active substance would be needed for drawing a firm conclusion on whether the ED criteria are met for non-target organism other than mammals.

Overall, a slight majority of experts considered that on the basis of the available data and considering the studies on ETU for amphibians, metiram meets the ED criteria for non-target organisms other than mammals through the T modality.

It is noted that this conclusion on the ED properties for metiram is based only on a slight majority and the amphibian studies with ETU seem to be the only data used to conclude that the ED criteria are met for metiram related to non-target organism.

Overall, a clear conclusion on a potential ED effect of metiram on amphibians is not possible based on the available data. However, more importantly, it is not considered applicable to conclude on ED properties of metiram for non-target organisms by extrapolating data from ETU on metiram.

Thus, BASF disagrees with EFSA's conclusion that metiram meets the ED criteria, since this is not within the legal framework of Regulation (EC) No 1107/2009 and not in line with the ECHA/EFSA Guidance for the identification of endocrine disruptors (2018).

4 Negligible exposure

Despite the position outlined above regarding the correct application of the ED criteria for non-target organisms to metiram a justification for negligible exposure is presented below.

Within the draft guidance document on negligible exposure (European Commission 2015) no specific guidance has been developed for negligible environmental exposure. There is no technical definition of "negligible exposure" in the environment, for instance the populations to be considered, the potential routes of exposure and the risk thresholds for decision making.

In the absence of guidance, "negligible exposure" has been taken as exposure levels well below levels at which a substance might have endocrine disrupting properties that may cause adverse effects on non-target organisms. In this case, exposure levels of the metabolite ETU are compared to the level at which ETU may potentially have a thyroid effect in amphibians (as discussed above).

The RAR (November 2018) currently lists the lowest NOEC for effects on amphibians to be 1000 µg/L, based on thyroid effects (BASF ID: 2006/1051113), although it should be noted that this is taken as a worst-case value from a published paper of questionable reliability. The more reliable NOEC from the available GLP study was determined to be 10,000 µg/L based on development (BASF ID: 2002/1003402). However, taking the overall lowest NOEC, with a standard assessment factor (AF) of 10, gives a **precautionary regulatory acceptable concentration (RAC) for ETU of 100 µg/L for thyroid effects in amphibian**.

ETU ED-RAC compared to PEC_{sw} values:

Considering the representative use on potatoes, as assessed for active substance renewal, the overall maximum predicted environmental concentration in surface water (PEC_{SW}) of ETU was determined to be 5.40 µg/L, based on FOCUS Step 2 modelling (FOCUS Step 3 modelling was not conducted), for 1 - 3 x 1.26 kg a.s./ha (RAR, November 2018, Volume 3, Section B.8. CP, B.8.8.5.1). As the maximum PEC_{SW} is approximately 19 times lower than the precautionary RAC for potential thyroid effects it is clearly demonstrated that exposure is much lower than the levels which may potentially have a thyroid effect in amphibians.

Furthermore, even if a worst-case assumption of 100% conversion of the active substance to the metabolite ETU was to be used, the overall maximum PEC_{SW} for the parent substance at FOCUS Step 3 is 6.61 µg/L. This PEC_{SW} is reduced to 0.985 µg/L when risk mitigation measures in the form of a 20 m no spray buffer zone and vegetative filter strip are applied, and which are required to demonstrate an acceptable risk from the active substance. Thus, even **based on this worst-case assumption of 100% conversion of the active substance, the precautionary ED RAC for ETU is over 100 times greater than the overall maximum PEC_{SW} with the required risk mitigation measures for the active substance.** A summary of these PEC_{SW} values and RACs are presented in the following table for clarity.

Table 1: ETU ED-RAC compared to PEC_{SW} values for metabolite and parent

Endpoint	Value	AF	RAC	PEC_{SW} [b]	PEC/RAC ratio
Amphibian ED NOEC	1000 µg/L [a]	10	100 µg/L	ETU: 5.40 µg/L (FOCUS Step 2)	0.05
				Metiram: 6.61 µg/L (FOCUS Step 3)	0.07
				Metiram: 0.985 µg/L (FOCUS Step 4 [c])	0.01

AF: assessment factor; RAC: regulatory acceptable concentration; PEC_{SW}: predicted environmental concentration in surface water

[a] Overall lowest endpoint based on published paper of questionable reliability (BASF ID: 2006/1051113)

[b] Overall worst-case PEC_{SW} values considering the representative GAP for potatoes for active substance renewal (1 – 3 x 1.26 kg a.s./ha)

[c] Inclusion of 20 m no spray buffer zone and vegetative filter strip

ETU ED-RAC compared to acute effects from the parent:

Although acute toxicity data are not available for amphibians it is possible to extrapolate based on data for fish. As discussed in the RAR (November 2018, Volume 3, Section B.8. CP, B.9.1.3), with regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic phases of amphibians (Fryday and Thompson, 2012; Weltje *et al*., 2013). Based on these extensive data reviews it can be concluded that the acute and chronic risk to amphibians is covered by the risk assessment for aquatic organisms.

The acute endpoints (LC_{50}) for fish for metiram and ETU are 336 µg/L and > 500,000 µg/L, respectively. As discussed, these endpoints cover effects on amphibians. Thus, it can be concluded that, ETU is essentially non-toxic to aquatic vertebrates. The RAC for acute effects of metiram on aquatic vertebrates is determined to be 3.36 µg/L (standard AF of 100 applied). In comparison to this, the lowest NOEC for potential ED effects of ETU in amphibians is 1000 µg/L, giving a precautionary RAC of 100 µg/L (standard AF of 10 applied). Therefore, mortality of aquatic vertebrates from exposure to metiram will occur at concentrations well below those that might cause any developmental effects from exposure to ETU.

Based on the precautionary RAC, for there to be a potential ED effect from exposure to ETU, a concentration of over 100 µg ETU/L would be required, which equates to an initial concentration of metiram of 412 µg/L (maximum 24.3% formation[i]). Thus, the concentration of metiram required to trigger a potential ED effect from exposure to ETU is 123 x greater than the concentration of the parent which would cause acute mortality.

Simplistically, based on the maximum FOCUS Step 3 PEC_{SW} for use of metiram on potatoes at 1 – 3 x 1.26 kg a.s./ha of 6.61 µg a.s./L, to achieve a concentration of ETU to potentially induce a thyroid effect an application rate of 1 – 3 x 79 kg a.s./ha would be required.

***Conclusion**:*

Based on a number of worst-case assumptions, the use of metiram still results in negligible exposure levels, which can be assumed greater than a factor of 100 protective of onset of adverse effects potentially observed in the AMA studies with ETU. Furthermore, the available aquatic toxicity data demonstrate that lethal effects from exposure to metiram would occur before there is the potential for an ED effect from exposure to ETU.

5 Overall conclusion

Based on the available data, the scientific criteria for the determination of ED properties are not met for metiram. However, further studies with metiram are required to address the uncertainties raised. Effects from exposure to ETU should not be taken as the sole information when assessing if metiram meets the ED criteria. Furthermore, there are also uncertainties with the available AMA studies conducted with ETU.

None-the-less, when taking into account the toxicity data, the use of metiram results in exposure levels at least a factor of 100 protective of onset of potential ED effects from ETU, based on worst case assumptions. Furthermore, the available aquatic toxicity data demonstrate that lethal effects from exposure to metiram would occur well before there is the potential for an ED effect from exposure to ETU. Thus, the proposed use of metiram on potatoes will lead to negligible exposure in the context of assessment of potential ED effects.

[i] Maximum observed occurrence of ETU in water/sediment of 64.7 %, corrected for the differences in molar masses (ETU = 408.6 g/mol; metiram = 1088.7 g/mol)

6 References

BASF SE (2018). Assessment of potential endocrine disruption in non-target organisms for the active substance metiram (BAS 222 F). BASF DocID 2018/1181256.

European Commission (2009). Regulation (EC) No 1107/2009 of the European parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

European Commission (2015). *Draft* technical guidance on the interpretation of points 3.6.3. to 3.6.5, and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, in particular regarding the assessment of negligible exposure to an active substance in a plant protection product under realistic conditions of use.

European Commission (2018). Regulation (EC) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties.

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Fryday S and Thompson, H (2012). Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural. Food and Environment research agency, UK.

OECD (2018). Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption.

Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013). Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. Environmental Toxicology and Chemistry, Vol. 32, No. 5, pp. 984-994.

7 Appendix: Assessment of ETU studies on amphibians

Four amphibian metamorphosis assay (AMA) studies are available with the metabolite ETU (common metabolite of metiram and mancozeb). These studies were not included in BASF's ED assessment in line with the ECHA/EFSA Guidance (2018; BASF DocID 2018/1181256), as this addressed the ED properties of the active substance only. An assessment of these studies is presented in the RAR for metiram (November 2018) and they were also discussed at the Expert Meeting on ED (EFSA Request, 22nd February 2019, Annex B).

One of these studies is a GLP/guideline study, the others are published papers. The RMS for metiram did not consider all these studies reliable. However, the experts agreed that the identified deficiencies were only minor and that these studies could be considered valid (EFSA Request, 22nd February 2019, Annex B). Subsequently, endpoints from all four studies have been listed in the List of Endpoints for metiram (November 2018).

The List of Endpoints for mancozeb (September 2017) only lists the endpoint from the GLP/guideline study (Zok, 2002; NOEC of 10 mg a.s./L) and the RAR Volume 3, B.9. states for all three of these published papers that *"The effects described for ETU are covered by the existing GLP study, 2002. Therefore, this paper provides additional data, but does not need to be considered in the risk assessment."*

Table 2: Available amphibian metamorphosis assay endpoints for the metabolite ETU

Test species	Test type	Endpoint	Value (mg/L)	Reference	Comments:
Xenopus laevis	Chronic 28 d (semi-static, nominal)	NOEC (develop-ment)	10	[redacted] (2002) 2002/1003402	GLP/guideline study
Xenopus laevis	Chronic 28 d (semi-static, nominal)	NOEC (develop-ment)	5	Opitz, R. *et al.* (2004) 2005/1043780	Relevant published paper of questionable reliability. [a] Endpoint amended from 10 mg/L to 5 mg/L during the peer review.
Xenopus laevis	Chronic 12 d (flow-through, nominal)	NOEC (thyroid alterations)	< 50	Opitz, R. *et al.* (2008) 2008/1102096	Relevant published paper of questionable reliability [a]
Xenopus laevis	Chronic 90 d (semi-static, nominal)	NOEC (thyroid alterations)	1	Opitz, R. *et al.* (2006) 2006/1051113	Relevant published paper of questionable reliability [a]

[a] Multiple factors affecting reliability, including non-GLP, unclear source and purity of test material, details of study method (e.g. no of replicates) not clearly reported and no chemical analysis or not clearly reported.