VSP rapportnummer 15065A01

Mesotrione

Afleiding van de JG-MKN en MAC-MKN voor oppervlaktewater

versie 23-08-2022

Opdrachtgegevens

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Opdracht	Dit adviesrapport betreft de afleiding van de waterkwaliteitsnormen JG-MKN en MAC-MKN (AA-EQS en MAC-EQS) voor mesotrione.
Versie	Aangepast advies voor Ctgb Dit is een herziening van advies 15065A00 van 7 oktober 2021 in reactie op een bezwaar van de aanvrager. Het commentaar van het Petit Comité is verwerkt in deze versie

Kwaliteitsprocedures en beoordelingskader

De afleiding van de waterkwaliteitsnormen in dit rapport is opgesteld in overeenstemming met de vigerende VSP kwaliteitsprocedures. De afleiding is beoordeeld door het “Petit Comité”, dat wordt gevormd door een aantal (agenda-)leden van de Wetenschappelijke Klankbordgroep normstelling water en lucht. De commentaren en reacties daarop zijn toegevoegd in Bijlage 5. Na aanbieding van advies 15065A00 aan het Ctgb heeft de aanvrager bezwaar gemaakt, waarna het herziene advies nogmaals is aangeboden aan het Petit Comité.

Contents

1 Introduction

1.1 General

Mesotrione is a herbicide that is authorised for use in maize. The current water quality standard is a Maximum Permissible Concentration (Maximaal Toelaatbaar Risiconiveau) of 0.077 µg/L. This value was originally derived in the context of the Pesticide Atlas and officially endorsed in 2014 (http://www.rivm.nl/rvs/). Syngenta, one of the registration holders of mesotrione in the Netherlands, requested an update of the water quality standards and submitted a statement and underlying data. The Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb) commissioned RIVM to evaluate the submitted dossier, check for additional data in the open literature and derive environmental quality standards (EQSs) according to the methodology of the Water Framework Directive (WFD). The first version of this advice was issued to Ctgb in October 2021. Ctgb received a rebuttal of the registration holder Syngenta concerning the recalculation of the lowest relevant chronic endpoint for macrophytes. This revised report includes RIVM's position on this rebuttal.

1.2 Standards considered

Under the WFD, two types of EQSs are derived to cover both long- and short-term effects resulting from exposure (EC, 2018):

- an Annual Average EQS (AA-EQS) – a long-term standard, expressed as an annual average concentration (AA-EQS) which should protect the ecosystem against adverse effects resulting from long-term exposure, and
- a Maximum Acceptable Concentration EQS (MAC-EQS) for aquatic ecosystems – the concentration protecting aquatic ecosystems from effects due to short-term exposure or concentration peaks.

The AA-EQS should not result in risks due to direct toxicity, secondary poisoning and/or risks for human health aspects. The latter two aspects are therefore also addressed in the AA-EQS, when triggered by the characteristics of the compound (i.e. human toxicology and/or potential to bioaccumulate). The MAC-EQS is based on direct ecotoxicity only. In the context of pesticide authorisation, only freshwater EQSs are used. However, since the values may be used for other purposes as well, standards for the saltwater environment are also derived in this report.

For authorisation of plant protection products, transient effects may be considered acceptable under certain conditions if the potential for recovery is demonstrated (EFSA, 2013). However, the quality standards in the context of the WFD refer to the absence of any impact on community structure of aquatic ecosystems. Hence, long-term undisturbed function is the protection objective under the WFD. Therefore, recovery in a test situation, after a limited exposure time, is not included in the derivation of the AA- and MAC-EQS (EC, 2018).

1.3 Methodology

1.3.1 Guidance documents

The methodology is in accordance with the European Technical Guidance for deriving Environmental Quality Standards under the Water Framework Directive (EC, 2018). This document is further referred to as the WFD-guidance. For those aspects that may not be fully covered by the WFD-guidance, additional information can be found in national guidance documents (Brock et al., 2011; RIVM, 2015; Smit et al., 2013).

1.3.2 Data sources

The applicant submitted a statement with an EQS-proposal for EQS ([redacted] 2020). This EQS-derivation was primarily based on data from the Renewal Assessment Report (RAR) that was prepared for mesotrione within the context of the European pesticides Regulation 1107/2009 and associated EFSA conclusion (EC, 2015; EFSA, 2016). The applicant also performed a literature search which resulted in a few relevant papers (Ni et al., 2014a; Ni et al., 2014b; Zhao et al., 2018). RIVM performed an additional search in SCOPUS (http://www.scopus.com/) using the search string 'mesotrione and aquatic' and the US EPA Ecotox Knowledgebase (US EPA, 2021) to check for any additional papers. This resulted in several additional potentially relevant studies.

1.3.3 Data evaluation and selection

In general, studies that were accepted in the RAR were not re-evaluated, but checked for adequate reporting of relevant endpoints. Where necessary, however, additional calculations were made, e.g. when statistical re-evaluation of the applicant only considered the EC_{10} and EC_{20}, but not the EC_{50}. The newly retrieved data, including the open literature data summarised by the applicant, were evaluated with respect to the validity (scientific reliability) of the study. Reliability indices (Ri) of 1 to 4 were assigned according to Klimisch et al. (1997), with Ri 1: fully reliable, Ri2: reliable with restrictions, Ri 3: not reliable and Ri 4: not assignable. A detailed description of the evaluation procedure is given in WFD-guidance (EC, 2018). Details concerning the validity assessment are listed for each study in the footnotes in Annex 1.

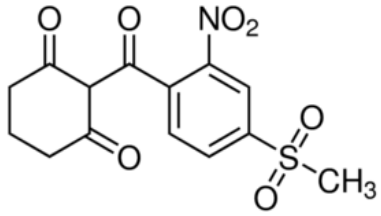
The lowest relevant endpoint per species is selected for EQS-derivation. In line with the WFD-guidance, preference is given to studies with the active substance over studies with formulated products. However, if for a species the only reliable endpoints are from a study with a formulation, this information is used.

According to the RAR, mesotrione is not susceptible to direct photolysis, but in natural water photodegradation can occur as a result of indirect photolysis (EC, 2015). Although the dissolved organic matter concentration in standard ecotoxicity tests is generally low, decline of test concentrations due to indirect photolysis cannot be ruled out. Therefore, studies without analytical measurements of mesotrione in test solutions were assigned Ri3. An exception was made for tests with bacteria, for which analytical measurements were not considered critical in view of the short test duration (max. 9 hours). For algae and macrophytes, no separate tests are available for acute and chronic exposure. Therefore, the EC_{50} is included in the acute dataset and the NOEC or EC_{10} in the chronic dataset. In line with the WFD-guidance, growth rate is selected as the most relevant endpoint.

2 Information on the substance

2.1 Identity

Table 1 Substance identification

Name	mesotrione
Chemical name (IUPAC)	2-(4-mesyl-2-nitrobenzoyl) cyclohexane -1,3-dione
CAS number	104206-82-8
EC number	609-064-00
Molecular formula	$C_{14}H_{13}NO_7S$
Molar mass	339.3
Structural formula	
SMILES code	CS(=O)(=O)C1=CC(=C(C=C1)C(=O)C2C(=O)CCCC2=O)[N+](=O)[O-]
Use class	systemic herbicide; controls most annual broadleaf and annual grass weed species
Mode of action	blocks the function of the essential plant enzyme 4-hydroxy-phenyl-pyruvatedioxygenase (4-HPPD) in the cytosol

2.2 Physico-chemical properties

Table 2 Physico-chemical properties. All data from EFSA (2016).

Parameter	Unit	Value	Remark
Water solubility	[mg/L]	160	unbuffered water, 20 °C
		1500	pH 6.9, 20 °C
		2200	pH 4.8 and 9.0, 20 °C
pK_a		3.12	20 °C
log K_{ow}		0.11	unbuffered water
		-1.1	pH 5
		< -1.0	pH 7, 9
Vapour pressure	[Pa]	$<5.7 \times 10^{-6}$	20 °C
Henry's law constant	[Pa.m^3/mol]	$<5.1 \times 10^{-7}$	20°C
Melting point	[°C]	165.3	with decomposition
Boiling point	[°C]		decomposes

2.3 Fate and behaviour

2.3.1 Behaviour in the environment

Selected environmental properties of mesotrione are given in Table 3.

Table 3 Selected environmental properties of mesotrione. All data from EFSA (2016).

Parameter	Name/Unit	Value	Remark
Koc	[L/kg]	14	lowest value 10 soils; pH 7.8; sorption decreases with increasing pH
Hydrolysis half-life	DT_{50} [d]	-	stable, pH 4, 5, 7, 9
Photolysis half-life	DT_{50} [d]	-	direct; no degradation
		12.8	indirect, natural water, continuous illumination
		20.1	at 30 °N
		19.5	at 40 °N
		20.5	at 50 °N
Biodegradation in water/sediment systems	DT_{50} [d]	5.6	whole system

2.3.2 Bioconcentration and biomagnification

Since log K_{ow} is < 3, the trigger for bioconcentration and biomagnification is not exceeded. A QS based on secondary poisoning of predators ($QS_{fw, sec\ pois}$ or $QS_{sw, sec\ pois}$) does not have to be derived.

2.4 Human toxicology

Mesotrione has a harmonised classification for Reprotoxicity Category 2, with hazard statement H361d “Suspected of damaging the unborn child” and for STOT Repeated Exposure 2, with hazard statement H373 “May cause damage to organs (eyes and nervous system) through prolonged or repeated exposure” (ECHA, 2021; HSE, 2017). Therefore, the $QS_{water, hh\ food}$ for human fish consumption should be included in the EQS-derivation. The ADI for mesotrione is set at 0.01 mg/kg bodyweight per day (EC, 2021).

3 Derivation of water quality standards

3.1 Laboratory ecotoxicity data

This section reports on the available acute and chronic laboratory ecotoxicity data for water organisms. Detailed toxicity data are presented in Annex 1 and the final data selection is given below in Table 4 and 5.

Mesotrione was originally approved in 2001 and studies with algae and macrophytes from that dossier report NOECs and EC_{50}-values obtained by linear regression. For the RAR-dossier, the applicant submitted statistical reports in which additional EC_{10} and EC_{20}-values were provided in line with current regulatory needs. The new effect values were estimated by non-linear regression, which is the preferred technique according to current guidelines. However, those reports only consider recalculated EC_{10} and EC_{20}-values, but no EC_{50}. For reasons of consistency, both EC_{50} and EC_{10} values were recalculated by RIVM by non-linear regression with GraphPad. The relevant studies are marked in Table 4 and 5, details can be found in the footnotes in Annex 1. For clarity the originally reported and recalculated values are summarised in Annex 2 for the lowest relevant endpoints and test durations.

3.1.1 Re-evaluation of *Myriophyllum*-endpoints

As explained above (see 3.1), RIVM recalculated effect values for algae and macrophytes in order to derive EC_{50} and EC_{10} values for these organisms in a consistent way. For *Myriophyllum spicatum*, derived E_rC_{50} and E_rC_{10} were 27.5 and 0.085 µg/L, respectively based on the study by [redacted] (2017). It was acknowledged in the original RIVM report that this was an extrapolated value and as such less reliable. However, because 31% and 54% effect was observed at the lowest test concentration for growth rate and yield, respectively (both based on total shoot length), it was not possible to follow the recommendation of the WFD-guidance and use a NOEC instead. Therefore, the E_rC_{10} value was used as a basis for the $QS_{fw, eco}$ in the initial assessment. As indicated in the introduction (see 1.1) the registration holder did not agree with the recalculated E_rC_{10} and argued that the next lowest reliable NOEC for *Lemna gibba* should be used with an assessment factor of 10. The evaluation of Syngenta's rebuttal by RIVM is included in Annex 4 of this advice. In summary, based on OECD and EFSA guidance on the use of extrapolated effect values, RIVM agrees that the E_rC_{10} cannot be used, because the E_rC_{10} is far below the lowest test concentration and the confidence interval indicates a high uncertainty (see further Annex 4). Therefore, the recalculated E_rC_{10} is rated as not reliable in Annex 1 and not included in the chronic dataset in Table 5. As a result, the NOEC for *L. gibba* is the only reliable chronic toxicity value for plants. The choice of the assessment factor for the $QS_{fw, eco}$ is further discussed in section 3.3.1.

3.1.2 Effects on algae

Some literature studies with algae investigated the direct effects of mesotrione on photosynthesis by measuring chlorophyll *a* fluorescence, e.g. by applying pulse amplitude modulated fluorometry (PAM) to assess the effect on photosystem II efficiency. This method is a quick and non-invasive method from which information on the toxicity of a contaminant can be obtained after several minutes to several hours, depending on the type of contaminant (Sjollema, 2014; Suresh Kumar et al., 2014). Most of the fluorescence studies with mesotrione were not considered valid because of the absence of analytical determination of test concentrations, but this was not the case for the study of Ni et al. (2014b) from which reliable

endpoints were derived for *Mycrocystis* sp. and *Scenedesmus quadricauda* (see Annex 1). Some authors point at a lack of proven ecological relevance of PAM-results, because a direct relationship between effects on photosynthesis and population growth is not demonstrated (Ralph et al., 2007). In a review, however, it is stated that photosynthesis related endpoints are highly relevant, because photosynthesis is the fundamental basis of the food chain (Suresh Kumar et al., 2014). The authors state that a comparison with traditional endpoints is necessary to conclude on the applicability of chlorophyll-a fluorescence based endpoints as biomarkers, although a correlation between photosynthesis inhibition and growth rate is demonstrated in some studies in which both endpoints were measured after a 3-days exposure period (Buma et al., 2009; Magnusson et al., 2008). Exposure duration in Ni et al. (2014b) was 96 hours, and the effect values are in line with results for other algae. Therefore, they are included in the dataset.

3.1.3 Selected ecotoxicity data

The selected acute and chronic ecotoxicity data are summarised in Table 4 and 5.

Table 4 Acute ecotoxicity of mesotrione for aquatic organisms.

Endpoints	$L(E)C_{50}$ [mg/L]	Remark	Ref.
Bacteria			
Vibrio fischeri	69.9	Microtox test; neutralised solution	Bonnet et al. (2008)
Protozoans			
Tetrahymena pyriformis	7728	study with active substance	Bonnet et al. (2008)
Diatoms			
Navicula pelliculosa	74	statistically re-evaluated	EFSA (2016)
Cyanobacteria			
Microcystis sp.	6.19	chlorophyll a fluorescence	Ni et al. (2014b)
Algae			
Raphidocelis subcapitata	4.5	statistically re-evaluated	EFSA (2016)
Scenedesmus quadricauda	4.41	chlorophyll a fluorescence	Ni et al. (2014b)
Macrophytes			
Lemna gibba	0.0211	growth rate (frond number), statistically re-evaluated	EFSA (2016)
Myriophyllum spicatum	0.0275	growth rate (shoot length), statistically re-evaluated	[redacted] (2017)
Crustaceans			
Daphnia magna	>622		EFSA (2016)
Fish			
Danio rerio	>0.0075	7-d test with larvae	Elskus (2007)
Lepomis macrochirus	>120		EFSA (2016)
Oncorhynchus mykiss	>120		EFSA (2016)

Table 5 Chronic ecotoxicity of mesotrione for aquatic organisms.

Endpoints	NOEC/EC_{10} [mg/L]	Remark	Ref.
Diatoms			
Navicula pelliculosa	40	statistically re-evaluated	EFSA (2016)
Cyanobacteria			
Microcystis sp.	0.5	chlorophyll a fluorescence	Ni et al. (2014b)
Algae			
Raphidocelis subcapitata	0.93	statistically re-evaluated	EFSA (2016)
Scenedesmus quadricauda	2	chlorophyll a fluorescence	Ni et al. (2014b)
Macrophytes			
Lemna gibba	0.002	growth rate (frond number), statistically re-evaluated	EFSA (2016)
Crustaceans			
Daphnia magna	180		EFSA (2016)
Fish			
Cyprinus carpio	≥0.180		Wang et al. (2018)
Pimephales promelas	12.5		EFSA (2016)

3.2 Derivation of the MAC-EQS

3.2.1 Deterministic approach

Valid acute toxicity data are available for 12 species from seven taxa: bacteria, protozoans, diatoms, cyanobacteria / algae, macrophyta, crustaceans and fish. A complete acute base set is available. All tests were performed in freshwater, including those with the diatom *Navicula pelliculosa* which is also found in marine environments[1]. The MAC-$QS_{fw, eco}$ is derived from the lowest relevant acute toxicity value available from the laboratory data, the EC_{50} of 21.1 µg/L for *Lemna gibba*. The LC_{50} of >7.5 µg/L for *Danio rerio* is not used, because at the highest test concentration no mortality was observed and the OECD-tests with other fish species indicate low sensitivity. An assessment factor of 10 may be applied because the substance has a known mode of action and representatives of the presumed most sensitive taxonomic groups (macrophytes; primary producers) are included in the dataset. The MAC-$QS_{fw, eco}$ is 21.1 / 10 = 2.1 µg/L.

No data for marine species are available and the the MAC-$EQS_{sw, eco}$ is derived on the basis of the freshwater dataset. Since there are no acute data from specific marine taxa, an additional assessment factor of 10 is applied to the MAC-$EQS_{fw, eco}$ (total AF=100). This results in a MAC-EQS_{sw} of 0.21 µg/L.

3.2.2 Statistical extrapolation

According to the WFD-guidance, statistical extrapolation using Species Sensitivity Distributions (SSD) may be performed when the database contains preferably more than 15, but at least 10 L(E)C_{50}-values, from different species covering at least eight taxonomic groups. Leaving the value for D. rerio out of consideration, the current acute dataset includes 11 species. The taxa to be included are indicated below, with the representative species in the current dataset.

[1] https://www.algaebase.org/search/species/detail/?tc=accept&species_id=31828

- Fish (species frequently tested include salmonids, minnows, bluegill sunfish, channel catfish, etc.); ➔ *Danio rerio*; family Cyprinidae
- A second family in the phylum Chordata (e.g. fish, amphibian, etc.); ➔ *Oncorhynchus mykiss*; family Salmonidae
- A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish, etc.); ➔ *Daphnia magna*
- An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.); ➔ no data
- A phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.); ➔ *Vibrio fischeri*; phylum Proteobacteria
- An order of insect or any phylum not already represented; ➔ *Tetrahymena pyriformis*; phylum Ciliophora
- Algae or Cyanobacteria; ➔ *Raphidocelis subcapitata*
- Higher plants. ➔ *Lemna gibba*

The requirements for the SSD are not fully met (insects are missing). Because insects are not expected to be sensitive to mesotrione, it was decided to explore the SSD for illustrative purposes using the EtX-programme (Van Vlaardingen et al., 2020). When using the >-values as such for fitting the SSD, the curve shows limited fit upon visual inspection (see Figure 1), although the Goodness of Fit is accepted in all cases (see Annex 3 for ETX-output). The HC_5 is 21.8~~3~~ µg/L which is similar to the EC_{50} for *L. gibba*. According to the WFD-guidance, an assessment factor of 10 is put on the acute HC_5, resulting in the same MAC-$QS_{fw, eco}$ of 2.1 µg/L as derived above with the deterministic approach. The limited fit may be explained by the specific mode of action (HPPD inhibition) which targets photosynthetic mechanism in higher plants in particular. More data for aquatic macrophytes would be needed to improve fitting of the lower left side of the SSD and/or to allow for construction of a specific SSD. Furthermore, it must be noted that two of the 11 endpoints for the SSD listed above, provide unbound values further limiting the number of relevant data. Therefore, for the present evaluation, the SSD-result can only be used as supportive for the deterministic approach.

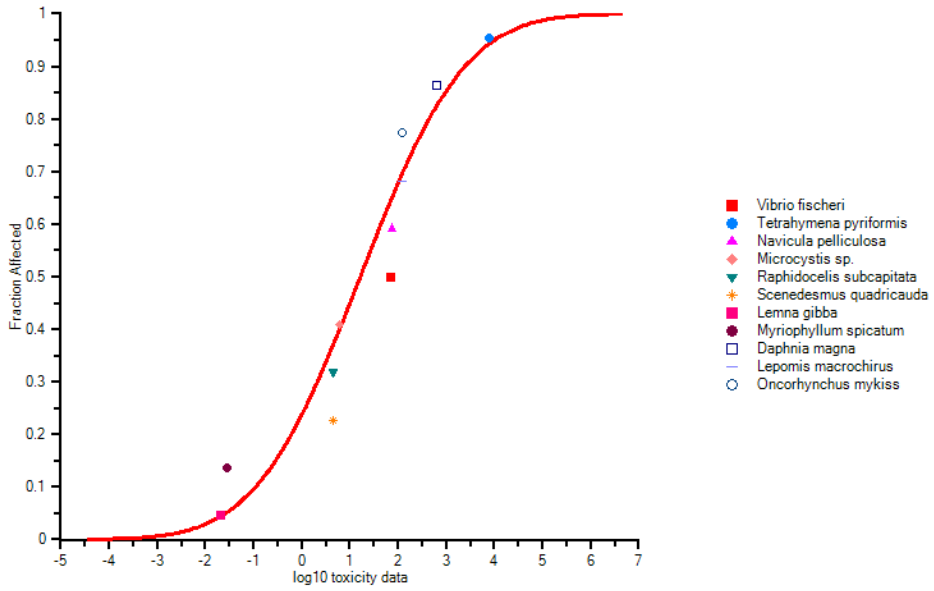


Figure 1. Sensitivity Distribution for mesotrione based on acute toxicity data for all available aquatic species. The X-axis represents log-transformed L(E)C50-values in mg/L, the Y-axis represents the fraction of species affected.

3.2.3 Selection of the MAC-EQS

The MAC-$EQS_{fw, eco}$ derived with the assessment factor approach is 2.1 µg/L, which is supported by a tentative evaluation using the SSD-approach.

The MAC-$EQS_{sw, eco}$ is derived from the MAC-$EQS_{fw, eco}$ with an additional assessment factor of 10 (total AF=100) and is 0.21 µg/L.

3.3 Derivation of the AA-EQS

3.3.1 Ecotoxicity - $QS_{fw, eco}$ and $QS_{sw, eco}$

NOEC/EC_{10}-values are available for 10 freshwater species from five taxa: diatoms, cyanobacteria / algae, macrophyta, crustaceans and fish. A complete base set is available, but there are not enough data for statistical extrapolation. Therefore, the $QS_{fw, eco}$ is derived from the lowest chronic toxicity value available from the laboratory data, the NOEC of 0.002 mg/L (2 µg/L) for *L. gibba*. According to the WFD-guidance, an assessment factor of 10 ~~is~~ may be applied if the substance has a known mode of action and representatives of the presumed most sensitive taxonomic groups are included in the dataset. Based on the comparable EC_{50}-values for *L. gibba* and *M. spicatum*, the registrant argues that the lowest assessment factor of 10 is sufficient, but this is not agreed upon by RIVM (see Annex 4). While acknowledging the fact that the derived chronic effect values for *M. spicatum* cannot be used, it is clear that the NOEC for *L. gibba* is not protective for this species. In the *Myriophyllum*-test, significant effects on all relevant parameters were observed at the lowest mean measured test concentration of 3.78 µg/L. Reduction in growth rate was 30.9% based on total shoot length, 19.5% based on mean shoot wet weight, and 28.1% based on mean shoot dry weight. According to the WFD-guidance, a NOEC can be calculated as LOEC/2, but only in case the effect at the level of the LOEC is between 10 and 20%. Given the fact that the least sensitive endpoints show 20% effect at 3.78 µg/L, the actual no-effect level for the most sensitive endpoint will be lower than the NOEC of 2 µg/L for *L. gibba*. Using this NOEC would thus overlook the fact that the QS-derivation should be based on the critical parameter observed in a macrophyte test. According to the WFD-guidance, the assessment factor of 10 should not be used when it is not possible to determine with high probability that the most sensitive species has been examined (footnote d to Table 3 of the WFD-guidance). In such case, an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity. Therefore, the $QS_{fw, eco}$ is derived with an assessment factor of 50 to the NOEC of 2 µg/L. The $QS_{fw, eco}$ is 2 / 50 = 0.040 µg/L = 40 ng/L.

The $QS_{sw, eco}$ is derived on the basis of the freshwater dataset. Since there are no chronic data from specific marine taxa, an additional assessment factor of 10 is applied to the $QS_{fw, eco}$. This results in a $QS_{sw, eco}$ of 4.0 ng/L.

3.3.2 Human fish consumption – $QS_{water, hh\ food}$

As indicated in section 2.3.2, a tentative calculation is made to assess whether human exposure via fish might be critical for EQS-derivation. Using the ADI of 0.01 mg/kg body weight per day, a maximum contribution of fish to the total intake of 20%, and assuming a default daily fish consumption of 115 g per day and a body weight of 70 kg, the fish-based $QS_{biota, hh\ food}$ is 1.2 mg/kg wwt fish. As no experimental BAF or BCF value is available, the upper-trophic level BAF of mesotrione is estimated to be 2.33 L/kg (assuming 10.7% lipid content in fish), using the BCFBAF QSAR module within the EpiSuite 4.11 programme. The corresponding 5% lipid

content BAF value of 1.09 L/kg is then used to calculate a $QS_{water,\ hh\ food}$ of 1.1 mg/L. This is much higher than the ecosystem based values, and further assessment of human fish consumption is not necessary.

3.3.3 Selection of the QS

Direct ecotoxicity and human fish consumption are the relevant routes for derivation of the AA-EQS for mesotrione. Secondary poisoning of birds and mammals is not relevant. As the QS value based on direct ecotoxicity ($QS_{fw,\ eco}$ or $QS_{sw,\ eco}$) is lower than the QS value based on human consumption of fishery products ($QS_{biota,\ hh\ food}$), the direct ecotoxicity is the critical route. Hence, the AA-EQS_{fw} is 40 ng/L and the AA-EQS_{sw} is 4.0 ng/L.

4 Discussion and conclusions

In this report, water quality standards for mesotrione are derived according to the methodology of the European Water Framework Directive. As expected for this herbicide, green algae and macrophytes are most sensitive. The applicant proposed a MAC-EQS of 0.45 µg/L ([redacted] 2020), based on a 7-day EC_{50} of 4.5 µg/L for yield of *L. gibba* from a reciprocal exposure test ([redacted] & [redacted], 2015). However, this EC_{50} is not mentioned in the study report and the applicant's study summary, so the origin of the reported effect values is not clear. The E_yC_{50} value may originate from a different study report not discussed in the applicant's document, but included in the CLH report (HSE, 2017) (see Annex 1 for details). Furthermore, as indicated in section 1.3.3., growth rate is the preferred endpoint for primary producers and with 28 µg/L, the E_rC_{50} from this study was higher than the 14-days E_rC_{50} of 21.1 µg/L from the other *Lemna*-test. Therefore, the latter was selected as the critical endpoint for derivation of the MAC-$EQS_{fw, eco}$.

For the AA-EQS, the appliclant proposed a value of 0.2 µg/L, based on the NOEC of 0.002 mg/L (2.0 µg/L) *for L. gibba*, as the study with *M. spicatum* did not deliver a reliable NOEC/EC_{10} value ([redacted] 2020). However, in the *Myriophyllum*-study, significant effects were already observed at the lowest test concentration of 4.04 µg/L nominal (3.76 µg/L actual), with 30.9, 19.1 and 28.1% reduction of growth rate based on shoot length, fresh weight and dry weight, respectively. The original study reports a LOEC of 4.04 µg/L and E_rC_{10}-values of 0.149 and 0.300 µg/L (nominal) for growth rate based on shoot length and fresh weight. The E_rC_{10} for dry weight was not calculated by the author ([redacted] 2017). RIVM recalculated the E_rC_{10}-values from the reported growth rate inhibition data using actual concentrations. The lowest E_rC_{10}-value was 0.085 µg/L for shoot length (see Annex 1 and 2). This value is well below the lowest test concentration and therefore not reliable. In line with the WFD-guidance the EQS is derived on the basis of the data from the *L. gibba* study with an assessment factor of 50. A lower assesment factor of 10 can only be considered when a reliable NOEC or E_rC_{10} value for *M. spicatum* becomes available.

The MAC-EQS_{fw} of mesotrione is 2.1 µg/L, MAC-EQS_{sw} is 0.21 µg/L.
The AA-EQS_{fw} of mesotrione is 40 ng/L, the AA-EQS_{sw} is 4.0 ng/L.

References

Reference list includes references in the Annexes.

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Annex 1 Aquatic toxicity data

Legend to column headings	
A	test water analysed Y(es)/N(o)
Test type	S = static; Sc = static closed; R = renewal; F = flow through; CF = continuous flow; IF = intermittent flow system
Test compound	ag = analytical grade; tg = technical grade; form = formulated product
Purity	refers to purity of active substance or content of active substance in formulation
Test water	am = artificial medium; dtw = dechlorinated tap water; dw = deionised/dechlorinated/distilled water; nw = natural water; rw = reconstituted water; rtw = reconstituted tap water; tw = tap water
T	temperature
Ri	reliability index according to Klimisch et al. (1997)
Ref.	reference
Original ref.	for studies from the RAR (EC, 2015), the original study reference is given

Table A1.1 Acute toxicity of mesotrione for freshwater organisms. Selected valid tests are given on a grey background (see section 1.3.3 for information on criteria).

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Vibrio fischeri		N	S	tg	99.9		neutralised			15 min	IC50	bioluminescence	69.9	2	2	Bonnet et al. (2008)	
Vibrio fischeri		N	S	Callisto	100 g/L		acidic			15 min	IC50	bioluminescence	1.1	2	1	Bonnet et al. (2008)	
Vibrio fischeri		N	S	Callisto	100 g/L		neutralised			15 min	IC50	bioluminescence	0.9	2	2	Bonnet et al. (2008)	
Protozoans																	
Tetrahymena pyriformis	amicronucleated strain GL	N	S	tg	99.9		6.5-7.0	28		9 h	IC50	generation time	7728	2	3	Bonnet et al. (2008)	
Tetrahymena pyriformis	amicronucleated strain GL	N	S	Callisto	100 g/L		6.5-7.0	28		9 h	IC50	generation time	4.0	2	4	Bonnet et al. (2008)	
Diatoms																	
Amphora coffeaeformis	1.0E+05 cells/mL	N	S	ag				17-19		96 h	IC50	generation time	13.1	3	5	Valiente Moro et al. (2012)	
Navicula pelliculosa	0.322E+04 cells/mL	Y	S	tg	95.1	am	6.3-8.3	24.0-24.2	14.9	96 h	ErC50	growth rate	74	2	6	EC (2015)	[redacted] (2012) [redacted] (1997)
Algae and cyanobacteria																	

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Ankistrodesmus fusiformis	1.0E+05 cells/mL	N	S	ag		am		17-19		96 h	IC50	generation time	56.1	3	7	Valiente Moro et al. (2012)	
Chlamydomonas reinhardtii		N	S	tg	99.6	am	6.8	24		24 h	EC50	chlorophyll content	<0.7	3	8	Xu et al. (2019)	
Chlorella vulgaris	2.0E+05 cells/mL	N	S	tg	94	am		24-26	55	48-72 h	ErC50	growth rate	18.86	3	9	Zhang et al. (2020)	
Chlorella vulgaris	2.0E+05 cells/mL	N	S	tg	94			24-26		72-96 h	ErC50	growth rate	18.80	3	9	Zhang et al. (2020)	
Microcystis sp.	2.3-3.0E+05 cells/mL	N	S	tg	99	am	7.1	24-26		72 h	EC50	yield	10.95	3	10	Ni et al. (2014a)	
Microcystis sp.	2.3-3.0E+05 cells/mL	N	S	tg	99	am	7.1	24-26		96 h	EC50	yield	9.76	3	10	Ni et al. (2014a)	
Microcystis sp.	2.3-3.0E+05 cells/mL	N	S	10% suspension	100 g/L	am	7.1	24-26		72 h	EC50	yield	0.26	3	10	Ni et al. (2014a)	
Microcystis sp.	2.3-3.0E+05 cells/mL	N	S	10% suspension	100 g/L	am	7.1	24-26		96 h	EC50	yield	0.24	3	10	Ni et al. (2014a)	
Microcystis sp.	10E+06 µm3/mL	Y	S	tg	99	am	7.1	24-26		96 h	EC50	chlorophyll a fluorescence	6.19	2	11	Ni et al. (2014b)	
Raphidocelis subcapitata	0.32E+04 cells/mL	Y	S	tg	95.1	am	6.2-10	24.1-24.2	14.9	72 h	ErC50	growth rate	4.5	1	12	EC (2015)	[redacted] (2013) [redacted] et al. (1997)
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	tg	79	am	8	22		48 h	ErC50	growth rate (fluorescence)	6786	3	13	(Cedergreen et al., 2008)	
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	form	100 g/L	am	8	22		48 h	ErC50	growth rate (fluorescence)	7810	3	13	(Cedergreen et al., 2008)	
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	tg	95			21-24		48 h	ErC50	growth rate	3.62	3	14	(Zhao et al., 2018)	
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	tg	95			21-24		72 h	ErC50	growth rate	3.35	3	14	(Zhao et al., 2018)	
Scenedesmus quadricauda	1.6-2.4E+05 cells/mL	N	S	tg	99	am	7.1	24-26		72 h	EC50	yield	11.19	3	10	Ni et al. (2014a)	
Scenedesmus quadricauda	1.6-2.4E+05 cells/mL	N	S	tg	99	am	7.1	24-26		96 h	EC50	yield	7.15	3	10	Ni et al. (2014a)	
Scenedesmus quadricauda	1.6-2.4E+05 cells/mL	N	S	10% suspension	100 g/L	am	7.1	24-26		72 h	EC50	yield	0.22	3	10	Ni et al. (2014a)	
Scenedesmus quadricauda	1.6-2.4E+05 cells/mL	N	S	10% suspension	100 g/L	am	7.1	24-26		96 h	EC50	yield	0.16	3	10	Ni et al. (2014a)	
Scenedesmus quadricauda	10E+06 μm^3/mL	Y	S	tg	99	am	7.1	24-26		96 h	EC50	chlorophyll a fluorescence	4.41	2	11	Ni et al. (2014b)	

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Crustaceans																	
Daphnia magna	<24 h	Y	S	tg	96.8	dct	5.6-8.5	20.2-20.6	178	48 h	EC50	immobility	>622	2	15	EC (2015)	[redacted] & [redacted] (1995)
Fish																	
Danio rerio	2-3 h post hatch	Y	R/F	Callisto	100 g/L	am		28		7 d	LC50	mortality	≥0.0075	2	16	Elskus (2007)	
Danio rerio	2-3 h post hatch	Y	R/F	Callisto	100 g/L	am		28		5 d	EC50	respiratory burst (innate immune response)	≥0.0075	2	16	Elskus (2007)	
Danio rerio	2-3 h post hatch	Y	R/F	Callisto	100 g/L	am		28		5 d	EC50	time to hatch	≥0.0075	2	16	Elskus (2007)	
Danio rerio	2-3 h post hatch	Y	R/F	Callisto	100 g/L	am		28		7 d	EC50	developmental abnormalities	≥0.0075	2	16	Elskus (2007)	
Danio rerio	2-3 h post hatch	Y	R/F	Callisto	100 g/L	am		28		7 d	EC50	swimming behaviour	≥0.0075	2	16	Elskus (2007)	
Danio rerio	6-8 h after fertilization	N	R	tg	≥90			25.9-26.1		120 h	AC50	lethality + hatch scores	16.0	3	17	Padilla et al. (2012)	
Geophagus brasiliensis		N	S					22		96 h	LC50	mortality	>0.460	3	18	Piancini et al. (2015)	
Lepomis macrochirus	1.12 g; 35 mm	Y	S	tg	95.1	dct	6.00-7.54	21.9-22.1	26.6	96 h	LC50	mortality	>120	1	19	EC (2015)	[redacted] et al. (1994b)
Oncorhynchus mykiss	1.75 g; 49 mm	Y	S	tg	95.1	dct	6.35-7.64	11.4-12.5	41	96 h	LC50	mortality	>120	1	20	EC (2015)	[redacted] et al. (1994a)
Oreochorimis niloticus		N	S					22		96 h	LC50	mortality	>0.460	3	18	Piancini et al. (2015)	
Rhamdia quelen	60-d old fingerlings	N	R	Callisto	100 g/L		6.2-7.0	22 ± 2	60-65	96 h	LC50	mortality	532	3	21	Kreutz et al. (2008)	
Macrophytes																	
Hydrilla verticillata	fluoridone resistant	N	S					25		14 d	EC50	phytoene	0.0118	3	22	Puri et al. (2009)	
Hydrilla verticillata	fluoridone resistant	N	S					25		14 d	EC50	ß-carotene content	0.0132	3	22	Puri et al. (2009)	
Hydrilla verticillata	fluoridone resistant	N	S					25		14 d	EC50	chlorophyll a	0.0046	3	22	Puri et al. (2009)	
Hydrilla verticillata	fluoridone susceptible	N	S					25		14 d	EC50	phytoene	0.0124	3	23	Puri et al. (2009)	
Hydrilla verticillata	fluoridone susceptible	N	S					25		14 d	EC50	ß-carotene content	0.0102	3	23	Puri et al. (2009)	

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Hydrilla verticillata	fluoridone susceptible	N	S					25		14 d	EC50	chlorophyll a	0.0031	3	23	Puri et al. (2009)	
Lemna gibba	3-4 plants (12 fronds)	Y	R	tg	97.6	am	4.5-5.9	24.7-25.2	700	14 d	ErC50	growth rate (frond number	0.0556	2	24	EC (2015)	[redacted] (2013a) [redacted] et al. (1996)
Lemna gibba	3-4 plants (12 fronds)	Y	R	tg	97.6	am	4.5-5.9	24.7-25.2	700	14 d	ErC50	growth rate (dry weight)	0.0211	2	24	EC (2015)	[redacted] (2013a) [redacted] et al. (1996)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.4-8.7	22-26	296	72 h	ErC50	growth rate (dry weight)	>0.020	2	25	[redacted] & [redacted] (2015)	
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	ErC50	growth rate (frond number)	0.028	2	26	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	ErC50	growth rate (dry weight)	0.028	2	26	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	EyC50	yield (frond number)	0.006	2	26	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	EyC50	yield (dry weight)	0.0052	2	26	HSE (2017)	[redacted] & [redacted] (2015)
Lemna minor	1 frond initiation	N	S	tg	79	am	5	24		7 d	ErC50	growth rate (frond area)	20	3	27	Cedergreen et al. (2008)	
Lemna minor	1 frond initiation	N	S	Callisto	100 g/kg	am	5	24		7 d	ErC50	growth rate (frond area)	40	3	27	Cedergreen et al. (2008)	
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC50	growth rate (shoot length)	0.0275	2	28	This evaluation	[redacted] (2017)
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC50	growth rate (fresh weight)	0.0928	2	28	This evaluation	[redacted] (2017)
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC50	growth rate (dry weight)	0.0443	2	28	This evaluation	[redacted] (2017)

1: MicroTox test in unbuffered solution (pH Callisto formulation 2.96; dilution factor of 0.45); no analytical verification of test concentration, but considered acceptable in view of short test duration
2: MicroTox test in neutralised solution; no analytical verification of test concentration, but considered acceptable in view of short test duration
3: solvent 0.5% DMSO, solvent control included; temperature reported in Bonnet et al 2007; no analytical verification of test concentration, but considered acceptable in view of short test duration; endpoint equivalent to doubling time (yield)

4: toxicity reportedly enhanced due to the surfactants in the formulation; temperature reported in Bonnet et al 2007; no analytical verification of test concentration, but considered acceptable in view of short test duration; endpoint equivalent to doubling time (yield)
5: endpoint equivalent to doubling time (yield); growth measured directly as absorbance at optical density at 750 nm wavelength; result not used because test concentrations were not measured
6: test according to FIFRA 123-2; test duration 120 h; mean measured concentrations 93-100% of nominal; hardness calculated from medium description; 72-120 h ErC50 reported as 66-96 mg/L based on linear regression; recalculation by applicant with non-linear regression only considers EC10 and EC20, but no EC50; for time windows 0-72, 0-96 and 0-120 h, mean coefficient of variation for section-by-section specific growth rates >35% due to lag phase, validity criteria are met when excluding the 0-24 h values and using cell numbers over 24-96 h and 24-120 h; effect values over 24-96 h are recalculated by evaluator using non-linear regression with GraphPad using growth rate of individual replicates
7: endpoint equivalent to doubling time (yield); growth measured directly as absorbance at optical density at 750 nm wavelength; result not used because test concentrations were not measured
8: >50% effect 14.7 µM; one concentration; no verification of test concentrations;
9: solvent DMSO and Triton X-100, solvent control included; hardness calculated from reported medium composition; reported ErC50 values are most likely the section-by-section growth rates over 48-72 h and 72-96 h instead of the growth rate over the whole period; concentrations not measured
10: results based on cell density; concentration-response curves in article show irregular growth pattern in control, validity criteria of OECD 201 regarding variation in day-to-day growth rate probably not met; test concentrations not measured
11: log-phase cultures exposed for 7 days, chlorophyll a fluorescence measured daily; mesotrione concentrations in algae-free medium in accordance with nominal concentrations
12: test according to FIFRA 123-2; duration 120 h; mean measured concentrations 100-109% of nominal; increase in pH >2 units, but this is a result of algae population growth; hardness calculated from medium description; 72-120 h ErC50 originally reported as 12-13 mg/L based on linear regression; recalculation by applicant with non-linear regression only considers EC10 and EC20, but no EC50; for EQS derivation, effect values are recalculated by non-linear regression with GraphPad using growth rate of individual replicates; EC10 is equal to applicant's value
13: algae test coherent with the ISO standards; result not used because test concentrations were not measured
14: solvent acetone, solvent control included; validity criteria (control performance) cannot be checked; concentrations not measured
15: test according to OECD 202 (1984); mean measured test concentrations 100-109% of nominal; cloudiness observed at highest test concentration 1000 mg/L; no mortality ≤600 mg/L, 90-100% mortality per replicate at 1000 mg/L; no toxic reference; EC50 reported by authors is 900 mg/L, included as >622 mg/L in RAR (highest test concentration without cloudiness and no mortality)
16: it is mentioned that analyses were performed, but results are not reported; renewal: day 0 - day 4, flow-through day 5 - 7 (post hatch). Publication also describes a preliminary study on immune function and several other parameters, on which mesotrione had no effects.
17: solvent control included (DMSO 0.4% v/v); endpoint recalculated from molar concentration; no analytical verification of test concentrations; AC50 is based on arbitrary scores for lethality and non-hatching and cannot be used for EQS derivation
18: test designed for biochemical assays, no mortality observed; no information on water quality; concentrations not measured
19: limit test according to FIFRA 72-1; loading 0.32 g/L; mean measured concentration 108% of nominal
20: limit test according to FIFRA 72-1; loading 0.66 g/L; mean measured concentration 108% of nominal
21: fish loading 1 g/L; water exchange rates of 20% were used each day; result not used because concentrations were not measured
22: plants collected from lake with fluridone-resistant population; no information on test compound and test conditions not reported; concentrations not measured
23: plants collected from private pond that was never treated with fluridone; no information on test compound and test conditions not reported; concentrations not measured
24: test according to FIFRA 123-2; hardness calculated by evaluator for M-Hoagland's medium; mean measured concentration 93-103% of nominal; doubling time 3.0 d, which is higher than validity criterion of OECD 221 (<2.5 d); reduced root growth, stunting at concentrations ≥4.0 µg/L; pale coloration at

≥8 µg/L, all new fronds affected at ≥16 µg/L; 14 d EC50 reported as 22 µg/L for increase in frond number and 7.7 µg/L for increase in dry weight, based on linear regression; recalculation of effect values for growth rate and yield by applicant with non-linear regression only considers EC10 and EC20, but no EC50; for consistency, all effect values for growth rate were recalculated by evaluator by non-linear regression with GraphPad using reported frond numbers and dry weight; bottom of the curve was forced through 0 because otherwise EC50 was not consistent with observed inhibition percentage

25: reciprocal test: exposure to 60 µg/L for 24 h, 30 µg/L for 48 h and 20 µg/L for 24 h, growth inhibition followed until day 7; hardness calculated by evaluator for AAP medium; mean measured concentration 91-97% of nominal, overall mean 94%; significant inhibition in growth rate 24.9% (frond number) and 29.1% (dry weight); higher concentrations in combination with shorter duration gave lower effect values; test is reliable, but not relevant for EQS derivation

26: report not available during assessment, the information has been retrieved from the CLH report on mesotrione. Although slightly outside of 80-120% (122% in one concentration at 7-days), nominal concentrations were used for the calculation and reporting of results

27: static test without analytical verification

28: test according to OECD 239 with rooted macophyte in presence of sediment; hardness calculated based on reported medium composition; mean measured concentrations in overlying water 83-110% of nominal, overall mean in fresh and aged solutions 95 and 97%, respectively (nominal corrected for purity); validity criteria met (CV growth rate <35% and at least doubling of parameter); authors report EC50s of 33.9, 108 and 53.3 µg/L for shoot length, fresh weight and dry weight, respectively; because authors did not provide EC10 for growth rate based on dry weight, all effect values were recalculated for reasons of consistency by non-linear regression with GraphPad using reported growth rate values for separate replicates; bottom of the curve was forced through 0 because otherwise EC50 did not match observed inhibition, this is most likely due to the fact that concentrations were not properly chosen (>10% effect at lowest test concentration)

Table A1.2 Chronic toxicity of mesotrione for freshwater organisms. Valid tests are given on a grey background.

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Diatoms																	
Amphora coffeaeformis	1.0E+05 cells/mL	N	S	ag				17-19		21 d	NOEC	growth	≥0.2	3	1	Valiente Moro et al. (2012)	
Navicula pelliculosa	0.322E4 cells/mL	Y	S	tg	95.1	am	6.3-8.3	24.0-24.2	14.9	72 h	ErC10	growth rate	40	2	2	EC (2015)	[redacted] (2012) S[redacted] et al. (1997)
Algae and cyanobacteria																	
Ankistrodesmus fusiformis	1.0E+05 cells/mL	N	S	ag		am		17-19		21 d	NOEC	growth	≥0.2	3	3	Valiente Moro et al. (2012)	
Microcystis sp.	10E+06 µm3/mL	Y	S	tg	99	am	7.1	24-26		96 h	NOEC	chlorophyll a fluorescence	0.5	2	4	Ni et al. (2014b)	
Nannochloris oculata		N	S	tg	≥95	am	?	25		48 h	NOEC	biomass	≥5	3	5	Deng et al. (2015)	
Pediastrum tetras	1.0E+05 cells/mL	N	S	ag		am		17-19		21 d	NOEC	growth	≥0.2	3	3	Valiente Moro et al. (2012)	
Rhapidocelis subcapitata	0.32E4 cells/mL	Y	S	tg	95.1	am	6.2-10	24.1-24.2	14.9	72 h	ErC10	growth rate	0.93	1	6	EC (2015)	[redacted] (2013) [redacted] et al. (1997)
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	tg	79	am	8	22		48 h	EC10	growth rate (fluorescence)	977	3	7	Cedergreen et al. (2008)	
Raphidocelis subcapitata	1.0E+04 cells/mL	N	S	Callisto	100 g/kg	am	8	22		48 h	EC10	growth rate (fluorescence)	1980	3	7	Cedergreen et al. (2008)	
Scenedesmus quadricauda	10E+06 µm3/mL	Y	S	tg	99	am	7.1	24-26		96 h	NOEC	chlorophyll a fluorescence	2	2	4	Ni et al. (2014b)	
Crustaceans																	
Daphnia magna	<24 h	Y	R	tg	96.8	am	3.83-8.15	19.6-20.6	238-240	21 d	NOEC	reproduction, length	180	2	8	EC (2015)	[redacted] (2013b) [redacted] et al. (1996)

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Daphnia magna	<24 h	Y	R	tg	96.8	am	3.83-8.15	19.6-20.6	238-240	21 d	NOEC	dry weight	<97	3	8	EC (2015)	[redacted] (2013b) [redacted] et al. (1996)
Fish																	
Cyprinus carpio	juvenile	Y	R	tg	96		6.8-7.5	21-23	260-300	28 d	NOEC	mortality	≥0.180	2	9	Wang et al. (2018)	
Pimephales promelas	eggs	Y	F	tg	97.6	dct	6.63-7.94	24.2-25.1	44.6	36 d	NOEC	survival, hatchability	≥200	2	10	EC (2015)	[redacted] & [redacted] (1997) [redacted] & [redacted] (2013)
Pimephales promelas	eggs	Y	F	tg	97.6	dct	6.63-7.94	24.2-25.1	44.6	36 d	NOEC	growth (weight)	25	3	11	EC (2015)	[redacted] & [redacted] (1997) [redacted] & [redacted] (2013)
Pimephales promelas	eggs	Y	F	tg	97.6	dct	6.63-7.94	24.2-25.1	44.6	36 d	NOEC	growth (length)	25	2	12	EC (2015)	[redacted] & [redacted] (1997) [redacted] [redacted] (2013)
Pimephales promelas	eggs	Y	F	tg	97.6	dct	6.63-7.94	24.2-25.1	44.6	36 d	NOEC	physical symptoms	12.5	2	13	EC (2015)	[redacted] & [redacted] (1997) [redacted] & [redacted] (2013)
Macrophytes																	
Lemna gibba	3-4 plants (12 fronds)	Y	R	tg	97.6	am	4.5-5.9	24.7-25.2	700	14 d	ErC10	growth rate (frond number)	0.008	2	14	EC (2015)	[redacted] (2013a) [redacted] et al. (1996)
Lemna gibba	3-4 plants (12 fronds)	Y	R	tg	97.6	am	4.5-5.9	24.7-25.2	700	14 d	NOEC	growth rate (dry weight)	0.002	2	14	EC (2015)	[redacted] (2013a) [redacted] et al. (1996)

Species	Species properties	A	Test type	Test comp.	Purity [%]	Test water	pH	T [°C]	Hardness $CaCO_3$ [mg/L]	Exp. time	Criterion	Test endpoint	Value [mg/L]	Ri	Notes	Ref.	Original ref.
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	NOErC	growth rate (frond number)	0.002	2	15	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	NOEyC	yield (frond number)	0.002	2	15	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	NOErC	growth rate (dry weight)	0.002	2	15	HSE (2017)	[redacted] & [redacted] (2015)
Lemna gibba	12 fronds	Y	R	tg	86.1	am	7.5-9.0	23-24		7 d	NOEyC	yield (dry weight)	0.002	2	15	HSE (2017)	[redacted] & [redacted] (2015)
Lemna minor	1 frond initiation	N	S	tg	79	am	5	24		7 d	EC10	growth rate (frond area)	6.8	3	16	Cedergreen et al. (2008)	
Lemna minor	1 frond initiation	N	S	Callisto	100 g/kg	am	5	24		7 d	EC10	growth rate (frond area)	4.7	3	16	Cedergreen et al. (2008)	
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC10	growth rate (shoot length)	0.000085	3	17	This evaluation	[redacted] (2017)
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC10	growth rate (fresh weight)	0.000166	3	17	This evaluation	[redacted] (2017)
Myriophyllum spicatum		Y	R	tg	84.6	am	7.48-9.84	18.0-21.7	90.3	14 d	ErC10	growth rate (dry weight)	0.000116	3	17	This evaluation	[redacted] (2017)

1: growth measured directly as absorbance at optical density at 750 nm wavelength, cell counts included to validate growth measurement; one concentration tested; control with 0.2 mg/L without algae included to check that photodegradation did not occur, but no results reported; total test duration too long, but exponential growth apparent from graph

2: test according to FIFRA 123-2; test duration 120 h; mean measured concentrations 93-100% of nominal; hardness calculated from medium description; 72-120 h ErC50 reported as 66-96 mg/L based on linear regression; recalculation by applicant with non-linear regression only considers EC10 and EC20; for time windows 0-72, 0-96 and 0-120 h, mean coefficient of variation for section-by-section specific growth rates >35% due to lag phase, validity criteria are met when excluding the 0-24 h values and using cell numbers over 24-96 h and 24-120 h; effect values over 24-96 h are recalculated by non-linear regression with GraphPad using growth rate of individual replicates

3: growth measured directly as absorbance at optical density at 750 nm wavelength, cell counts included to validate growth measurement; one concentration tested; control with 0.2 mg/L without algae included to check that photodegradation did not occur, but no results reported; total test duration too long, irregular growth pattern in control

4: log-phase cultures exposed for 7 days, chlorophyll a fluorescence measured daily; mesotrione concentrations in algae-free medium in accordance with nominal concentrations

5: concentrations not measured; control growth not reported

6: test according to EPA guidelines; duration 120 h; mean measured concentrations 100-109% of nominal; increase in pH >2 units, but this is a result of algae population growth; hardness calculated from medium description; 72-120 h ErC50 originally reported as 12-13 mg/L based on linear regression;

recalculation by applicant with non-linear regression only considers EC10 and EC20, but no EC50; for consistency, all effect values are recalculated by evaluator by non-linear regression with GraphPad using growth rate of individual replicates; EC10 is equal to applicant's value

7: algae test coherent with the ISO standards; result not used because test concentrations were not measured

8: test according to FIFRA 72-4; test solutions were increasingly yellow coloured at 100 mg/L nominal and higher; measured concentration at 100, 180 and 320 mg/L in accordance with nominal (overall average 94-100%), low recovery at 560 mg/L (59-61%) and 1000 mg/L (35-39%); result based on measured concentration; 100% mortality at 320 mg/L nominal and higher, probably due to low pH 3.83-4.67 which was outside recommended range of OECD 211 (pH 6-9); difference in weight is not related to concentration and NOEC is not used in RAR because the effect on dry weight was not considered biologically relevant

9: no mortality observed

10: test according to FIFRA 72-4; mean measured concentrations 88-98% of nominal, results based on nominal; no significant effect on hatching/survival at highest test concentration

11: test according to FIFRA 72-4; mean measured concentrations 88-98% of nominal, results based on nominal; significant decrease in length at 50 mg/L and higher, significant decrease in weight at 50 and 200 mg/L; statistical re-evaluation revealed slight overall correlation between weight and concentration, but not significant and non-monotonous; EC10 not reliable and relevance of NOEC questionable

12: test according to FIFRA 72-4; mean measured concentrations 88-98% of nominal, results based on nominal; significant decrease in length at 50 mg/L and higher, significant decrease in weight at 50 and 200 mg/L; statistical re-evaluation significant overall negative correlation between concentration and length, but wide confidence intervals and scattering around the curve; EC10 not reliable

13: test according to FIFRA 72-4; mean measured concentrations 88-98% of nominal, results based on nominal; as from day 28, increasing numbers of fry with loss of balance, spinal deformities and skin lesions; endpoint used in RAR

14: test according to FIFRA 123-2; hardness calculated by evaluator for M-Hoagland's medium; mean measured concentration 93-103% of nominal; doubling time 3.0 d, which is higher than validity criterion of OECD 221 (<2.5 d); reduced root growth, stunting at concentrations ≥4.0 µg/L; pale coloration at ≥8 µg/L, all new fronds affected at ≥16 µg/L; 14 d EC50 reported as 2.2 µg/L for increase in frond number and 7.7 µg/L for dry weight, based on linear regression; recalculation by applicant with non-linear regression only considers EC10 and EC20, but no EC50; for reasons of consistency, all effect values for growth rate are recalculated by evaluator by non-linear regression with GraphPad using reported frond numbers and dry weight, bottom of the curve was forced through 0 because otherwise a poor fit was obtained and EC50's did not match with calculated inhibition; ErC10 calculated as 5.9 µg/L (frond #) and 1.3 µg/L (dwt), but NOEC's eventually selected because 0% inhibition was observed at 8 (frond #) and 2 µg/L (dwt), 67 and 46% at 16 and 4 µg/L

15: report not available during assessment, the information has been retrieved from the CLH report on mesotrione. Although slightly outside of 80-120% (122% in one concentration at 7-days), nominal concentrations were used for the calculation and reporting of results

16: static test without analytical verification

17: test according to OECD 239 with rooted macrophyte in presence of sediment; hardness calculated based on reported medium composition; mean measured concentrations in overlying water 83-110% of nominal, overall mean in fresh and aged solutions 95 and 97%, respectively (nominal corrected for purity); validity criteria met (CV growth rate <35% and at least doubling of parameter); authors report ErC10 0.149 for shoot length and 0.300 µg/L for fresh weight; because authors did not provide EC10 for growth rate based on dry weight, all effect values were recalculated for reasons of consistency by non-linear regression with GraphPad using reported growth rate values for separate replicates; bottom was of the curve was forced through 0 because otherwise EC50 did not match observed inhibition, this is most likely due to the fact that concentrations were not properly chosen (>10% effect at lowest test concentration); ErC10-values are not reliable because they are far below the lowest test concentration (3.76 µg/L mean measured) and confidence intervals are large

Annex 2 Summary of reported and recalculated effect values for algae and plants

Studies with algae and macrophytes from the DAR report NOECs and EC_{50}-values obtained by linear regression. For the RAR-dossier, the applicant submitted statistical reports in which additional EC_{10} and EC_{20}-values were estimated by non-linear regression. However, those reports only consider recalculated EC_{10} and EC_{20}-values, but no EC_{50}. For reasons of consistency, both EC_{50} and EC_{10} values were recalculated by RIVM by non-linear regression with GraphPad. This Annex provides and overview of the reported and recalculated values. Only lowest relevant endpoints and test durations are shown. Selected values indicated in bold on a gray background, all values in mg/L.

Table A2.1 Summary of effect values for algae and macrophytes (in mg/L). Selected values are given on a grey background.

Species	Test Endpoint	Time	NOEC	LOEC	EC10	EC20	EC50	Remark	Reference
Navicula pelliculosa	growth rate	0-72 h	48	96			66	linear regression	Smyth et al. (1997)
					51.0	53.2		non-linear regression	[redacted] (2012)
		24-96 h			**40.0**		**74**	non-linear regression; curve forced through 0	this evaluation
Rhapidocelis subcapitata	growth rate	0-72 h	0.75	1.5			13	linear regression	[redacted] et al. (1997)
					0.93	1.66		non-linear regression	[redacted] (2013)
					0.93		**4.5**	non-linear regression	this evaluation
Lemna gibba	growth rate (dwt)	0-14 d	0.002	0.004			0.0077	linear regression	[redacted] et al. (1996)
					0.002	0.0047		non-linear regression	[redacted] (2013a)
			0.002		0.0013		**0.021**	non-linear regression; curve forced through 0; NOEC selected, growth rate was not inhibited at 2 µg/L	this evaluation
Myriophyllum spicatum	growth rate (shoot length)	0-14 d	-	0.00477	0.000149 (0.149 µg/L)		0.0339	3-param. Normal CDF (cumulative distribution function)	[redacted] (2017)
					0.000085 **(0.085 µg/L)**		**0.0275**	non-linear regression; curve forced through 0	this evaluation

Annex 3 ETX-output

Parameters of the normal distribution

Name	Value	Description
mean	1.218807799	mean of the log toxicity values
s.d.	1.698131318	sample standard deviation
n	11	sample size

HC5 results

Name	Value	log10 (Value)	Description
LL HC5	0.000274523	-3.561421293	lower estimate of the HC5
HC5	0.021838079	-1.660785569	median estimate of the HC5
UL HC5	0.282227816	-0.549400186	upper estimate of the HC5
sprHC5	1028.066262	3.012021107	spread of the HC5 estimate

FA At HC5 results

Name	Value	Description
FA lower	0.695	5% confidence limit of the FA at standardised median logHC5
FA median	5	50% confidence limit of the FA at standardised median logHC5
FA upper	18.964	95% confidence limit of the FA at standardised median logHC5

HC50 results

Name	Value	log10 (Value)	Description
LL HC50	1.953516522	0.290817089	lower estimate of the HC50
HC50	16.55037349	1.218807799	median estimate of the HC50
UL HC50	140.216302	2.146798509	upper estimate of the HC50
sprHC50	71.77635837	1.85598142	spread of the HC50 estimate

FA At HC50 results

Name	Value	Description
FA lower	30.99676526	5% confidence limit of the FA at standardised median logHC50
FA median	49.99999998	50% confidence limit of the FA at standardised median logHC50
FA upper	69.00323477	95% confidence limit of the FA at standardised median logHC50

Anderson-Darling test for normality

Sign. level	Critical	Normal?		
0.1	0.631	Accepted		
0.05	0.752	Accepted	AD Statistic:	0.46482485
0.025	0.873	Accepted	n:	11
0.01	1.035	Accepted		

Kolmogorov-Smirnov test for normality

Sign. level	Critical	Normal?		
0.1	0.819	Accepted		
0.05	0.895	Accepted	KS Statistic:	0.674045549
0.025	0.995	Accepted	n:	11
0.01	1.035	Accepted		

Cramer von Mises test for normality

Sign. level	Critical	Normal?		
0.1	0.104	Accepted		
0.05	0.126	Accepted	CM Statistic:	0.064747367
0.025	0.148	Accepted	n:	11
0.01	0.179	Accepted		

Annex 4 Evaluation of rebuttal

Opdrachtgegevens

VSP rapportnummer	15065B00
Projectnummer	E/124016/07/AA
Opdrachtgever	Ctgb
Datum opdracht	10-01-2022
Datum rapportage	28-01-2022
Auteur(s)	[redacted]
Toetser	
Opdracht	Dit advies betreft een reactie op het bezwaar tegen de afleiding van de JG-MKN en MAC-MKN voor mesotrione in Adviesrapport 15065A00
Versie	CONCEPT voor Ctgb

Expert judgment on the derivation of the AA-EQS for surface water of mesotrione

1. Introduction and background

In a response to the rebuttal issued by Syngenta, this report presents an expert judgment concerning the derivation of the AA-EQS for surface water of the herbicide mesotrione, as described in RIVM-Report No. 15065A00.

Mesotrione is a herbicide that is authorised for use in maize. The current water quality standard is a Maximum Permissible Concentration (Maximaal Toelaatbaar Risiconiveau) of 0.077 µg/L. This value was originally derived in the context of the Pesticide Atlas and officially endorsed in 2014 (http://www.rivm.nl/rvs/). Syngenta, one of the registration holders of mesotrione in the Netherlands, requested an update of the water quality standards and submitted a statement and underlying data. The Ctgb commissioned RIVM to evaluate the submitted dossier, check for additional data in the open literature and derive an AA-EQS and a MAC-EQS for aquatic ecosystems according to the methodology of the Water Framework Directive (WFD), which were presented in the RIVM-report (Report No. 15065A00).

Following the Technical Guidance Document for deriving environmental quality standards in the context of the WFD (further referred to as WFD TG #27), the AA-EQS was derived deterministically by selecting the lowest relevant chronic endpoint and applying the corresponding assessment factor. The lowest relevant chronic endpoint selected was the E_rC_{10} value of 0.078 µg/L for *Myriophyllum spicatum* based on total shoot length. This value was not presented in the study report [redacted] 2017), but was recalculated by RIVM. In fact, for reasons of consistency, all EC_{50} and EC_{10} values from that study were recalculated by RIVM by non-linear regression using GraphPad. An assessment factor of 10 is applied because the substance has a known mode of action and representatives of the presumed most sensitive taxonomic groups (macrophytes; primary producers) are included in the dataset. The AA-$QS_{fw, eco}$ was therefore derived as 0.078 / 10 = 0.0078 µg/L = 7.8 ng/L. It was acknowledged in the RIVM report that this is an extrapolated value and as such less reliable. However, because 31% effect was observed at the lowest test concentration, it was not possible to follow the recommendation of the WFD TG#27 to use a NOEC in case a reliable EC_{10} cannot be estimated (see further below, section 2).

In the rebuttal, Syngenta argues that the data point used for the derivation of the AA-EQS (presented as 0.078 µg/L) is not credible or reliable because the E_rC_{10} concentration has been extrapolated well below the lowest test concentration (3.78 µg/L; mean measured). Syngenta therefore believes that this E_rC_{10} value for total shoot length (along with the similarly derived E_rC_{10} values for fresh weight and dry weight) should be regarded unreliable with a reliability score of 3 (RI=3) instead of 2 (Ri=2) and should therefore not be suitable for derivation of an AA-EQS.

Syngenta alternatively proposes to use the NOEC/EC_{10} value from the *Lemna gibba* study (Smyth et al. 1996) of 2.0 µg/L based on frond number with an assessment factor of 10 to get to an AA-EQSfw, eco of 0.2 µg/L. Syngenta justifies the use of an assessment factor of 10 as the standard prescribed assessment factor for 3 chronic data points.
This approach was also suggested in the RIVM-report as an alternative derivation of the AA-EQS, but with a higher assessment factor (than 10) due to the higher sensitivity of *M. spicatum* and uncertainty about the sensitivity of other macrophyte species. However, the choice of the factor would not be straightforward due to substantial effects at the lowest test concentration.

In their rebuttal, Syngenta disagrees with this approach on the assessment factor:
The “apparent much higher sensitivity of M. spicatum” claimed by Ctgb is based on extrapolated, unreliable data. Indeed based on the reliable EC50 values from Table 4 of 0.0211 and 0.0258 mg/L for L. gibba and M. spicatum, respectively, there is little difference in their sensitivity, with L.gibba, having a slightly lower endpoint. Therefore, the need to apply an additional AF is questioned. Furthermore,, as to “the uncertainty about the sensitivity of other macrophyte species”, uncertainty about the sensitivity of other species is always present in evaluation of QSs and precisely what the prescribed AF is there to cover.
The original table presenting the effect values derived for algae and aquatic plants in the RIVM report has been copied below (Table 1).

Table 6: Summary of effect values for algae and macrophytes. Selected values are given on a grey background (copied from RIVM Report No. 15065A00). All values in mg/L

Species	Test Endpoint	Time	NOEC	LOEC	EC_{10}	EC_{20}	EC_{50}	Remark	Reference
Navicula pelliculosa	growth rate	0-72 h	48	96			66	linear regression	[redacted] et al. (1997)
					51.0	53.2		non-linear regression	[redacted] (2012)
		24-96 h			**40.0**		**74**	non-linear regression; curve forced through 0	recalculated
Rhapidocelis subcapitata	growth rate	0-72 h	0.75	1.5			13	linear regression	[redacted] et al. (1997)
					0.93	1.66		non-linear regression	[redacted] (2013)
					0.93		**4.5**	non-linear regression	recalculated
Lemna gibba	growth rate (dwt)	0-14 d	0.002	0.004			0.0077	linear regression	Smyth et al. (1995)
					0.002	0.0047		non-linear regression	[redacted] (2013)
			0.002		0.0013		**0.021**	non-linear regression; curve forced through 0; NOEC selected (instead of the EC_{10} of 0.0013 mg/L) as growth rate was not inhibited at 2 µg/L	recalculated
Myriophyllum spicatum	growth rate (shoot length)	0-14 d	-	0.00477	0.000149 (0.149 µg/L)		0.0339	3-param. Normal CDF (cumulative distribution function)	[redacted] (2017)
					0.0000779 **(0.0779 µg/L)**		**0.0258**	non-linear regression; curve forced through 0	recalculated

2. Response RIVM

a. Reliability of the data point in question

The RIVM has re-evaluated the reliability of the recalculated E_rC_{10} values on the basis of several relevant guidelines and guidance documents. Specific sections within these documents have been highlighted in the table below (Table 2).

Table 7: Sections on ECx reliability in relevant guidelines and guidance documents

OECD TG 210: Fish, Early-life Stage Toxicity Test OECD (2013) Page 23	*… ECx should not require extrapolation outside the range of positive concentrations (Draper and Smith 1999, OECD 2006). For example, a general guide might be for ECx to be no more than about 25% below the lowest tested concentration or above the highest tested concentration.*
OECD TG 239: Water-Sediment Myriophyllum spicatum toxicity test OECD (2014) Page 6	*35. To determine an ECx, test concentrations should bracket the ECx to ensure an appropriate level of confidence. For example, if estimating the EC50, the highest test concentration should be greater than the EC50 value. If the EC50 value lies outside of the range of test concentrations, associated confidence intervals will be large and a proper assessment of the statistical fit of the model may not be possible. The use of more test concentrations will improve the confidence interval around the resulting ECx value.*
OECD Series on Testing and Assessment (54): Current Approaches In The Statistical Analysis Of Ecotoxicity Data: A Guidance To Application (OECD, 2006) Page 22	*29. Several limitations of concentration-response modelling are:* *• Estimation of ECx values outside the concentration range introduces a great deal of uncertainty (i.e., extrapolation outside the range of the data).*
OECD Series on Testing and Assessment (54): Current Approaches In The Statistical Analysis Of Ecotoxicity Data: A Guidance To Application (OECD, 2006) Page 88	*341. Because of the fact that a fitted statistical model only reflects the information in the data, extrapolation outside the range of observation is usually unwarranted. Therefore, estimating an ECx that is much lower than the lowest applied (nonzero) dose or concentration should be avoided.*
Technical guidance for deriving environmental qualilty standards. Guidance Document No. 27 (EC, 2018) Page 144 (WFD TG#27)	*For similar reasons, the data from tests resulting in an effect at the lowest test concentration should be tabulated as NOEC < or L(E)C50 <, followed by the value of the lowest test concentration. Although these values cannot be used directly for the derivation of EQSs, useful information can be obtained from comparing the sensitivity of that species with the EQS. This comparison may permit an adjustment to the AF. In the case of NOEC <, an attempt should be made to calculate the EC10, if possible.*

Technical guidance for deriving environmental qualilty standards. Guidance Document No. 27 (EC, 2018) Page 145 (WFD TG#27)	*When the growth rate ErC10 and ErC50 are not reported, these values should be re-calculated based on the raw data. Resulting values can be pooled to derive one value per species.* *For deriving the AA-EQS, the ErC10 as well as the NOEC can be used. For reliable estimates of ErC10 (i) the concentration-response curve needs to be consistent with a sigmoidal concentrationresponse relationship and (ii) sufficient concentrations should be used to define the ErC10 with an appropriate level of confidence, i.e. according to OECD 201 the concentration series should preferably cover the range causing 5-75 % effect.* *If it is not possible to recalculate the ErC10 because of missing data or estimates of the ErC10 are not reliable, preference should be given to the NOEC. Due to typical spacing of test concentrations (spacing factor <3.2 according to OECD 201), NOECs based on growth rate or yield are often identical. Pooling of NOECs for either growth rate or yield from different studies on the same species might be justified for AA-EQS derivations.*

Taking the above sections into consideration, the E_rC_{10} values indeed bear a considerable uncertainty as they are extrapolated outside of the concentration range.
To further investigate the suitability of the concerned E_rC_{10} value, RIVM assessed its reliability using the normalised width of confidence interval (NW) as reliability indicator, as proposed by EFSA in the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology; Appendix E Section 2.1 and 4.1 (EFSA, 2019). It should be noted that this document was developed in the framework of PPP authorisation and has not been discussed in the context of WFD TG#27. The NW is an indicator based on the relative width of the 95 % confidence interval around an EC10 value. It is calculated as the ratio between the width of the EC10 confidence interval and the median value of EC10:

$$NW = \frac{(EC_{10,upp} - EC_{10,low})}{EC_{10,med}}$$

An EC10 value with an NW value of > 2 is considered to offer a low reliability.
Below, multiple extrapolated E_rC_{10} values from the *M. spicatum* study based on total shoot length are presented (Table 3). One E_rC_{10} value is from the original report (A), one from the RIVM-report (B) and another one which was recalculated in an additional exercise by the RIVM for this purpose (C). The latter E_rC_{10} value is slightly higher due to the use of mean measured concentrations instead of nominal concentrations and the use of a lower zero-concentration within GraphPad that represents the negative control. Other than that, the statistical approach for the E_rC_{10} values of B and C are equal.

Table 3: Evaluation of EC_{10} according to EFSA

	Mesotrione 14-d E_rC_{10} (shoot length) on *M. spicatum* [µg/L]		
	A: study report	B: 1st recalculation[a]	C: 2nd recalculation[b]
EC10,med	0.149	0.07786	0.08451
EC10,low	0.024	0.00305	0.007389
EC10,upp	0.93	0.4289	0.4012
NW	**6.080536913**	**5.469432314**	**4.659933736**

a: recalculation by non-linear regression with GraphPad using growth rate of individual replicates.
b: In addition to (a), also includes further statistical refinement and corrections on mean measured concentrations.

Even though the NW from the second recalculation is lower than from the original report, the NW remains well above 2. Given the fact that the NW values are well above 2, and that the E_rC_{10} values are extrapolated well below the concentration range, the extrapolated E_rC_{10} would not be considered reliable for the derivation of an AA-EQS according to the EFSA-guidance.

b. Assessment factor on the *L. gibba* NOEC

Syngenta questions the need for a higher assessment factor than 10 as they argue that there is little difference in sensitivity to Mesotrione between *L. gibba* and *M. spicatum*, given the E_rC_{50} values 0.0211 and 0.0258 mg/L, respectively.
RIVM disagrees with this statement.

First of all, Syngenta argues there is little difference in sensitivity to Mesotrione between *L. gibba* and *M. spicatum*, given the E_rC_{50} values 0.0211 and ~~0.0258~~ 0.0275 mg/L, respectively. Indeed, the difference is minor, with the *L. gibba* E_rC_{50} value being the lowest. However, these values indicate the sensitivity of the macrophytes measured at the 50%-effect level, and not the sensitivity at the no-effect level - which is relevant in this case.

Secondly, even though we acknowledge the low reliability of the derived chronic effect values (as discussed above), it is clear that significant effects on all relevant parameters (**30.9%** reduction in total shoot length growth rate; **19.5%** reduction in mean shoot wet weight growth rate; and **28.1%** reduction in mean shoot dry weight growth rate) in *M. spicatum* are observed at the lowest test concentration (3.76 µg/L; mean measured). The study reports also indicate that the NOECs could not be determined and that the LOECs are the lowest test concentration. These are clear indications that the actual chronic no-effect values (represented by NOEC or E_rC_{10}) for *M. spicatum* are likely to be below the NOEC of 2.0 in *L. gibba*; and simply cannot be ignored in this AA-EQS derivation.

The WFD TG #27 describes that a LOEC could be used in case no NOEC is available and when percentage of effect of the LOEC is > 10 and < 20%. Only then, a NOEC can be calculated as LOEC/2. This is only the case for reduction in growth rate based on mean shoot wet weight, at which the LOEC concerns 19.5% effect. The NOEC for this parameters could then be calculated as 3.76/2 = 1.88 µg/L. For the other two parameters, the % effect in the LOEC concentration is higher than 20%, which would result in lower NOEC values, further indicating a higher chronic sensitivity in *M. spicatum* over *L. gibba*. Using this NOEC would thus overlook the fact that the assessment should be based on the critical parameter observed in a macrophyte test.

All of this uncertainty could have been (partially) avoided if more care was taken for the design of the toxicity study of *M. spicatum*. No reasoning is provided for the selection of the (too high) concentration range. Likewise, no range-finding study was conducted prior to the test to get an idea of the toxicity of the substance to *M. spicatum* and select a proper concentration range.

Furthermore, two toxicity studies on another macrophyte *L. gibba* have been completed prior to the start of the *M. spicatum* study. These studies also provide useful information on the general sensitivity of water plants to the test substance (NOEC/EC10 of 2.0 ug/L). With this in mind, a reliable chronic effect value for *M. spicatum* could have been derived if the study was designed differently.

As noted in Table 2 above, the WFD TG #27 also has a specific section on 'Dealing with toxicity values higher or lower than range of test concentrations' (Section A1.3.2.9.), which describes the following:
'[…] *For similar reasons, the data from tests resulting in an effect at the lowest test concentration should be tabulated as NOEC < or L(E)C50 <, followed by the value of the lowest test concentration. Although these values cannot be used directly for the derivation of EQSs, useful information can be obtained from comparing the sensitivity of that species with the EQS. This comparison may permit an adjustment to the AF. In the case of NOEC <, an attempt should be made to calculate the EC10, if possible.*'

In the current situation, useful information on the chronic sensitivity of *M. spicatum* is obtained, which requires an adjustment of the assessment factor, in case the NOEC of 2.0 µg/L is chosen as the critical effect value. According to the WFD TG#27, an assessment factor of 10 should not be used when it is not possible to determine with high probability that the most sensitive species has been examined (footnote d to Table 3 of WFD TG#27). In such case, an assessment factor of 50 should be applied to take into account any interspecies variation in sensitivity.

Given the above, RIVM agrees to use the NOEC of 2.0 observed in the *L. gibba* study, but believes an assessment factor of 50 should be used (instead of 10) to compensate for the remaining uncertainty on the higher chronic sensitivity of *M. spicatum*. This would lead to a (realistic worst-case) $QS_{fw, eco}$ of 0.04 µg/L.

Within the framework of the Dutch national policy on water quality, RIVM also derives indicative EQS values, is based on the WFD derivation methodology. Within the guidance for derivation of indicative EQS values, a LOEC can be divided by 10 in case a NOEC cannot be determined. Using the current dataset, this would allow the use of the LOEC/10 of the *M. spicatum* study (3.78/10 = 0.378 µg/L) for an indicative AA-$QS_{fw, eco}$ with an assessment factor of 10; resulting in an indicative $QS_{fw, eco}$ of 0.0378 µg/L. Using this alternative approach leads to a similar $QS_{fw, eco}$ value.

3. Conclusion

The derivation of the $QS_{fw, eco}$ of mesotrione in RIVM-report 15065A00 has been reevaluated. Based on the above, the $QS_{fw, eco}$ is derived from the lowest reliable chronic toxicity value available from the laboratory data; the NOEC value of 2 µg/L for *L. gibba*. An assessment factor of 50 is applied because reliable long-term toxicity results are available from at least three species across three trophic levels, but it is not possible to determine with high probability that the most sensitive species are included within this dataset. An assessment factor of 50 is therefore required to take into account any interspecies variation in sensitivity. The $QS_{fw, eco}$ is 2 / 50 = 0.040 µg/L = 40 ng/L. The $QS_{sw, eco}$ is 0.0040 µg/L = 4.0 ng/L.

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Annex 5 Commentaren Petit Comité WK normstelling

versie A00

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
7	2.4 (Human toxicology)	The Harmonized Classification also reports STOT Re 2 (specific taret organ toxicity through prolonged or repreated exposure), for eyes and the nervous system. According to the RIVM 2015 guidance, part 3, chapter 1.3.2. this is also considered a trigger, so we have two triggers for including the QSwater, hh food (human assessment and secondary poisoning).	Added
10	Table 5	The Fathead minnow (Pimephales promelas) endpoint for mesotrione in the EFSA conclusion is listed with NOEC (physical symptoms) = 12.5 mg test item/Lnom (36d study flow through). Also, in Table A1.2 in the Appendix you ist the 12.5 mg/L endpoint (NOEC) as the selected one. Are you sure >200 is correct? If so, where does the difference come from (the reason should be explained in a footnote). (It does not have an impact on the derivation of the quality standards.)	Checked. NOEC ≥200 included because of no significant difference in hatch/survival at highest test concentration. 12.5 selected for Table 5.

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
17	Annex I, Legend	Reference [7] is the EFSA conclusion and [6] is the RAR.	Changed
17ff	Table A1.1	Footenote variant X^a is missing. The meaning of *a* could not be found.	X^a stands for the description of the given note for the formulated product. As this is also highlighted under the 'Test comp.' column, it becomes redundant and will be deleted.
17ff	Table A1.1 and A1.2	We agree in principle that recalculations of EC50 values were required as different fitting models for the dose response were used for the new EC10/20 calculations. Was the impact on the endpoint value structually more or less conservative across the dataset? For transparency reasons, it would be great, if recalculated (differing as compared to the EFSA conclusion) EC50 values could be somhow marked (with a * or similar) in the table (e.g. the acute 4.5 mg/L value for Raphidocelis, if the study is from the RAR/ EFSA conclusion.)	The recalculated acute values for R. subcapitata and L. gibba do not have an exact RAR/EFSA conclusion-equivalent (72-h ErC50 and 14-d ErC50, respectively). The values given the EFSA conclusion report are either derived at 120 hours (R. subcapitata) or based on biomass (L. gibba). The N. pelliculosa values are not mentioned in the EFSA Peer review conclusion. **N. pelliculosa** Original report: 96-h ErC50: 88 mg/L EFSA LOEP: 120-h ERC50: >96 mg/L Recalc: 96-h ErC50: 74 mg/L **R. subcapitata:** EFSA conclusion: 120-h ErC50: 13 mg/L Recalc: 72-h ErC50: 14.5 mg/L

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
			L. gibba EFSA conclusion: 14-d EbC50 (for dry weight): 0.0077 mg/L Recalc: 14-d ErC50: 0.0211 mg/L We added an annex with the original and recalculated values, but only for the lowest relevant endpoints and test durations
20	Table A1.1 footnotes	Footnote 16: 'analytical method described but not reported' – what does this mean? And in what way are the results preliminary? Is it useful to incude this endpoint?	In the publication by Elskus *et al*. (2007), the materials and methods describe the following: "*To determine if nominal dosing concentrations reflect actual dosing concentrations, we are optimizing protocols for analyzing the concentration of active ingredients in our dosing solutions at the start and conclusion of the 5-d (day) exposure periods.*" However, results on measured concentrations at start and end of the 5-day exposure are not provided. Perhaps this is because the analytical method was not finalised yet. The study also included a preliminary study on the effects of mesotrione (and other compounds) on immune function and several other parameters. In

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
			this preliminary study, no significant effects on mesotrione were observed. The wording in our report will be made more clear.
22 10 17	Table A1.2 versus Table 5 versus Table A1.1	The diatom *Amphora coffeaeformis* is missing from the chronic evaluation table, while an endpoint of >0.2 mg/L is reported as being used in Table 5 for chronic effects. In Table A1.1 for the acute studies the study [24] is listed with a differentt endpoint, but is considered unreliable Ri3. Can you check and correct accordingly?	Corrected
21/22	Table A1.2	Study [24]: *Ankistrodesmus* has two data entries in this table (it is not a diatom, but a green alga by the way, one record is listed under diatoms). For the first record (endpoint generation time), the study received an Ri2 for the other record the study received and Ri3, the notes do not distinuguish why there is a difference in acceptability of the same study. From note 3 it is understood that the control showed irregular growth pattern and that the exposure sort of has been checked but nothing is	This is indeed an error. Study [24] is not reliable. Corrected.

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
		reported? Its a bit unclear from the notes in how far the results can be used as supporting information or are invalid. Please check and clarify or correct.	
21/22	Table A1.2	Note 8 (Daphnia dry weight endpoint): While I appreciate that the chosen approach is in line with the RAR and the EFSA conclusion, it is not clear on which basis the dry weight effect NOEC <97 mg/L is condidered unrelated to the treatment and is this not considered. Could the note contain this explanation?	Explanation added: dry weight was not considered a biologically relevant endpoint. Main reason is that there is no relationship with concentration
21/22	Table A1.2	Note 16[a]: *a* not denoted in footnotes	Deleted, see previous comment.
Reference List	Ref [1]	The WFD GD #27 has a new version from 2018. Please check and potentially update accordingly.	The correct version of the guidance was followed
10	3.2.1	MAC-EQS derivation, AF aproach: there is basically only one ('sort of') marine species included in the dataset (*Navicula*). That means separate analyses for salt- and freshwater indeed do not make sense and the data should be pooled. That was done for the MAC-EQS, but its not mentioned anywhere in this	A total AF of 100 for the MAC-EQS(sw) is already applied, but indirectly. For the MAC-EQS(sw), an AF of 10 is applied on the MAC-EQS(fw), which, on its own, is derived on an AF of 10. (=factor of 100 on the lowest exp. EC_{50}/LC_{50}) In the current derivation of the EQS, the N. pelliculosa species is

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
		section. Only in the 3.2.3 'Selection of MAC-QS' it is mentioned. I think the reasoning for the derivation of the saltwater MAC-QS could be improved: in my view the total AF should be 100, as we have no 'additional specific marine species', but the MoA is known and representative species of the most sensitve group have been tested (see 2018 WFD guidance doc nr 27, Table 4, p51). I agree with using the *Lemna* endpoint for the deterministic approach and also with reducing the assessment factor for freshwater to 10 due to known MoA and due to the coverage of the most sensitve groups. Please add the saltwater MAC-EQS here as well for reasons of completeness.	considered a freshwater diatom as the results from this species are from study performed in freshwater. Comment added in text MACsw added in text
10	3.2.2	Please add in the third paragraph that 3 out of 7 endpoints were unbound values. This really decreases the value of the SSD further.	Added. (in fact it's 2 because the Danio endpoint was not included)
11	3.2.2	MAC-QS statistical extrapolation, SSD approach:	There are insufficient reliable experimental information on

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
		-as mesotrione has a known MoA and there is a clear difference in the acute toxicity to primary producers as compared to the other tested decomposers / heterotroph organisms. So, according to the nr27 guidance, an SSD approach for this type of compound would anyway only be acceptable, if sufficient data on primary producers would be available (i.e. min 10 data points). This is clearly not the case, and therefore cannot be used, but we should also at least mention that in the context of the presented SSD not having so much value for the MAC-QS derivation. The snetence '*The limited fit may be explained by the specific mode of action (HPPD inhibition) which targets photosynthetic mechanism in higher plants in particular. More data for algae and macrophytes would be needed to improve fitting of the lower left side of the SSD and/or to allow for construction of a specific SSD.' t*ouches upon that, but actually a separate SSD should be contructed only including primary producers, so	specifically primary producers to derive an such an SSD. As described in the text, the SSD-result can only be used as supportive for the deterministic approach.

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
		it should be modifed accordingly. - the statistics for the goodness of fit should be reported.	
12	3.3.1	I agree with the derivation of the QS fw,eco and QS sw,eco! Maybe be more clear on the reasoning for the total AF ater (similar to comment above, reference WFD GD nr 27, Table 6, p57).	
12	3.3.2	Secondary poisoning assessment is missing (QSbiota,fw and QSbiota,sw). Not triggered due to log pow < 3, but should be mentioned.	New header under 2.3. and lines added in text under 3.3.3
12	4	I agree with both the elaboration on the derivation of the MAC-QS and the AA-QS, in relation to the applicants proposal.	
General	Footnotes to evaluation table	In some footnotes it is mentioned that the *'bottom was set to 0 because otherwise EC50 did not match observed inhibtion'* its nto really clear what is meant by that. I am guessing, but maybe something like: data were normalized and the lowest and highest response was fixed (or contrained) to 0 and 1) respectively?	Text changed into 'curve forced through 0'.

D. ten Hulscher			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
2	-	De afleiding is beoordeeld door de leden van de Wetenschappelijke Klankbordgroep normstelling water en lucht Moet dat zijn: petit comité of gaat het nog naar alle leden	vervangen door het 'Petit comité van de WK normstelling water en lucht'.
8	3.1 2e alinea	In de tekst staat: …….EC_{50}-values obtained by linear regression. For the RAR-dossier, the applicant submitted statistical reports in which additional EC_{10} and EC_{20}-values were estimated by non-linear regression. **Vraag:** is non-linear regression gebruikelijk om EC10 en EC20 waarden te verkrijgen? Is er iets bijzonders aan de hand met de experimenten?	Statistisch afleiden van EC_x waarden voor algen (en macrophyten) op basis van non-lineaire regressie heeft de voorkeur. Dit staat ook beschreven in de OECD TG 201: '*The aim is to obtain a quantitative concentration-response relationship by regression analysis. It is possible to use a weighted linear regression after having performed a linearising transformation of the response data - for instance into probit or logit or Weibull units (8), but non-linear regression procedures* *are preferred techniques that better handle unavoidable data irregularities and deviations from smooth distributions.*' we voegen opmerking toe

D. ten Hulscher			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
12	3.3.1 laatste zin	MAC-EQS_{sw} moet zijn MAC-$EQS_{sw, eco}$	Eens. Wordt aangepast.

W. Peijnenburg			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
5	1.3.3	Hier wordt aangegeven dat voor testen met bacteriën het accoord is om geen analytische verificatie van de blootstellingsconcentratie te hebben vanwege de korte duur van de experimenten. Hier ben ik het niet per se mee eens: het gaat immers om het bevestigen van de actuele blootstellingsconcentratie en die zou bij het begin van de test gemeten moeten worden. Verdere verificatie na 9 uur lijkt me dan inderdaad niet nodig. Toevoeging: Op pagina 8 staat vervolgens dat alle bacterie-testen op een na zijn afgewezen vanwege dit aspect. Hier is niet duidelijk of in de ene studie die wel meegenomen is, de exposure concentratie is gemeten. Wellciht kan dit aspect op beide plaatsen nog eens bekeken worden.	- Verificatie van de test concentraties zou inderdaad helpen bij het beoordelen van de studie. Echter, met een photolytische halfwaardetijd van ~13 dagen, wordt er in 15 minuten en 9 uur geen significante degradatie verwacht. In de photolyse studie wordt ook nog amper degradatie gezien na 24 uur (~98,5% mesotrione nog aanwezig). Ondanks deze beperking zien wij deze resultaten waardevol genoeg om mee te nemen in de beoordeling. - Die zin op pagina 8 is niet helemaal kloppend inderdaad. De [8] studie heeft wel gemeten

W. Peijnenburg			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
			concentraties (en is dus wel 'reliable' en geen uitzondering. Aangepast.
10	3.2.1	21.2 moet 21.1 zijn	Aangepast.
11	3.2.2	Ik vraag me af of meer algen data daadwerkelijk gaan helpen: de algendata die er zijn, wijzen er op dat algen veel minder gevoelig zijn dan de macrofyten.	Wel mee eens. Ik heb 'algen' in de tekst nu weggelaten.

versie A01

Willie Peijnenburg (RIVM): no comments (email message 22-06-2022)
Dorien ten Hulscher (RWS): no comments (email message 23-06-2022)

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
General comment on Annex 4	n.aa	Op 20th April 2022 the ctgb sent comments on the RIVM evaluation of the Syngenta rebuttal. These seem to not have been included into the Annex 4 and neither clarified why they were naken up nor addressed to my current knowledge. Those comments will be reiterated below in reference to Annex 4 (textual comments were not repeated).	Our apologies for missing your comments. Thank you for rephrasing them here.

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
8 9 12 14	3.1.1 Table 4 3.3.1 4	We agree with the principal text anc content, however please check reconsider the Myriophyllum endpoints after having addressed below comments regarding the quesiton of nominal and mean measured concentrations.	See below
14	4	The last concluding sentecne should mark the changed EQS in red too: 'The AA-EQS_{fw} of mesotrione is **40** ng/L, the AA-EQS_{sw} is **4.0** ng/L.'	Red text was only added for convenience and is removed in the final version.
21 and 26	Annex 1, Table A1.1. and A1.2	In the interest of transparancey please explain why the Myriohyllum endpoint was changed since the last time. Also, please see comment below on Annex 4 regarding the choice of mean measued versus nominal concentrations.	The first recalculation was based on the nominal concentrations of mesotrione instead of the nominal concentrations of the **test item**. The second recalculation was done on the actual measured mesotrione concentrations in the overlying water instead of the nominal concentrations. This is explained on page 35.
28	Annex 2	Consider adjustment of Myriophylum endpoint after considerint below comment on nominal versuus mean measured conc (annex 4)	See below
31	Annex 4 - rebuttal	Please add the part in italics: 'However, because 31% *(growth*	Added. But why italics?

Mascha Rubach, Ctgb			
Pagina	**Paragraaf**	**Opmerking**	**Reactie**
		rate, TSL) and 54% (yield, TSL) effect was observed at the lowest test concentration […].	
32	Annex 4 – rebuttal First paragraph, first sentence	The sentence refers to the lowest concnetration being 3.78 µg/L mean measured. In the MKN report it is stated that the lowest actual concentration was 4.04 µg a.s./L. I checked the study report and 4.04 µg a.s./L is related to pure mesotrione nominal. As the concentrations remained within the 80-120% range the nominal concentrations would be acceptable and therefore the mean measured value is not relevant. I suggest to delete therefore and use the 4.04 µg/L nominal value).	Indeed, the (individual) measured concentrations of mesotrione remain within 80-120% of the nominal concentrations of mesotrione. However, we believe that the effect values based on the measured concentrations provide a more accurate representation of the true toxicity of mesotrione. Therefore, RIVM uses actual measured concentrations where available. Furthermore, the effect values based on the mean measured concentrations are more conservative compared to the nominal-based effect values. In Table 3, the 1st recalculation (B) is now presented as the lowest EC10 value; but this is biased as the use of a lower zero-concentration within GraphPad representing the negative control was also not taken into account here. In the end; the effect values based on the mean measured

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			concentrations are more conservative. The main point remains: the values (whatever the calculation used) is well below the lowest test concentration and therefore not reliable.
34	Annex 4 – rebuttal Table 2	Between OECD54 and WFD GD 27 I would add the EFSA EN1673 here too as follows: Column 1: Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology 2019, Appendix E, in general and in particular sections 2.1 and 4.1.; (EFSA Supporting publication 2019: EN-1673) Column 2: 2.1 Normalised width of confidence interval The normalised width of confidence interval (NW) is an indicator based on the relative width of the 95 % confidence interval around the EC10 value. It is calculated as the ratio between the width of the EC10	We are not convinved that this should be added in Table 2 as it does not touch on how to deal with reliability of extrapolated ECx values. The suggested addition only specifies the NW and how to use it. This is already covered on page 35.

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		confidence interval and the median value of EC10. $NW = (EC10,upp - EC10,low)/EC10,med$ Please note that this indicator is unrelated to the shape of the dose–response curve. The relevance of this estimation for the hazard characterization is not immediately interpretable. In principle, this indicator is applicable to any ECX estimation, not just EC10. 4.1 NW-based classification To implement this classification, it was considered that a NW < 0.2 should be considered as ideal. In this situation, we have 95 % confidence in saying that the true EC10 will not be outside the estimated EC10 ± 10 %. In the database, around 10 % of studies satisfied this condition. At the other end of the range, it was considered that when NW > 2, EC10 estimations are likely to offer rather low reliability. In the database, this situation occurred in 12 % of cases.	

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		Intermediate scenarios and their relative occurrence in the database are detailed in Table E9. Table E9: Normalised width-based classification (non relevant information deleted from table) NW Rating < 0.2 Excellent 0.2-0.5 Good < 1 Fair < 2 Poor ≥ 2 Bad	
35	Annex 4 1st pgf after table	'It should be noted that this document was developed in the framework of PPP authorisation and has not been discussed in the context of WFD TG#27.' Comment: That's formally correct, but it would only be problematic if the 95% CIs were not acceptable in the context of the WFD I think.	
35	Annex 4 2nd pgf after table	'The latter E_rC_{10} value is slightly higher due to the use of **mean measured concentrations instead of nominal concentrations** and the use of a lower zero-concentration within GraphPad that represents the negative control.' Comment:	See previous reaction

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		Why were mean measured concentrations used for this exercise? Can a justification be given on why this was done? [The concentrations in the study remained within the required range of 80-120% of nominal and therefore results can and should be based on nominal (pure) a.s. content. I also thought that the original recalculation, leading to 0.0779 µg/L was based on nominal.] Its not immediately evident that the EC50 would be higher instead of lower when mean measured would be used.	
36ff	Table 3, foot notes and following text	Please conside the point of nominal versus mean measured concnetrations made above and clarify in the text. In relative sense it does make a difference for the NW and it also does not impact the AA-EQS. I only feel that we should be correct in derving the endpoints based on nomincal or mean measured and accordint to guidance.	See previous reaction