Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Chlorsulfuron (ISO); 2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulphonamide

EC number: 265-268-5
CAS number: 64902-72-3

CLH-O-0000001412-86-48/F

Adopted

4 December 2014

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.
All attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website.

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Substance name: **chlorsulfuron (ISO); 2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] benzenesulphonamide**
CAS number: **64902-72-3**
EC number: **265-268-5**
Dossier submitter: **Poland**

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	Germany		MemberState	1
Comment received				
The German CA supports the proposed environmental classification and labelling as Aquatic acute 1 (H400) Aquatic chronic 1 (H410). We do not support the chronic M-factor of 1000 (acute) and 100 (chronic), because recalculation of the relevant ErC50 and NOEC is necessary. From our point of view also human health endpoints should be evaluated for possible classification.				
Dossier Submitter's Response				
ECHA comment: The human health hazard classes were not addressed by the dossier submitter and thus not open during public consultation or for RAC assessment. However, we have taken note of your comment and will consider possible actions. **Dossier Submitter**: We agree with German CA comments concerning recalculation of the relevant ErC_{50} and NOEC. 7 and 14 day growth endpoints, based on previously reported data (Boeri et al., 2002) have been re-calculated and included in this document (see the section "Supplemental information - In depth analyses by RAC" in Annex 1). The re-calculated value of ErC50 and NOEC was used in the classification and labelling of chlorsulfuron (ISO).				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	France		MemberState	2
Comment received				
There is a typo in the CA substance name. It should be written benzenesulFonamide (not benzene sulphonamide).				
Dossier Submitter's Response				
We would like to thank France CA for indicating these typo in substance name.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2014	France		MemberState	3
Comment received				
France thinks that the proposal Carc. Cat. 2; H351 for chlorsulfuron should be discussed.				
Dossier Submitter's Response				
ECHA comment: The human health hazard classes were not addressed by the dossier submitter and thus not open during public consultation or for RAC assessment. However, we have taken note of your comment and will consider possible actions.				
RAC's response				
Noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	Germany		MemberState	4
Comment received				
During the pesticides peer review of chlorsulfuron, a classification with R40 (equivalent to Carc. Cat 2/H351) was proposed based on testicular interstitial cell tumours in rats (EFSA Scientific Report (2008) 201, 1-107). The relevant effects were described and discussed more detailed in the DAR, which is publically available from EFSA. Please address the need for classification regarding carcinogenic properties.				
Dossier Submitter's Response				
ECHA comment: The human health hazard classes were not addressed by the dossier submitter and thus not open during public consultation or for RAC assessment. However, we have taken note of your comment and will consider possible actions.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2014	France		MemberState	5
Comment received				
P8: A proposal for Carc. Cat. 2; H351 (limited evidence of carcinogenic response) was made in the EFSA review. It is stated on page 8 that the DS thinks that the proposed classification is not warranted because there was no statistically increase in the overall (unilateral and bilateral) Leydig cell tumour incidence. Nevertheless, the carcinogenicity of chlorsulfuron is not discussed in the CLH report. Therefore, the conclusion in Table 3 page 6 "conclusive but not sufficient for classification" is not adequate. It is to be noted that, in an addendum (January 2012) to the monograph, a new study on the active substance chlorsulfuron was examined and it was concluded that chlorsulfuron was a weak aromatase inhibitor. Moreover, it has been shown that a structurally similar compound, namely triflusulfuron-methyl, was an aromatase inhibitor and it was classified as Carc. Cat. 2; H351 based on an increased in Leydig cell tumours in rats. So, France thinks that the proposal Carc. Cat. 2; H351 for chlorsulfuron should be discussed.				
Dossier Submitter's Response				
ECHA comment: The human health hazard classes were not addressed by the dossier submitter and thus not open during public consultation or for RAC assessment. However, we have taken note of your comment and will consider possible actions.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	Germany		MemberState	6
Comment received				
Please discuss whether the findings observed in the 2-generation study in rats (Mylchreest, 2005) which were described in the DAR trigger classification for effects on reproduction. Effects were summarised as follows: Effects on the reproductive organs of males (decreased weight of epididymides and testes, increased number of epididymal sperm) and females (decreased number of ovarian follicles), and increased male ratio of f1 offspring. The relevant effects were described and discussed more detailed in the DAR, which is publically available from EFSA. In the past, similar findings led to classification proposals by RAC.				
Dossier Submitter's Response				
ECHA comment: The human health hazard classes were not addressed by the dossier submitter and thus not open during public consultation or for RAC assessment. However, we have taken note of your comment and will consider possible actions.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the aquatic environment

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	Germany		MemberState	7
Comment received				
The use of data from aquatic plant tests instead of algae tests is usual for classification and labeling purposes. As a general remark we suggest using EC50 values at day 7 (if available) instead of data at day 14 from aquatic plant toxicity tests for classification and labeling of acute effects of the substance. page 36: Blasberg, J.; Hicks, S.L.; Stratton, J.L. (1991) This study with Selenastrum capricornutum was run over 5 days (120 hours). The tested concentrations given in study description on page 37 (0.01, 0.03, 0.06, 0.12 and 0.24 mg chlorsulfuron/L) are not the same as given in table 17 (0.01, 0.018, 0.032, 0.058 and 0.103 mg/L). We would prefer to give all raw data (cell counts at 0, 24 and 48 hours) for completion of table 17 and for better evaluation. We cannot find data for growth rate calculation in table 17. For classification and labelling purposes ErC50 values (if possible to obtain) are preferred. page 37: Boeri, R.L.; Wyskiel, D.C.; Ward, T.J. (2000) This study with Anabaena flos-aquae was run over 5 days (120 hours). We would prefer to give all raw data (at 0, 24, 48, 72 and 96 hours) for completion of table 18 and for better evaluation. We would prefer to give EC50 data for growth and growth rate at 72 and 96 hours and NOEC, additionally. page 38: Boeri, R.L.; Wyskiel, D.C.; Ward, T.J. (2002) This study with the duckweed Lemna gibba was run over a period of 14 days. The EC50 (14d) is given as 0.00042 mg/L (nominal) and the NOEC (14d) is given as 0.00024 mg/L (nominal) related to healthy frond count. The initially (0 hours) measured test concentrations were 93 -110 % of the nominal concentrations. But the measured test concentrations at the end of the study (day 14) were below LOQ (limit of quantification) of 0.0000132 mg/L at all concentrations. In that case the use of nominal concentrations for calculation of EC50/NOEC is not in compliance with the test guideline. We therefore suggest to recalculate all EC50/NOEC data under consideration of mean measured test concentrations (geometric mean of initially measured concentration and one half of LOQ) to correct these data to real measured concentrations.				

The cited paper of McKelvey (2011) where a detailed calculation of average specific growth rate was provided and the ErC50 (14d) = 0.00069 mg/L has been determined is not available in the DAR addendum (last addendum was January 2012). Please indicate the year of the respective addendum in order to ensure traceability of the presented data. We assume that the calculation has been done with nominal concentrations from the original test data. In our opinion the ErC50 value should therefore also be recalculated based on mean measured concentrations.
For classification and labelling of the acute hazard we suggest using a newly calculated ErC50 (7d) based on measured concentration instead of ErC50 (14d) = 0.00069 mg/L (nominal).

Page 40: Comparison with criteria for environmental hazards
Please correct the relevant data for classification and labelling according to the suggested recalculated ErC50 and NOEC values of the 3 tests mentioned above.

Page 41: Conclusions on classification and labeling for environmental hazards:
Please use the correct values for acute and chronic aquatic classification (ErC50 (7d) and NOEC (14d). We do not support the chronic M-factor of 1000 (acute) and 100 (chronic) based on study with Lemna gibba (ErC50 (14d) of 0.00069 mg/L and NOEC (14d) of 0.00024 mg/L), because a recalculation of relevant ErC50 (7d) and NOEC (14d) related to mean measured concentration is necessary and these results are not yet available.

Dossier Submitter's Response

Comment*: The use of data from aquatic plant tests instead of algae tests is usual for classification and labeling purposes. As a general remark we suggest using EC50 values at day 7 (if available) instead of data at day 14 from aquatic plant toxicity tests for classification and labeling of acute effects of the substance.*

Response: We agree with German CA comments concerning recalculation of the relevant ErC_{50} and NOEC.
7 and 14 day growth endpoints, based on previously reported data (Boeri et al., 2002) have been re-calculated (see the section "Supplemental information - In depth analyses by RAC" in Annex 1) for use in the classification and labelling of chlorsulfuron (ISO).

Comment*: page 36: Blasberg, J.; Hicks, S.L.; Stratton, J.L. (1991)*
This study with Selenastrum capricornutum was run over 5 days (120 hours). The tested concentrations given in study description on page 37 (0.01, 0.03, 0.06, 0.12 and 0.24 mg chlorsulfuron/L) are not the same as given in table 17 (0.01, 0.018, 0.032, 0.058 and 0.103 mg/L). We would prefer to give all raw data (cell counts at 0, 24 and 48 hours) for completion of table 17 and for better evaluation. We cannot find data for growth rate calculation in table 17. For classification and labelling purposes ErC50 values (if possible to obtain) are preferred.

Response: The tested concentrations given in the study description on page 37 of the CLH report (0.010, 0.03, 0.06, 0.12, and 0.24 mg/L) are incorrect, and most likely a typographical error. The concentrations tested in the study are correctly mentioned in Table 17 (0.01, 0.018, 0.032, 0.058 and 0.103 mg/L) of the CLH report.
The following table presents the 0, 24 and 48 hour cell count data that was requested, in addition to the previously submitted 72, 96, and 120 hour cell counts (Blasberg et al., 1991; AMR 2081-91).

Nominal concentration (mg/L)	**Mean (3 flasks) cell counts (cells x 10^4/mL)**					
	0-hour	**24-hour**	**48-hour**	**72-hour**	**96-hour**	**120-hour**
Blank control	0.26	1.1	2.7	14	39[a]	110
Vehicle control	0.33	0.63	2.4	9.7	27	94
0.010		1.0	2.2	13	36	100
0.018		0.78	2.3	7.9	30	72*
0.032		0.70	1.7	7.7	30	59*
0.058		0.70	1.4*	5.6*	17*	40*
0.103		0.41	1.3*	3.1*	12*	28*

* Significantly different from the control by Dunnet's test criteria, $\alpha = 0.05$
[a] Reported incorrectly as 29 in original submission.

Although it is agreed to report the E_rC_{50} for classification and labelling, this study did not calculate these endpoints. Re-calculation of the growth endpoint is possible if the raw data for cell counts per replicate is available. However, this data is not available in the report, and only treatment means are given. Although it may be possible to fit a curve to the reported treatment means, the confidence bounds would be incorrect and checks for normality and variance homogeneity will be compromised. Therefore, re-calculation of growth endpoints was not considered necessary.

***Comment**: page 37: Boeri, R.L.; Wyskiel, D.C.; Ward, T.J. (2000)*
This study with Anabaena flos-aquae was run over 5 days (120 hours). We would prefer to give all raw data (at 0, 24, 48, 72 and 96 hours) for completion of table 18 and for better evaluation. We would prefer to give EC50 data for growth and growth rate at 72 and 96 hours and NOEC, additionally.

Response: From Boeri et al., 2000 (DuPont-4466):
The average specific growth rate was calculated using the following equation:

$$\text{Growth Rate} = \frac{\ln N_n - \ln N_0}{t_n}$$

where t_n is the time of observation in hours measured from the initiation of the test N_0 and N_n are the initial and subsequent densities (cells/mL) corresponding to the observation time. The table below provides the requested raw data (with the exception of the 0 hour data).

Mean, measured concentration (mg/L)	Average specific growth rate (% of control)				
	24-hour	48-hour	72-hour	96-hour	120-hour
Control	0.150	0.103	0.073	0.058	0.050
0.236	0.121 (81)	0.089 (86)	0.067 (92)	0.058 (100)	0.050 (100)
0.485	0.120 (80)	0.085 (83)	0.067 (92)	0.055 (95)	0.047 (94)
0.961	0.125 (83)	0.069 (67)	0.057 (78)	0.042 (72)	0.042 (84)
1.92	0.118 (79)	0.064 (62)	0.042 (58)	0.030 (52)	0.020 (40)
3.95	0.108 (72)	0.054 (52)	0.037 (51)	0.015 (26)	0.010 (20)

The requested 72, 96, and 120 hour EC_{50} values based on growth rate are provided below. In addition, the 120 hour NOEC value is also provided.
72 hour E_rC_{50} = 3.46 mg/L (95% Confidence Interval: 2.55 mg/L to > 3.95 mg/L)
96 hour E_rC_{50} = 1.92 mg/L (95% Confidence Interval: 1.64 mg/L to 2.25 mg/L)
120 hour E_rC_{50} = 1.77 mg/L (95% Confidence Interval: 1.55 mg/L to 2.02 mg/L)

120 hour NOEC = 0.485 mg/L (average specific growth rate)

***Comment**: page 38: Boeri, R.L.; Wyskiel, D.C.; Ward, T.J. (2002)*
This study with the duckweed Lemna gibba was run over a period of 14 days. The EC50 (14d) is given as 0.00042 mg/L (nominal) and the NOEC (14d) is given as 0.00024 mg/L (nominal) related to healthy frond count. The initially (0 hours) measured test concentrations were 93 -110 % of the nominal concentrations. But the measured test concentrations at the end of the study (day 14) were below LOQ (limit of quantification) of 0.0000132 mg/L at all concentrations. In that case the use of nominal concentrations for calculation of EC50/NOEC is not in compliance with the test guideline.
We therefore suggest to recalculate all EC50/NOEC data under consideration of mean measured test concentrations (geometric mean of initially measured concentration and one half of LOQ) to correct these data to real measured concentrations.
The cited paper of McKelvey (2011) where a detailed calculation of average specific growth rate was provided and the ErC50 (14d) = 0.00069 mg/L has been determined is not available in the DAR

addendum (last addendum was January 2012). Please indicate the year of the respective addendum in order to ensure traceability of the presented data. We assume that the calculation has been done with nominal concentrations from the original test data. In our opinion the ErC50 value should therefore also be recalculated based on mean measured concentrations.
For classification and labelling of the acute hazard we suggest using a newly calculated ErC50 (7d) based on measured concentration instead of ErC50 (14d) = 0.00069 mg/L (nominal).

Response: This study was performed under static conditions, conducted over a 14 day period, with analytical determination of test concentrations performed at test initiation, and at the end of the 14 day test period. The initial measured concentrations of chlorsulfuron ranged from 93 to 110% of the targeted nominal concentrations. Final, measured concentrations of chlorsulfuron in all test treatments were less than the limit of quantitation (LOQ). Hydrolysis of chlorsulfuron has been found to be only significant at a pH of 5, with chlorsulfuron essentially stable at a pH 7 and pH 9 (Dietrich and McAleer, 1989; AMR 1455-89.161-1). In addition, photolysis is not a major degradation process for chlorsulfuron at pH 5, pH 7, or pH 9 at 25°C (Dietrich and McAleer, 1989; AMR 1455-89.161-1). pH values for the *Lemna gibba* study discussed here ranged from 7.5 at initiation to 9.8 at the conclusion of the study, indicating stability of chlorsulfuron throughout the study period. Previous chronic studies indicate that chlorsulfuron is stable in the test medium over the duration of the study. For example, a static, 21 day chronic study with *Daphnia magna* reported final, mean measured concentrations that were 75 to 100% of the targeted nominal concentrations (Hutton, 1989; HLR 35-89). For the *L. gibba* study, the decrease in test concentrations observed at the end of the study is due to the macrophytes taking up the compound during the study period, decreasing the concentrations of the test material over time to concentrations below the LOQ, but above the LOD. Given the known stability of chlorsulfuron, it was determined in the *L. gibba* study that analytical verification of the test concentrations was only necessary at the initiation (to verify test concentrations) and conclusion of the study (to verify loss of chemical concentrations through uptake). However, to verify the stability, a stability study was conducted, with measured concentrations over 14 days ranging from 95 to 104% of the targeted nominal concentration.
It is suggested that geometric mean measured concentrations be used in the re-calculation of all endpoints. However, we feel that the use of the geometric mean measured concentrations is not necessary, as this study can be considered more environmentally relevant than a static renewal test, as chlorsulfuron has a maximum seasonal application of one. The most recent EU test guideline (OECD, 221 – *Lemna* sp. Growth Inhibition Test) suggests using geometric mean measured concentrations if the concentrations are < 80% of nominal by the end of the test. This recommendation is typically used for unstable compounds or compounds prone to degradation through hydrolysis or photolysis. Chlorsulfuron does not rapidly undergo either of these processes under standard laboratory conditions used in aquatic ecotoxicology testing, and a decrease in concentration is simply due to the uptake of chlorsulfuron into the organisms. Therefore, we do not feel that it is necessary, or justified, to use geometric mean measured concentrations based on initial measured concentrations and 1/2LOQ.
In addition, the endpoints from this study have been previously agreed at the level of the EU (DAR Chlorsulfuron, Vol. 3, Annex B9, 2007) and by EFSA (EFSA Scientific Report (2008) 201), and should be considered scientifically valid and justified in the classification and labeling of chlorsulfuron.
Frond count data from the *L. gibba* study was used to calculate the 14 day E_rC_{50} based on average specific growth rate (McKelvey, 2011; DuPont-31183). This data is presented in the Chlorsulfuron Addendum following the evaluation of new Annex II data, Post Approbation Addendum to Volume 3-Annex B.9, 2012, prepared by Evaluating Member State, France. Although this calculation used nominal concentrations, it is simply a calculation from data previously reported, reviewed and agreed, and therefore should be considered scientifically valid and justified for use in the classification of chlorsulfuron. Re-calculation of the 7 and 14 day EC_{50}s and NOECs based on geometric mean measured concentrations is not considered necessary. However, we do agree with the commenter that additional growth endpoints are needed for classification of chlorsulfuron. Therefore, the calculation of a 7 day EC_{50} and NOEC, as well as the 14 day NOEC based on average specific growth rate and nominal concentrations was completed and is summarized separately (see the section "Supplemental information - In depth analyses by RAC" in Annex 1).

RAC's response

RAC agrees with the comment, that the EC50 values defined for day 7 (if available) instead of at the values defined for day 14 in aquatic plant toxicity tests are more appropriate for estimating aquatic acute effects in classification.

Date	Country	Organisation	Type of Organisation	Comment number
02.07.2014	France		MemberState	8

Comment received

The classification and M factors are agreed:
Aquatic acute 1 , M (acute) = 1000
Aquatic chronic 1, M (chronic) = 100

Some minor comments are identified:

- Aerobic soil degradation (laboratory conditions) Tunink, A. (2010 a) DuPont-27603 reviewed in DAR Addendum, Vol. 3, Annex B8, B.8.2.2.1.1:
In table page 23, the values of DT50 and DT90 do not reflect the values from the study summary page 26, could you please clarify?
DT50 values ranging from 12.4 to 71.2 days (study summary) instead of 13.5 to 72 days (table page 23) and DT90 values from 51.0 to 255.9 days (study summary) instead of 44.8 to 239.1 days (table page 23).
- Vapour pressure values page 28:
The values reported in the table (2.3 x 10-11 Pa and 1.7 x 10-13 mm Hg) are not the reported endpoints under point 5.3.3 (3.1 x 10-9 Pa and 2.3 x 10-11 mm Hg), could you please clarify?

- Endpoints for Selenastrum capricornutum page 30:
In the table page 30, the EC50 value of 0.050 mg/L seems to be the 120 hour EC50 instead of the 72 hour EC50, could you please clarify?
In page 40 point 5.5, the 72 hour EC50 of 0.068 mg/L is cited and agreed.

- Justification for a chronic factor-M of 100 page 41:
The justification is based on a 48 hour NOEC of 0.00036 mg/L for Lemna. However, the 14 d NOEC of 0.00024 mg/L for Lemna should be considered more appropriate for classification purpose. It is noted that both endpoints lead to the same chronic M factor.

Dossier Submitter's Response

Comment: *Aerobic soil degradation values from Tunink, A. (2010a) DuPont-27603:*
In table page 23, the values of DT50 and DT90 do not reflect the values from the study summary page 26, could you please clarify?
DT50 values ranging from 12.4 to 71.2 days (study summary) instead of 13.5 to 72 days (table page 23) and DT90 values from 51.0 to 255.9 days (study summary) instead of 44.8 to 239.1 days (table page 23).

Response: The correct values that should be presented in the CLH report (table page 23 and summary page 26) are:

SOIL (TYPE)	CHLORSULFURON DT_{50} (DAYS)	CHLORSULFURON DT_{90} (DAYS)
Mattapex #25 (sandy loam)	13.5	44.8
Lleida (heavy clay)	72.0	239.1
Nambsheim (sandy clay loam)	68.7	228.3
Goch (sandy loam)	27.6	91.5
Suchozebry (sandy loam)	25.9	85.9

Comment: *Vapour pressure values page 28:*
The values reported in the table (2.3 x 10-11 Pa and 1.7 x 10-13 mm Hg) are not the reported endpoints under point 5.3.3 (3.1 x 10-9 Pa and 2.3 x 10-11 mm Hg), could you please clarify?

Response: The correct vapour pressure values should be 3.1 x 10-9 Pa and 2.3 x 10-11 mm Hg as

reported in DuPont study no. G/PC-22-CA, Revision No. 1: *Vapor pressure of chlorsulfuron at 25°C* (1992) by Schmuckler, M. and also found in the EFSA Scientific Report (2008) 201, 1-107.

Comment: *Endpoints for Selenastrum capricornutum page 30:*
In the table page 30, the EC50 value of 0.050 mg/L seems to be the 120 hour EC50 instead of the 72 hour EC50, could you please clarify?
In page 40 point 5.5, the 72 hour EC50 of 0.068 mg/L is cited and agreed.

Response: The endpoint for *S. capricornutum* from the summary of aquatic toxicity values on page 30 is incorrectly labelled as the 72 hour endpoint. The EC_{50} of 0.050 mg/L is the 120 hour endpoint as the commenter noted.

Comment: *Justification for a chronic factor-M of 100 page 41:*
The justification is based on a 48 hour NOEC of 0.00036 mg/L for Lemna. However, the 14 d NOEC of 0.00024 mg/L for Lemna should be considered more appropriate for classification purpose. It is noted that both endpoints lead to the same chronic M factor.

Response: It is agreed that a 14 day NOEC should be used as the more appropriate endpoint for classification. A 14 day NOEC based on average specific growth rate has been re-calculated based on previous data and is summarized in a separate document.

RAC's response

We agree that the 14-d NOEC of 0.00024 mg/L for *Lemna* should be considered as a more appropriate value for classification than the 48-hour NOEC of 0.00036 mg/L.

Date	Country	Organisation	Type of Organisation	Comment number
03.07.2014	United Kingdom		MemberState	9

Comment received

The environmental information presented in the CLH report is taken directly from the DAR. The UK CA feels that the environmental endpoints need clearer evaluation with reference to classification criteria. This is especially relevant for the most sensitive trophic level – algae and aquatic plants.

The Boeri et al, 2002 Lemna gibba study is a 14 day test but a 7 day growth endpoint is preferred in the CLH guidance. Are 7 day values available from the study or can 7 day values be calculated from the raw study data? Given the 14 day duration, the validity of the controls should be verified as over such timescale the controls may no longer reflect exponential growth due to nutrient depletion. The results are based on nominal concentrations but it is unclear if there was analytical verification to support this – this should be clarified. Following the original study results, the CLH report includes a recently derived (unclear duration but presume 14 day) ErC50 frond number of 0.00069 mg/l based on average specific growth rate (McKelvey, 2011) but a NOEC for this endpoint is not included - a NOEC (or EC10/EC20) should be included for consideration of chronic classification. In addition, it would be useful to present ErC50 values based on total frond area, dry weight and fresh weight as different substances impact measured variables differently.

The DAR and EFSA Conclusion refer to a second 14 day Lemna study using Lemna minor (Douglas et al, 1988) but this was considered invalid due to lack of analytical support. Given chlorsulfuron shows limited degradation / photolysis potential over the test duration and appears to be stable in other ecotoxicity media at similar pH (including algal and Lemna tests and a longer 21 day Daphnia study), the UK CA thinks the study should be evaluated for classification.

The aquatic acute classification conclusion refers to a 14 day Lemna gibba EC50 of 0.00035mg/l. This value relates to frond biomass (unclear if dry or wet weight) and is not an appropriate endpoint for classification. It would be more appropriate to present and consider all EC50 values based on growth.

The aquatic chronic classification conclusion refers to a 48 hour Lemna gibba NOEC of 0.00036mg/l.

The UK CA does not think that this study method is applicable for the classification endpoint given the short length of exposure.

Overall, the UK CA does not think the data is adequately evaluated to agree aquatic toxicity and M Factors.

Dossier Submitter's Response

The validity of the control data can be calculated and exponential growth of the controls can be verified over the 14 day period. According to the most recent EU test guideline (OECD, 221 - *Lemna* sp. Growth Inhibition Test) the validity of the test can be determined through the calculation of the doubling time. For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days. To determine the doubling time of frond number, the following formula is used:

$$T_d = \ln 2 / \mu$$

where μ is the average specific growth rate of the controls. The average specific growth rate can be calculated by the following formula:

$$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t}$$

where:

- μ_{i-j} : average specific growth rate from time i to j
- N_i : measurement variable in the test or control vessel at time i
- N_j : measurement variable in the test or control vessel at time j
- t : time period from i to j

The average specific growth rate and doubling time over the 14 day testing period can be calculated. In addition, data for day 6 and 8 (since data on day 7 was not recorded) is also presented in the table below.

Day (t)	Control frond count, initial (mean of three replicates)	Control frond count at time, t (mean of three replicates)	Average specific growth rate (μ)	Doubling time (T_d)
6	15	143	0.376	1.8
8	15	307	0.377	1.8
14	15	837	0.287	2.4

A *Lemna minor* study was conducted on technical chlorsulfuron (Douglas et al., 1988; DPT 186(a)/881172), and was reviewed in the Chlorsulfuron DAR (DAR Chlorsulfuron, Vol. 3, Annex B9, 2007) and EFSA Conclusion (EFSA Scientific Report (2008) 201). Although we feel that the commenter's point of view is valid that even without analytical support this study could be considered for evaluation for classification (based on known chemical and physical properties of chlorsulfuron), there are a number of issues with this particular study that prevents us with agreeing that this study should be used. The lack of analytical testing prevents demonstration that nominal concentrations prepared at initiation, or during renewals of test solutions, were prepared accurately. Without this analytical data, there is little confidence in the nominal values reported (regardless of stability). In addition, according to the most recent EU test guideline (OECD, 221 – *Lemna* sp. Growth Inhibition Test), different media are recommended for *Lemna minor* and *Lemna gibba.* Testing and culturing of *L. minor* is performed under a lower pH (pH adjusted to 6.5 ± 0.2) than that of *L. gibba* (pH adjusted to 7.5 ± 0.1). The results from Douglas et al., 1988 indicate that the pH of the initial and freshly prepared media used for test solution renewal was adjusted to a pH of 5.0. Hydrolysis of chlorsulfuron has been found to be only significant at a pH of 5, with chlorsulfuron essentially stable at a pH 7 and pH 9 (Dietrich and McAleer, 1989; AMR 1455-89.161-1). The lack of analytical data during this study does not allow for confidence that (a) nominal concentrations of chlorsulfuron were prepared correctly at initiation and during periods of test solution renewal, and (b) degradation of chlorsulfuron through hydrolysis was not occurring throughout the study period. Taken together, we disagree that this study should be considered for evaluation in the classification of chlorsulfuron, as more reliable studies are available (e.g., Boeri et al., 2002).

However, we do agree that growth endpoints should be used for classification of chlorsulfuron. The 7 and 14 day growth E_rC_{50} values and NOECs based on the average specific growth rate have been re-

calculated based on the data from the original report (Boeri et al., 2002), and is summarized separately (see the section "Supplemental information - In depth analyses by RAC" in Annex 1).

RAC's response

The DAR and EFSA conclusion refer to a second 14 day *Lemna* study using *Lemna minor* (Douglas et al, 1988), however, this study was not considered to be valid due to lack of analytical support. Since chlorosulfuron shows limited degradation / photolysis potential over the test duration and appears to be stable in other ecotoxicity media at similar pH (including algal and *Lemna* tests and a longer 21 day *Daphnia* study), RAC sees no reason to exclude this study from evaluation for classification.

Date	Country	Organisation	Type of Organisation	Comment number
04.07.02014	Belgium		MemberState	10

Comment received

Based on the results of the aquatic toxicity test on the most sensitive species Lemna gibba, the fact that the substance is considered as no rapidly degradable it is justified to classify, following the classification criteria of the regulation 1272/2008, as Aquatic acute 1, H400 and Aquatic Chronic 1, H410.

In view of the proposed classification and toxicity band for acute toxicity between 0.0001 mg/l and 0.001mg/l, an M-factor for acute toxicity of 1000 could be assigned.

In conclusion : we agree with the proposed environmental classification by Bureau for Chemical substances of Poland. However before supporting the proposed Mchronic = 100, we need a clarification of following items :

Key study chronic toxicity : Lemna gibba, (Porch et al, 2010a)
- 4 different exposure intervals are reported : was the exposure duration of the test 7days? If this is the case why a 7d NOEC wasn't recorded? Or were these intervals followed by a clearance period until the end of the 7-day test duration?
- A 48h exposure NOEC of 0.00036 mg/l is considered to determine the chronic M-factor. As the exposure time is limited, this value should rather be considered as an acute value instead of a chronic one. If a 7d NOEC is not available, we propose to use the study of Boerie et al (2002) as key study for chronic toxicity, with recalculation of the 14d NOEC to a 7d exposure.

Could you also provide further info on the key study for acute toxicity : Lemna gibba (Boeri et al, 2002)
- Was the test concentration maintained (>80% of nominal) during the test?
- Is there a reference substance used?
- What testing procedure is used? Static, semi-static or flow through?
- The test duration recommended according to OECD 221 test guideline is 7 days. Please recalculate the 14d EC50 to this exposure duration.

Dossier Submitter's Response

***Comment**: Key study chronic toxicity: Lemna gibba, (Porch et al, 2010a)*
- 4 different exposure intervals are reported: was the exposure duration of the test 7days? If this is the case why a 7d NOEC wasn't recorded? Or were these intervals followed by a clearance period until the end of the 7-day test duration?
- A 48h exposure NOEC of 0.00036 mg/l is considered to determine the chronic M-factor. As the exposure time is limited, this value should rather be considered as an acute value instead of a chronic one. If a 7d NOEC is not available, we propose to use the study of Boerie et al (2002) as key study for chronic toxicity, with recalculation of the 14d NOEC to a 7d exposure.

Response: This *Lemna gibba* test (Porch et al., 2010a; DuPont-28843) consisted of four treatment exposure intervals (4, 8, 24 and 48 hours), each with six nominal concentrations. During each exposure interval (for example, 4 hours), the organisms were exposed to various concentrations of chemical for 4 hours, and then removed from the test medium and placed into fresh, clean culture medium for the remainder of the 7 day testing period. This same method was used for each exposure interval (8, 24 and 48 hours), and a NOEC for each interval is presented in the report.

A 7 day E_rC_{50} and both 7 and 14 day NOECs have been re-calculated based on previous data and are summarized in a separate document. These newly calculated endpoints can be used for classification and labeling of chlorsulfuron.

***Comment**: Could you also provide further info on the key study for acute toxicity: Lemna gibba (Boeri et al, 2002)*
- Was the test concentration maintained (>80% of nominal) during the test?
- Is there a reference substance used?
- What testing procedure is used? Static, semi-static or flow through?
- The test duration recommended according to OECD 221 test guideline is 7 days. Please recalculate the 14d EC50 to this exposure duration.

Response: This study was performed under static conditions, conducted over a 14 day period, with analytical determination of test concentrations performed at test initiation, and at the end of the 14 day test period. The initial measured concentrations of chlorsulfuron ranged from 93 to 110% of the targeted nominal concentrations. Final, measured concentrations of chlorsulfuron in all test treatments were less than the limit of quantitation (LOQ). Hydrolysis of chlorsulfuron has been found to be only significant at a pH of 5, with chlorsulfuron essentially stable at a pH 7 and pH 9 (Dietrich and McAleer, 1989; AMR 1455-89.161-1). In addition, photolysis is not a major degradation process for chlorsulfuron at pH 5, pH 7, or pH 9 at 25°C (Dietrich and McAleer, 1989; AMR 1455-89.161-1). pH values for the *Lemna gibba* study discussed here ranged from 7.5 at initiation to 9.8 at the conclusion of the study, indicating stability of chlorsulfuron throughout the study period. Previous chronic studies indicate that chlorsulfuron is stable in the test medium over the duration of the study. For example, a static, 21 day chronic study with *Daphnia magna* reported final, mean measured concentrations that were 75 to 100% of the targeted nominal concentrations (Hutton, 1989; HLR 35-89). For the *L. gibba* study, the decrease in test concentrations observed at the end of the study is due to the macrophytes taking up the compound during the study period, decreasing the concentrations of the test material over time to concentrations below the LOQ, but above the LOD. Given the known stability of chlorsulfuron, it was determined in the *L. gibba* study that analytical verification of the test concentrations was only necessary at the initiation (to verify test concentrations) and conclusion of the study (to verify loss of chemical concentrations through uptake). However, to verify the stability, a stability study was conducted, with measured concentrations over 14 days ranging from 95 to 104% of the targeted nominal concentration.
It is suggested that geometric mean measured concentrations be used in the re-calculation of all endpoints. However, we feel that the use of the geometric mean measured concentrations is not necessary, as this study can be considered more environmentally relevant than a static renewal test, as chlorsulfuron has a maximum seasonal application of one. The most recent EU test guideline (OECD, 221 – *Lemna* sp. Growth Inhibition Test) suggests using geometric mean measured concentrations if the concentrations are < 80% of nominal by the end of the test. This recommendation is typically used for unstable compounds or compounds prone to degradation through hydrolysis or photolysis. Chlorsulfuron does not rapidly undergo either of these processes under standard laboratory conditions used in aquatic ecotoxicology testing, and a decrease in concentration is simply due to the uptake of chlorsulfuron into the organisms. Therefore, we do not feel that it is necessary, or justified, to use geometric mean measured concentrations based on initial measured concentrations and 1/2LOQ.
In addition, the endpoints from this study have been previously agreed at the level of the EU (DAR Chlorsulfuron, Vol. 3, Annex B9, 2007) and by EFSA (EFSA Scientific Report (2008) 201), and should be considered scientifically valid and justified in the classification and labeling of chlorsulfuron.
Frond count data from the *L. gibba* study was used to calculate the 14 day E_rC_{50} based on average specific growth rate (McKelvey, 2011; DuPont-31183). This data is presented in the Chlorsulfuron Addendum following the evaluation of new Annex II data, Post Approbation Addendum to Volume 3-Annex B.9, 2012, prepared by Evaluating Member State, France. Although this calculation used nominal concentrations, it is simply a calculation from data previously reported, reviewed and agreed, and therefore should be considered scientifically valid and justified for use in the classification of chlorsulfuron. Re-calculation of the 7 and 14 day EC_{50}s and NOECs based on geometric mean measured concentrations is not considered necessary. However, we do agree with the commenter that additional growth endpoints are needed for classification of chlorsulfuron. Therefore, the calculation of a 7 day EC_{50} and NOEC, as well as the 14 day NOEC based on average specific growth rate and nominal concentrations was completed and is summarized separately (see

the section "Supplemental information - In depth analyses by RAC" in Annex 1).
RAC's response
Noted.