Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile

EC Number: -
CAS Number: 131341-86-1

CLH-O-0000001412-86-162/F

Adopted

9 June 2017

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUDIOXONIL (ISO); 4-(2,2-DIFLUORO-1,3-BENZODIOXOL-4-YL)-1H-PYRROLE-3-CARBONITRILE

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 comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. 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. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile
EC number: -
CAS number: 131341-86-1
Dossier submitter: Denmark

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
31.08.2016	France		MemberState	1
Comment received				
p.17: Part B. 1.3 Table 9. Stability in organic solvents: Please note that data are available on solubility in organic solvents in the DAR (2005) of fludioxonil (in the Volume 3, Annex B2, point B.2.1.12) and reported in the RAR of Fludioxonil (in the Volume 3, Annex B2, point B.2.6).				
Dossier Submitter's Response				
We can include the data from the DAR if you find the need for this.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
22.07.2016	Spain		MemberState	2
Comment received				
The Spanish CA supports not classification regarding human health				
Dossier Submitter's Response				
Thank you for your evaluation and support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
02.09.2016	Belgium		MemberState	3
Comment received				
A DAR is available for Fludioxonil. We regret that the relevant Volumes of the DAR were not annexed to the CLH report since this had allowed a thorough evaluation of the				

environmental data without search for other sources of information than the CLH dossier.

BECA welcomes the proposal for classification from the Danish Environmental Protection Agency. Unfortunately, reliabilities of study were unknown and it is not easy to assess the quality of the studies with the few information available in data tables.
According to the available data, BECA agrees that there appears to be no evidence of toxicity for human health.

The BE CA supports the proposed classification as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410.

Lowest acute toxicity values for the 3 trophic levels are all in the same range, with LC50 between 0.1mg/l and 1 mg/l, justifying an Macute =1.
Also all reliable NOEC values for the 3 trophic levels are between 0.01 and 0.1 mg/l.
Combined with the fact that the substance is not rapidly degradable a Mchronic=1 applies

Some editorial or/and minor comments :
The substance is not listed in annex XV of regulation 1272/2008. So it is not clear why an number is given in table 1.

Dossier Submitter's Response

Thank you for your support. In S-CIRCABC the biocide IUCLID file is available with all the studies handed in for the biocide evaluation. Also annotations are given with reliability index for all the studies. The toxicological studies from the DAR are also included in S-CIRCABC. Whereas the ecotoxicological studies can be found in this link:
http://dar.efsa.europa.eu/dar-web/provision/request/subid/86

Regarding the number in Table 1, then it is the future number for Annex VI that is listed. We were told to include this number.

RAC's response

Noted. No classificataion for human health is supported. RAC has also considered data and information from the DAR, available from EFSA's website.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	4

Comment received

The German CA supports that fludioxonil does not require classification and labelling for human health hazards.
We support the proposed environmental classification and labelling as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) and an acute M-factor of 1. We do not support the chronic M-factor of 1. We propose to change the chronic M-factor to 10.

Dossier Submitter's Response

Thank you for your evaluation and support for the human health part.
Regarding the environmental part then we have argued why we do not find the old daphnia study (Rufli, 1989) as reliable. This is also described in the annotations in the biocide IUCLID file under point 9.1.6.2-02. This procedure has been accepted during the biocide substance evaluation. Find later more argumentation concerning this.

RAC's response

Noted. No classification for human health is supported.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUDIOXONIL (ISO); 4-(2,2-DIFLUORO-1,3-BENZODIOXOL-4-YL)-1H-PYRROLE-3-CARBONITRILE

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	5
Comment received				
Section 4.10.6 Lanxess Deutschland GmbH agrees with the conclusion for no classification based on the submitted data.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	6
Comment received				
Fludioxonil was not carcinogenic in rats or mice in lifetime bioassays. Therefore, Syngenta agrees that no carcinogenicity classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted. Confidential attachments were taken into consideration.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	7
Comment received				
The RMS position is supported, not to classify fludioxonil for carcinogenicity.				
Dossier Submitter's Response				
Thank you for your evaluation and support for the human health part.				
RAC's response				
Noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	8
Comment received				
Section 4.9.6 Lanxess Deutschland GmbH agrees with the conclusion for no classification based on the submitted data.				
Dossier Submitter's Response				
Thank you.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	9
Comment received				
For completeness two new Ames tests (Bowles, 2009 and Chang, 2016) with fludioxonil are being provided which have not been considered in the CLH report. The studies do not impact the proposed classification and both studies confirm that fludioxonil is not mutagenic. In *in vitro* mutagenicity assays Fludioxonil gave negative results. *In vitro* clastogenicity assays gave a positive result but no clastogenicity activity was shown *in vivo* (the validity of this result was confirmed by the presence of blue stained urine which is indicative of systemic exposure of technical material in the mouse micronucleus assay therefore, it can be deduced that the bone marrow was also exposed). No DNA damage was observed *in vivo*. Syngenta therefore supports the position that no classification is necessary for mutagenicity. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
These studies were not originally submitted for the CLH or CAR evaluation (biocides) but submitted during the public consultation period. However the dossier submitter/eCA DK has requested study summaries (now attached as an **ANNEX A** to this RCOM table). Both Ames tests were negative , there were no evidence of mutagenicity under the conditions of these studies. The studies do not impact the proposed classification and both studies confirm that fludioxonil is not mutagenic.				
RAC's response				
Noted. The submitted information has been considered.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	10
Comment received				
The RMS position is supported, not to classify fludioxonil for mutagenicity				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding classification proposal for the human health part.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
31.08.2016	France		MemberState	11
Comment received				
Rat teratology study (Page 62) As regards the non-statistically significant increased foetal and litter incidences of dilated renal pelvis and dilated ureter observed in high dose group, the validity of the HCD cited				

in the study report could not have been checked. Is there any available HCD for this strain (SD), this source (Charles River) and for the time period the study was carried out, in order to ensure that the observed increases are not treatment related?
Dossier Submitter's Response
As indicated in the CLH-report (p. 62 and p.88 & p. 95; HCD data position paper) and in the CAR for biocides (page 127) this was aready investigated by the dossier submitter DK. Given the arguments stated in the position paper p.88 (general remarks on HCD data) and p.95 for this specific rat teratology study, it seems plausible that the same laboratory and strain has been used, however as previously stated it could not be firmly concluded. However it should be noted that the incidence of foetuses with dilatation of the ureter and/or renal pelvis was slightly (but not significantly) increased only in the top dose given rise to maternal toxicity and it is additionally noted that the concurrent control incidences for these findings are at the lower end of the laboratory's historical range. These variations/retardations are categorized of low or moderate concern in ECETOC "Guidance on Evaluation of reproductive toxicity data" monograph No.31. Maternal toxicity in the form of of reduced food consumption (10% compared to control) and mean bodyweight gain (21% compared to control) on days 6-11 in the high dose were observed. If more relevant data exists regarding HCD data on the Charles River rat, applicant are encouraged to submit these. However since HCD should be specific data for a given laboratory, concurrent time periode and strain it might be less relevant to make a more general search.
RAC's response
The HCD position paper from industry, which indicates that it is plausible that the same strains in the same laboratory at the same timepoint has been used to generate the HCD, was considered by RAC. For additional information, see the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	12
Comment received				
Section 4.11.6 Lanxess Deutschland GmbH agrees with the conclusion for no classification based on the submitted data.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	13
Comment received				
No evidence of reproductive toxicity or teratogenicity were observed, and there was no effects of fludioxonil on offspring in multigeneration studies. Therefore, Syngenta agrees that no classification for reproduction is necessary.				

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip
Dossier Submitter's Response
Thank you.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	14
Comment received				
The RMS position is supported, not to classify fludioxonil for reproductive toxicity.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding classification proposal for the human health part.				
RAC's response				
Noted.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	15
Comment received				
Section 4.6.2.5 Lanxess Deutschland GmbH agrees with the conclusion for no classification based on the lack of structural alerts and the lack of data highlighting a concern.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	16
Comment received				
Based on the acute inhalation study where no respiratory tract irritation was observed and the lack of evidence of respiratory sensitisation in humans, Syngenta agrees that no classification for respiratory sensitisation is warranted. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	17
Comment received				
The RMS position is supported, not to classify fludioxonil for respiratory sensitization.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding the classification proposal for the human health part.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	18
Comment received				
Fludioxonil shows low oral, acute and inhalation toxicity. Therefore Syngenta agrees that no classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	19
Comment received				
The RMS position is supported, not to classify fludioxonil for acute toxicity.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	20
Comment received				
Fludioxonil was found to be non-irritant in one study. It was found to be a mild irritant in another study but does not trigger classification. Therefore Syngenta agrees that no classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
No comment.				

RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	21
Comment received				
The RMS position is supported, not to classify fludioxonil for skin irritation or corrosion.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	22
Comment received				
Fludioxonil was found to be a mild irritant in a study but does not trigger classification. Therefore Syngenta agrees that no classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	23
Comment received				
The RMS position is supported, not to classify fludioxonil for eye irritation or serious damage to eyes.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
31.08.2016	France		MemberState	24
Comment received				
(Page 28-30) In the skin sensitisation Maximization test carried out with fludioxonil, both the topical induction and the challenge concentrations are two low. Therefore the reliability of the negative results obtained in this study are questionable. The negative results obtained in a skin sensitisation Maximization test performed with the				

formulated product Celest 025 FS (26 g fludioxonil /L) cannot support the absence of intrinsic sensitising potential of fludioxonil. Indeed the topical induction and the challenge concentrations are 2% and 0.6% respectively (when expressed in fludioxonil). Furthermore since this formulated product is red, it was not possible to document irritation by the test material during topical induction. Is there any further data available on fludioxonil (other in vivo studies performed with the technical product or formulated products containing fludioxonil, in vitro studies or in silico data), that could support the absence of sensitising potential of fludioxonil?
Dossier Submitter's Response
Applicant has submitted the below mentioned information (no study or study summaries have been provided at this stage) "A further M&K study is available with the fludioxonil solo formulation A8240B (GEOX WG50 – Fludioxonil concentration 49.6%) – the concentration at induction was 1% and the challenge concentrations were 25% and 50%. There were no signs of a reaction after challenge (nothing was reported regarding colouration and inability to inspect application sites) and therefore A8240B was considered to be a non-sensitiser in this test and as such could support the absence of sensitisation potential." The two skin-irritation studies performed with pure fludioxonil (moisted with water/saline) did not results in dermal reactions leading to classification for skin irritation. Taking into account all the available information, no sensitising potential is expected of fludioxonil.
RAC's response
Noted. Remaining uncertainties in relation to applied doses have been discussed during the RAC consultation. Further information has been provided by industry after the end of the public consultation. Information from 19 skin sensitisation studies with fludioxonil technical and formulations containing fludioxonil in different concentrations was summarised by industry. Out of these 19 studies, 18 were negative. The only weak positive response was seen in a 3 induction Buehler assay (25%) with A8240B (500 g/L) – induction concentration formulation applied 50%, challenge 10% (equivalent to 0.5% induction/ 5% challenge Fludioxonil). A more robust study with the same formulation, a guinea pig maximization test (induction concentration 1%/ challenge concentration 100% of formulation applied, equivalent to 0.5% induction/50% challenge Fludioxonil) was clearly negative. Industry concluded that overall these data provide very clear support for fludioxonil having no sensitisation potential. The presence of this further information has been indicated in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	25
Comment received				
Fludioxonil did not show any sensitisation potential. Therefore, Syngenta agrees that no classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	26
Comment received				
The RMS position is supported, not to classify fludioxonil for skin sensitization.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding the classification proposal for the human health part.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	27
Comment received				
Fludioxonil shows low oral, acute and inhalation toxicity. Therefore, Syngenta agrees that no STOT-SE classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	28
Comment received				
The RMS position is supported, not to classify fludioxonil for STOT-SE.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding the classification proposal for the human health part.				

RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	29
Comment received				
Fludioxonil was well tolerated in repeat dose studies in multiple species with treatment related findings only at doses above the triggers for STOT-RE classification. Therefore, Syngenta agrees that no STOT-RE classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	30
Comment received				
The RMS position is supported, not to classify fludioxonil for STOT-RE.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding the classification proposal for the human health part.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	31
Comment received				
Syngenta agrees that no classification is necessary. There is no evidence that fludioxonil causes human aspiration toxicity hazard. Additionally the hazard based on viscosity (>20.5 mm2/s) is not applicable as fludioxonil technical is a solid with a melting point of 198°C and as such it is not possible to determine viscosity. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLUDIOXONIL (ISO); 4-(2,2-DIFLUORO-1,3-BENZODIOXOL-4-YL)-1H-PYRROLE-3-CARBONITRILE

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	32
Comment received				
The RMS position is supported, not to classify fludioxonil for aspiration hazard.				
Dossier Submitter's Response				
Thank you for your evaluation and support regarding the classification proposal for the human health part.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
31.08.2016	France		MemberState	33
Comment received				
The endpoints available in the pesticide Monograph of Fludioxonil (2005-2006) and summarized in the EFSA scientific report on the conclusion of the peer review of the active substance (EFSA scientific report (2007) 110, 1-85) on marine species are not listed in the CLH report. This will not change the outcome of the proposed classification. For information, new studies have been submitted in the Annex I Renewal dossier of Fludioxonil submitted under Regulation (EC) 1107/2009. The assessment is currently ongoing. This will not change the outcome of the proposed classification. Regarding the algae studies presented in the CLH report, they should be considered invalid due to important pH variation at the end of the test. The recalculation at 48 hours is questionable since it cannot be proven that the pH will be at an acceptable range. Additionally 72 or 96 hours EC50 are requested for algae according to Regulation (EC) 1272/2008. However new data on algae are available in the Annex I renewal dossier for the pesticide use of fludioxonil that may be used for classification. After first review, these new studies are not expected to change the outcome of the proposed classification. Regarding the acute fish and Daphnia studies of Bievers (1997a, IUCLID 9.1.1-02) and Surprenant (1990 IUCLID 9.1.2-01), they are not considered valid as the amount of solvent in the control is different to the one in the tested concentrations. However this issue did not seem to be raised with the acute toxicity study on fish performed by Holmes & Swigert (1993a, IUCLID 9.1.1-01) and with the acute study on Daphnia realised by Holmes & Swigert (1993a, IUCLID 9.1.2-02) in which L(E)C50 are found to be lower than 1 and greater than 0.1 mg/L Thus this will not change the outcome of the proposed classification : H400 with acute M factor of 1. Regarding the chronic Daphnia magna study of Putt (1991) study that gave the lowest NOEC on which is based the chronic M factor of 1, France as RMS of the pesticide dossier has some concerns regarding the reliability of this study. These concerns are mainly due to the differences between solvent control and negative control (35% less offspring/female in the solvent control compared to negative control despite a better percentage of survival of the adults in the solvent (100% vs. 93%). Additionally, the concentration of the solvent in the solvent control is not identical to the ones in the tested concentrations. Thus the reliability of the study is questionable. This is however not expected to change the outcome of the chronic classification and chronic M factor proposed as there are other available NOEC lower than 0.1 mg a.s./L and greater than				

0.01 mg/L : H410 with chronic M factor of 1

Dossier Submitter's Response

Thanks for your evaluation and support regarding the classification proposal for the environmental part .

We agree that the endpoints from the pesticide Monograph of fludioxonil will not change the outcome of the proposed classification of fludioxonil.
Regarding the new studies for the pesticide evaluation then we have not seen these and have no knowledge about these endpoints. However we appreciate that FR can update us on this.
Regarding the algae studies then we do not find that these should be disregarded. We agree that in the studies by (Rufli, H., 1989a) and (Hoberg, J.R., 1992/2005), then pH is not measured at 24 and 48 hours. However normally pH drifting happens after 48 hours. This has been investigated and reported in a number of articles by Niels Nyholm, e.g. in Nyholm and Källqvist (1989), ET&C, Vol8. During biocide evaluation this is a procedure which is often applied. We are however happy to hear that the new studies do not change the conclusion of the proposed classification.

Regarding the high concentration of solvent then we have noted this in the annotations for the studies (Holmes &Swigert, 1993a), (Holmes &Swigert, 1993b) and (Holmes &Swigert, 1993c) and further we have made the following remark together with a RI of 2: *TS solution was made with a 1.0 ml/L acetone concentration. This is 8 times of what is given as the maximum solvent concentration in the guideline, which must be considered a mayor deviation. However there were no effects on the solvent control, so this is not found to have had unacceptable effects on the results.*

Regarding the study by Putt (1991) then the NOEC is equal to the lowest tested concentration and we found the study acceptable for this endpoint, we had the following remarks for the study together with a RI of 2: *There is a high variation in the measured test concentrations, especially in the high concentrations. This is probably a result of material not being dissolved. The mean measured concentration is used for the endpoint derivation. The test is performed under flow-through conditions and degradation therefore would be expected not to occur, the TWA approach for calculating the concentrations is therefore not used. Problems with undissolved substance are not considered to influence on the result as the NOEC is obtained at a low concentration.*

The light intensity is given in foot candles. 25-50 foot candles is equal to 250-500 lux (1 foot candle is equal to 10.76 lux). This is quite low compared to the requirement in the OECD 211 guidance of1000-1500 lux.

The variation around the mean number of living offspring produced per parent animal in the control should be less than 25%, this is not reported in the study summary.

The study was found acceptable for the derivation of the NOEC, which is the relevant endpoint for the chronic toxicity.

RAC's response

RAC agrees that the Acute toxicity tests on the marine invertebrates from the pesticide Monograph of fludioxonil do not change the outcome of the proposed classification for the acute aquatic hazard, however they provide the lowest EC50 for invertebrates (96h LC50 was 0.27 mg/L – Holmes & Swigert 1993d; 96h EC50 was 0.37 mg/L – Surprenant 1990b), therefore should be reported in the report.

RAC is grateful for the information on the new studies and would appreciate knowing them as they become available.

Regarding the algae studies presented in the CLH report, in particular the key study Hoberg, J.R., 1992/2005, RAC notes that *"The pH ranged from 7.6 – 7.8 at test start and increased to 9.2 to 10.6 at termination of the test* (120h)", but pH is not measured at 24 and 48 hours. RAC is aware that 72 or 96 hours EC50 are requested for algae according to Regulation (EC) 1272/2008. However, according to the TG OECD 201 *"the test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached."*
Taking into account the few information available to RAC, it is just possible to take note of what the DS stated.

Regarding the high concentration of solvent used in the two acute studies on fish (Bievers, 1997a) and Daphnia (Surprenant, 1990), RAC could not find this information in the summaries of the studies reported in the DAR 2005 and in the CAR 2015.
Regarding the three acute studies of Holmes & Swigert (1993a, 1993b, 1993c) on fish and Daphnia, RAC agrees with the DS response related to the acute test on Daphnia, while no information are available from the summaries of the studies on fish.

Regarding the study of Putt (1991), RAC agrees that the differences between solvent control and negative control in the number of offspring are relevant, however the solvent control fulfilled the validity criteria of the test (OECD 211) and the NOEC was based on comparison with the solvent control.
The data on the concentration of the solvent in the control and in the test concentration were not reported in the summary of the test. RAC takes note.
RAC agrees with DS that the undissolved substance at the high concentrations do not influence the results. However, the light intensity is quite low compared to the requirement in the OECD 211 guidance. The calculation of the coefficient of variation around the mean number of living offspring produced per parent animal is less than 25% in the solvent control, although it is not reported in the summary of the study.
Taking into account the available information, the study was found acceptable.

Date	Country	Organisation	Type of Organisation	Comment number
31.08.2016	Finland		MemberState	34
Comment received				
FI CA supports the proposed environmental classification Aquatic Acute 1, H400 with M-factor of 1 and Aquatic Chronic 1, H410 with M-factor of 1 for Fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile.				
Dossier Submitter's Response				
Thanks for your evaluation and support regarding the classification proposal for the environmental part				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	35
Comment received				
Section 5.6 Lanxess Deutschland GmbH agrees with the conclusion for environmental classification				

(aquatic acute 1 (H400), aquatic chronic 1 (H410), M-factor = 1), based on the submitted data.
Dossier Submitter's Response
Thanks for your evaluation and support regarding the classification proposal for the environmental part
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
26.08.2016	United Kingdom		MemberState	36

Comment received

Classification:
We agree with the proposed classification of Aquatic Chronic 1. However, we consider further information is required to confirm appropriate M Factors (see ecotoxicity comments).

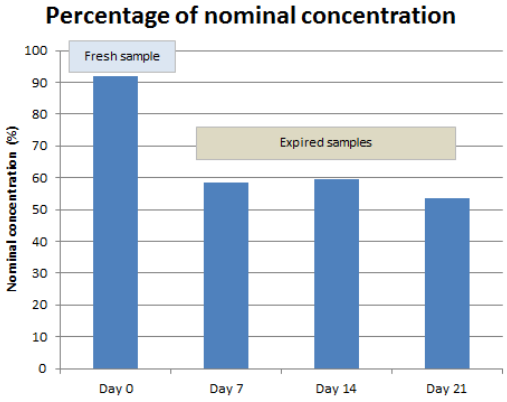
Chronic toxicity to invertebrate endpoints:
The Rufi, 1989 study was considered valid for review under Dir. 91/414/EEC with the 21-d NOEC of 0.005mg/l used in the risk assessment. Given the study limitations highlighted in the CLH report, please can it be confirmed if the NOEC is based o comparison with a solvent control? This is important to aid interpretation of the NOECs reliability. In addition, it would be useful to present further details of measured concentrations as the CLH report notes high variation.

Toxicity to algae endpoints:
The two available algal growth inhibition studies were considered valid in the DAR with 72, 96 or 120 hour duration endpoints. We feel additional information is required to conclude on reliable endpoints for classification given the proposed non-standards duration endpoints.

The CLH proposal presents 48 hour endpoints 'due to excessive pH variation at 72 and 120 hours'. Please can it be confirmed if this relates to the controls and/or the exposure treatments? In addition, were control validity criteria met? If so, it is unclear why an increase pH would invalidate study results. We feel ErC50 and ErC10/NOErC values for standard duration endpoints such as 72 or 96 hours, should be presented in order to consider the implications for classification. This is important for the chronic endpoint where a 48 hour endpoint is unlikely to represent exponential growth.

We note the CLH proposal presents a 48-h ErC50 of 0.21 mg/l (based on geometric mean measured concentrations) for the Hoberg, 1992 study. However, the DAR presents a 48-h ErC50 of 0.25 mg/l (based on geometric mean measured concentrations). Please can the correct value be clarified?

Bioaccumulation in fish:
It is unclear if the presented BCF is lipid normalised. Please can this be confirmed as lipid normalised data is preferred. In this instance we note a BCF > 500 would not impact the classification given the substance is considered non-rapidly degradable.

Dossier Submitter's Response
Please find below our response to the UK comments Chronic toxicity to invertebrate endpoints (Rufli, 1989): UK comment: confirmation required that the NOEC is based on comparison with the solvent control. **DK answer:** The raw data has been examined and it is difficult to ascertain whether the original statistical analysis was conducted with comparison to the control or the solvent control. However throughout the statistics sections in the report and raw data it was stated that there was no significant difference reported between the control and solvent control. The reported re-analysis of the data (Taylor & Allen 2016) suggested that in the original report the statistical comparison was made using the pooled control data. In the reanalysis of the data, Taylor & Allen (2016) verified that there was no difference between the control and solvent control, and then made the comparisons for adult mortality and cumulative number of live young per female against the solvent control only. UK comment: present further detail of the measured concentrations as the CLH report notes high variation **DK answer:** The test medium was renewed three times each week as recommended by the current TG. Usually analytical sampling is conducted on 3 pairs of samples (3 fresh and 3 expired). However in Rufli (1989) the concentrations were measured in samples collected on day 0 in fresh solutions, and days 7, 14 and 21 in old solutions. OECD 211 requires that the assessment of fresh and expired solutions are made on matched pairs '(i.e. analyses should be made on a sample from the same solution – when freshly prepared and at renewal). Therefore, as only one fresh solution was sampled, and fresh and expired samples were not taken from the same solutions, the measured concentrations have not been accurately analysed as required by the GD; and so are not reliable.      The OECD 211 GD requires something which looks more like this:

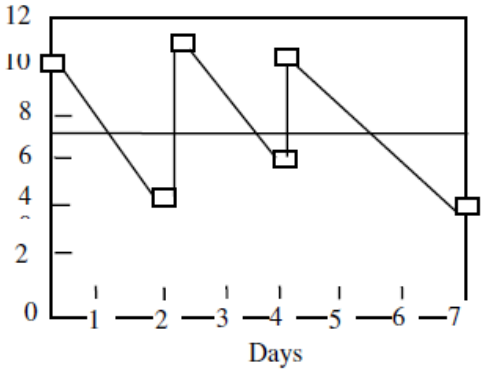


Figure 1: Example of time-weighted mean

The mean measured concentrations of the test item were in the overall range of 41 to 78 % of the nominal values. The variability within each concentration individually ranged between 34-103% of nominal. Therefore each concentration was highly variable and therefore the results from such dosing are not reliable. And a reliability factor of 3 was given.

Toxicity to algae endpoints:
DK answer: We know that the algae tests were considered valid in the DAR, however we do not agree to this conclusion as there is too high pH drift after 72 and 120 hours. This problem is very often seen in algea tests and a common solution is to use results from shorter periods. We however know that we do not have pH measurements after 24 and 48 hour. But as explained in the answer to comment 33, pH drift normally occurs after 48 hours. This is also explained in the review article by Nyholm & Källqvist (1989). In the algae studies exponential growth was occurring at 48 h. FR also state in comment 33 that new algae studies were submitted for renewal of the pesticide and endpoints are close to the ones calculated for 48 hour. We have not seen the studies for the renewal as a pesticide as these were not sent to us during the biocide evaluation.
The pH did vary during the study Hoberg (1992) in both the controls and test concentrations, see pH measurements below.

Table 2. Conductivity, pH, temperature and light int
120-hour exposure of *Selenastrum capricc*

Nominal Concentration (mg A.I./L)	pH	
	0–hour	120–hour
0.80	7.8	9.2
0.40	7.8	10.5
0.20	7.7	10.6
0.10	7.6	10.5
0.050	7.6	10.5
0.026	7.6	10.6
Solvent Control	7.6	10.2
Control	7.6	10.4

The endpoints are shown below based on geometric mean measured concentrations.

RESULTS

Endpoint	Geometric Mean Measured Concentration (mg a.i./L)		
	EC50	95% confidence intervals	NOEC
Total Biomass			
48-Hour	0.019	0.018 - 0.032	0.014[a]
72-Hour	0.029	0.020 - 0.041	0.014[a]
96-Hour	0.024	0.022 - 0.026	0.014[a]
120-Hour	0.024	0.023 - 0.026	0.014[a]
Average Growth Rate			
48-Hour	0.21	0.074 - 0.46	0.027[a]
72-Hour	0.41	0.32 - 0.48	0.014[b]
96-Hour	0.31	0.30 - 0.33	0.014[a]
120-Hour	0.33	0.32 - 0.34	0.014[a]

[a] The NOEC was determined by Williams' Test.
[b] Kruskal-Wallis' Test did not determine a reasonable NOEC. Therefore, the NOEC was empirically estimated to be the highest concentration with less than 10% inhibition.

UK comment: confirmation of discrepancy between the 48hr ErC50 of 0.25 mg/L presented in the DAR and the 48h ErC50 of 0.21 mg/L presented in the CLH report. Related to study by Hoberg (1992)

DK answer: The value in the DAR comes from a memo provided by the notifier during registration review to recalculate the endpoints based on the geometric mean measured concentrations. The CLH reported value comes from the GLP report amendment and the data from that table is shown below. The 0.21 mg/L should be considered the correct value as it comes from a GLP certified source.

Statistical analyses of the available data for yield revealed that the following EyC10 and EyC20 values were reliably calculated by Priestly & Allen 2015:

Table 7: Statistical analysis of yield for EC10 and EC20 endpoints for the fludioxonil toxicity to Freshwater Green Alga, Pseudokirchneriella subcapitata (Hoberg, 1992; Syngenta file number CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

Parameter	24 hours		48 hours		72 hours	96 hours	120 hours	
	EyC10	EyC20	EyC10	EyC20	EyC20	EyC20	EyC10	EyC20
Value [mg a.s./L]	0.015	0.017	0.0093	0.0175	0.0097	0.0118	0.0085	0.0129
lower 95%-cl	0.015	0.016	0.0005	0.0023	0.0090	0.0104	0.0078	0.0122
upper 95%-cl	0.015	0.017	0.0211	0.0340	0.0104	0.0132	0.0091	0.0135

cl: confidence limits
No EyC10 values at 72 or 96 hours could be reliably determined.

Statistical analyses of the available data for average specific growth rate revealed that the following ErC10 and ErC20 values were reliably calculated:

Table 8: Statistical analysis of average specific growth rate for EC10 and EC20 endpoints for the fludioxonil toxicity to Freshwater Green Alga, Pseudokirchneriella subcapitata (Hoberg, 1992; Syngenta file number

CGA173506/0243 using mean measured concentrations from Hoberg, 2005; Syngenta file number CGA173506/6950)

Parameter	72 hours		96 hours		120 hours	
	ErC10	ErC20	ErC10	ErC20	ErC10	ErC20
Value [mg a.s./L]	0.017	0.047	0.0286	0.0610	0.0456	0.0809
lower 95%-cl	0.009	0.025	0.0262	0.0574	0.0406	0.0744
upper 95%-cl	0.032	0.091	0.0311	0.0646	0.0505	0.0872

cl: confidence limits

No ErCx values at 24 or 48 hours could be reliably determined.

Bioaccumulation in fish:
The following BCF values were found:

Edible fish : BCF=58 L/kg wet fish; non-edible fish : BCF=741 L/kg wet fish; whole fish : BCF=366 L/kg wet fish

There has been no correction/normalisation of BCF according to fish with 5% lipid content as it is found that the tested fish Lepomis macrochirus has a lipid concentration that do not deviate significantly from the recommended 5%. However the actual lipid concentration of the tested fish is not reported. Further information regarding this can be found in the annotations of the biocide IUCLID dossier.

RAC's response

Classification:
RAC agrees on the need for additional information to conclude on reliable endpoints for classification, please see the response to the comment 3.

Chronic toxicity to invertebrate endpoints:
RAC takes note of what the DS stated about the statistical difference between the control and the solvent control, on the base of the analysis conducted by Taylor & Allen, 2016. However these information are not available to RAC.

As clarified by the DS, the solutions were renewed three times a week and samples were analysed at the beginning and during the test, as recommended by the OECD TG 202, part II. RAC agrees with DS on the difference in the frequency of the analytical determinations between the old guide OECD 202-II and the new one OECD 211, however the test was performed according to the old guide and, in this case, the analytical requests are fulfilled.

Regarding the measured concentrations, high variation was observed compared to the nominal concentrations, therefore results were based on measured concentrations. No information was given about the reasons of the observed decrements, however at the test concentration of 0.005 mg/L, at which the NOEC was obtained, the mean measured concentration was 78% of the nominal and the variability within the test concentration was 70-94%.
Taking into account the available information the study was found acceptable.

Toxicity to algae endpoints:
The DS clarified that the pH increase is related to controls, however we have pH measurements only at 0 and 120 hour.
RAC is aware that 72 or 96 hours EC50 are requested for algae according to Regulation (EC) 1272/2008. However, according to the OECD TG 201 the test period may be

shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached.

UK comment: confirmation of discrepancy between the 48hr ErC50 of 0.25 mg/L presented in the DAR and the 48h ErC50 of 0.21 mg/L presented in the CLH report. Related to study by Hoberg (1992).
RAC notes that in the Addendum 1 to DAR (October 2006) there is some more information on the study describing effects on algae growth (Hoberg, J.R.,1992), in particular:

Table 9.2.64: *Growth inhibition of Selenastrum capricornutum by fludioxonil (CGA 173506).*

Time interval	EC_{50} (mg/L) for cell density Based on initial measured conc.	**E_rC_{50} (mg/L) [95% conf. int.]** **Based on mean measured conc.***	E_bC_{50} (mg/L) Based on mean measured conc.*
0-48	0.14	**0.21 [0.074-0.46]**	0.19 [0.018-0.032]
0-72	0.098	**0.41 [0.32-0.48]**	0.020 [0.020-0.041]
0-96	0.10	**0.31 [0.30-0.33]**	0.024 [0.022-0.026]
0-120	0.092	**0.33 [0.32-0.34]**	0.024 [0.023-0.026]

* Based on the values calculated in the supplemental report

In this table, values at 48, 72h, 96 and 120h are reported for the relevant endpoint ErC50.
In its response to comments, DS referred to additional information from documents unfortunately not available to RAC, therefore RAC can just take note of what DS stated.

RAC additional observations:
- Taking into account the information on the study to Effects on algae growth (Hoberg, J.R., 1992) in the Addendum 1 to DAR (October 2006), RAC notes that exponential growth seems guaranteed at 48h: Algal volume values in the control cultures (negative and solvent controls) increased by a factor of 20-40 over 48h and by a factor 83-93 ca. over 72 hours. Thus, it fulfilled the criterion for increasing by a factor of at least 16.

Bioaccumulation in fish:
RAC takes note of the DS response. Information in the annotations of the biocide IUCLID dossier are not available to RAC.

Date	Country	Organisation	Type of Organisation	Comment number
02.09.2016	Sweden		MemberState	37
Comment received				
The Swedish CA supports classification of Fludioxonil (CAS No. 131341-86-1) in Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) as specified in the proposal. This conclusion is based on: - the lowest reliable short term LC/EC50 endpoints for the three main trophic groups of aquatic organisms (fish, invertebrates and algae) are all <1 mg/l and >0.1 mg/l, - reliable chronic NOECs endpoints for fish, invertebrates and algae are <0.1 mg/l, - the substance is not rapidly biodegradable. The SE CA agrees with the rationale for setting of M-factors of 1 for both acute and chronic toxicity for the aquatic organisms.				

Minor comments: The water/sediment study mentioned in "5.1.2.3 Simulation test" is missing in Table 21. Also it would have been helpful with the reliability of the studies indicated in all tables.
Dossier Submitter's Response
Thanks for your evaluation and support regarding the classification proposal for the environmental part. We can include the water/sediment study in Table 21. With regard to the reliability of the studies then these values can be added as well.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	38
Comment received				
For completeness Syngenta is providing a new aqueous photolysis study which has not been considered in the CLH report. The results from this study do not impact the proposed classification. However, this study is considered more appropriate because it was conducted in quartz vessels since fludioxonil has negligible absorbance above 340 nm. The DT50 in the study was 0.28 – 0.33 days and there are no breakdown products observed at levels above 10 %. For completeness a new fish full life cycle study is being provided which has not been considered in the CLH report. The results from this study do not impact the proposed classification. The resulting NOEC was 0.018 mg a.s./L. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
We have received study summaries for the two new studies on October 28, 2016. The two new studies are found reliable. As stated by Syngenta then a lowest NOEC of 0.018 mg/L was obtained in the full life-cycle toxicity test with Fathead Minnow (pimephales promelas). Reference is Marini (2015). This endpoint will not change the conclusion and the final outcome of the proposal for classification. The new photo-degradation study is now evaluated and the results from the study will not change the conclusion of the proposed classification				
RAC's response				
RAC takes note of the additional information available on the full life-cycle toxicity test with Fathead Minnow (*Pimephales promelas*) (Marini, 2015). This endpoint will not change the conclusion and the final outcome of the proposal for classification, but the study provides a NOEC of 0.018 mg/L, lower than the reliable values reported in the CLH report for chronic toxicity to fish.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Germany		MemberState	39
Comment received				
The use of data from tests with saltwater organisms is as well suitable as the data from fresh water organism tests usually used for classification and labeling purposes for the				

environment. We suggest using the lowest available EC/LC50 or NOEC values of valid studies for classification and labeling of acute or chronic effects of the substance.

- page 76 Short-term toxicity to aquatic invertebrates and page 74 table 23:
There exists an additional valid study report for acute toxicity for fludioxonil with lower EC/LC50 data:
Holmes,C.M. and Swigert,J.P. (1993) report 108A-134:
This valid study run in accordance with EPA 72-3 and ASTM guidelines with the saltwater mysid Mysidopsis bahia with fludioxonil in a flow-through system over a period of 96 hours. The LC50 (96 h) is 0.27 mg/L based on mean measured concentrations.
For classification of the acute risk of fludioxonil we suggest to use this lowest LC50 (96 h) of 0.27 mg/L (mean measured) for invertebrates instead of the study results for Daphnia magna EC50 (48 h) = 0.4 mg/L.

- page 76 Long-term toxicity to aquatic invertebrates and page 75 table 23
Putt,A.E. (1991) report 91-2-3672
The NOEC (21 d) = 0.019 mg/L (lowest test concentration in the study) based on reproduction (number of offspring) of Daphnia magna is presumably not the relevant endpoint for classification and labeling, because mortality of the offspring daphnids seems to be the relevant endpoint. This was obvious in the semi-static study of Rufli, H. (1989c) with Daphnia magna. The NOEC (21d) = 0.005 mg/L based on survival of young daphnids (F1-generation) and time for appearance of first brood is one order of magnitude lower than the NOEC (21 d) = 0.03 mg/L based on reproduction (number of offspring).
Unfortunately the study of Rufli, H. (1989c) was made only in compliance with the old guideline OECD 202 part II und does not fulfill criteria of the new guideline OECD 211 (validity criteria: number of 60 live juveniles per parent).
The study is approved as relevant in the list of endpoints for fludioxonil (EFSA conclusion, 2007) and should be reliable.
The semi-static study of Fournier, A.E. (2014) with Daphnia magna has only supplementary information. There are not sufficient data given in the CLH report and no data are available from the IUCLID5 dossier.

- page 76 Long-term toxicity to fish and page 75 table 23
There exists an additional valid study report for chronic toxicity for fludioxonil with lower NOEC data:
Surprenant, D.C. (1992) report 90-5-3319
In this ELS toxicity test with Pimephales promelas over 35 days under flow-through conditions according to EPA 850.1400 (OECD 210) growth of fish and survival were the most sensitive biological parameters. The relevant NOEC (35 d) for fludioxonil was determined to be 0.019 mg/L (mean measured) for mean weight (growth) and 0.07 mg/L for mortality of fish. The study is valid and reliable. The NOEC of 0.019 mg/L is the lowest value for long-term toxicity to fish.

- page 77 Algae and aquatic plants and page 75 table 23
It is not in line with the criteria of classification and labelling to use ErC50 and NOEC values of 48 hours instead of 72 or 96 hours from studies with algae. Additionally the growth of algae in the test with Pseudokirchneriella subcapitata (former: Selenastrum capricornutum) Hoberg, J. (1992) was not sufficient after 48 hours (factor of 16) for validity of the test already after 48 hours. The explanation to use the results of both tests already after 48 hours "due to excessive pH variation at 72 hours and 120 hours" in the two studies with algae Hoberg, J. (1992) and Rufli, H. (1989a) is not plausible. The growth of algae in both studies was sufficient after 72 hours and the validity criteria were met.

In the study of Rufli, H. (1989a) with Desmodesmus subspicatus the pH variation was only 1.7 units after 72 hours in the control instead of not more than 1.5 units (only recommendation of the OECD 201 guideline). In the study of Hoberg, J. (1992) with Pseudokirchneriella subcapitata the pH variation was 2.6 units after 120 hours in the control but no pH-values between 0 hours and 120 hours are available in the raw data. We suggest to use all ErC50, ErC10 and NOEC results of 72 hours for both tests, because they are valid and reliable and in line with the criteria for classification and labelling. The relevant values for algae Pseudokirchneriella subcapitata are:
NOEC (72 h) = 0.027 mg/L (geometric mean measured)
ErC50 (72 h) = 0.353 mg/L (geometric mean measured)

- Page 80 Conclusion on classification and labelling for environmental hazards
The conclusion on C&L should be based on the relevant data for acute and chronic classification and labeling according to the above test results.
The acute M-factor of 1 will not be changed, because the new relevant value LC50 (96 h) of 0.27 mg/L (mean measured) for Mysidopsis bahia is in the same range as the relevant value cited in the CLH-report for invertebrates Daphnia magna EC50 (48 h) of 0.4 mg/L (mean measured). The lowest reliable endpoint for algae is ErC50 (72 h) of 0.353 mg/L (geometric mean measured).
The relevant value NOEC (35 d) of 0.019 mg/L (mean measured) for fish Pimephales promelas is in the same order of magnitude as the lowest value cited in the CLH-report for fish Pimephales promelas NOEC (28 d) of 0.039 mg/L (mean measured) and does not influence the chronic M-factor.
The lowest reliable endpoint for algae is NOEC (72 h) of 0.027 mg/L (geometric mean measured) of Pseudokirchneriella subcapitata.
The lowest reliable endpoint for invertebrates is the NOEC (21 d) of 0.005 mg/L (mean measured) related to survival of young daphnids instead of NOEC (21 d) of 0.019 mg/L (mean measured) related to reproduction of Daphnia magna.

Therefore the chronic M-factor should be changed to 10, because the lowest NOEC = 0.005 mg/L is in the range of 0.001 and 0.01 mg/L and fludioxonil is not rapidly degradable.

Dossier Submitter's Response

Short term toxicity to aquatic invertebrates:
The study with the mysid Mysidopsis bahia was not submitted for the biocide evaluation. However it was found in the pesticide evaluation. We agree that this endpoint reflect the lowest value for short term toxicity to aquatic invertebrates.
For short term effects lower values are found (e.g. in Biever (1997a) with LC50 of 0.23 mg/L (fish) and for algae with an EC50 of 0.21 mg/L) than for the study with invertebrates.

Long term toxicity to aquatic invertebrates, related to study by Rufli (1989):
The table below shows that there is no dose response of for the cumulative number of dead neonates per female in the study as there are fewer dead neonates in the highest concentration than in all the other lower test concentrations and both controls. Therefore this is not a valid endpoint from which to calculate a NOEC. It is also not consistent with current OECD 211 GD which uses the cumulative number of live neonates per female as the parameter.

Table 4: Mortality of adults and neonates (Rufli 1989 *Daphnia* reproduction study)

Nominal concentrations (mg a.s./L)	Original mean measured concentrations (mg a.s./L)	Immobilised daphnids at Day 21 (n = 10)	Mean cumulative juveniles per female at Day 21		
			Dead and alive	Live	Dead
Control	-	1	62	55	7
Solvent control	-	1	54	47	7
0.00074	0.00038	5	53	36	17
0.0022	0.0009	1	70	57	13
0.0067	0.0052	2	68	51	17
0.020	0.0109	0	59	39	20
0.060	0.033	5	52	40	12
0.18	0.110	4	7	4	3
NOEC	-	No dose response	0.03	-	No dose response
EC_{10}		Not calculable	-	0.0276	-

DE comment: The NOEC (21d) = 0.005 mg/L based on the survival of young daphnids (F1-generation) and time to appearance of first brood is one order of magnitude lower than the NOEC (21d) = 0.03 mg/L based on reproduction (number of offspring).

DK answer: Time to first brood as an endpoint (Daphnia reproduction study Rufli 1989) The number of offspring were not recorded daily in this. The table below shows when observations were made. Data has not been recorded for days 8, 10, 11, 13 (the study was not observed or maintained on these days and neonates were not removed). Therefore this is not a true pattern of when first broods were actually produced as 57% of this data was not observed. On this basis this parameter should not be used as an endpoint as it is unreliable as it is missing data from crucial times during the study. Time to first brood is also not a main parameter in the current OECD 211 GD.

Table 5: Time to first brood data (Rufli 1989 *Daphnia* reproduction study)

Mean measured concentrations (mg a.s./L)	Number of *Daphnia* with first brood							
	Day 8 [b]	Day 9	Day 10 [b]	Day 11 [b]	Day 12	Day 13 [b]	Day 14	≥Day 14
Control	No observations	9	No observations	No observations	1	No observations	0	0
Solvent control		7			3		0	0
0.00038		7			3		0	0
0.0009		8			2		0	0
0.0052		6			4		0	0
0.0109		1			9		0	0
0.033		0			10		0	0
0.110		0			3		1	6 [a]

[a] Three of the parental daphnids had died before offspring production had started

[b] Neonates were not counted or removed from the test vessels on these days

The original of the results of the study without the inappropriate parameters are shown in the table below. The recalculated mean measured concentrations from Table 2 are shown in brackets.

Table 6: Summary of appropriate endpoints and recalculated measured concentrations (Rufli 1989 *Daphnia* reproduction study)

Endpoint	LOEC (mg a.s./L)	NOEC (mg a.s./L)
Immobilisation	0.1 (0.13)	0.1 (0.13)
Reproduction: Total cumulative number of young	0.1 (0.13)	0.03 (0.042)
~~Reproduction: Fraction of cumulative dead neonates~~	~~0.01~~	~~0.005~~ [a]
~~Appearance of first brood~~	~~0.01~~	~~0.005~~ [b]

The vehicle and the blank were not significantly different in respect to the above parameters
[a] Not dose responsive
[b] Data not recorded on 4 out of the 7 days when first broods may have occurred

DK CA consider that the Rufli (1989) study is no longer reliable as the methodology used was prior to ring testing and significant recommendations were made for critical factors (dosing, feeding, culture medium, use of surfactant and solvent use) to improve study design and reliability. The Rufli 1989 study departs substantially from current test guidelines for these aspects. The expectation of the appearance of male neonates is a strong indication that the culturing procedures at that time were insufficient to produce a reliable study. As the guideline criteria have moved substantially since this study was conducted it is no longer be used for registration purposes in its original form. Using modern parameters and a re-interpretation of mean measured concentration it is useful as supporting evidence only.

Long term toxicity to fish: ELS test with pimephales promelas, this study was not handed in for the biocide evaluation and we can not find it in EFSAs endpointlist for fludioxonil as a pesticide (2007).

Algae study: See our answer to comment 33 and 36. Regarding the validity criteria that the cell concentration in control cultures should be increased at least by a factor of 16 within 3 days, then this is achieved. So in conclusion the test is valid and still results from 48 h can be used.

We do not find that the study by Rufli (1989c) should be considered reliable and we therefore are of the opinion that a chronic M-factor of 1 should be applied as we suggested.

RAC's response

Short-term toxicity to aquatic invertebrates:
Regarding the short-term toxicity studies on marine invertebrates, please see the response to the comment 33.

Long-term toxicity to aquatic invertebrates:
RAC agrees that the study of Rufli (1989c) should be considered reliable.
The test was performed according to the OECD TG 202, part II and the validity criteria were fulfilled. The mortality in the controls was lower than 20% (10% of mortality in both control and in solvent control), the number of live juveniles per parent after three broods (21d in this case) was higher than 20 (55 live juveniles in the control and 47 in the solvent control). Table 4, showed by the DS, indicates that there is still a dose response effect for cumulative juveniles per parent (total, live and dead) if the test concentrations where the mortality is higher than 20% are excluded (test concentrations of 0.00038, 0.033 and 0.11 mg/L). The cumulative live juveniles per female at the test concentrations of 0.0009, 0.0052, 0.0109

mg/L are 103.6%, 92.7% and 70.9% of the control. The NOEC for this endpoint is 0.0052 mg/L.
The endpoint time to first brood, see table 5 of DS response, is calculated at day 9 (time for appearance of first brood in the control). The number of daphnids with first brood in the test concentrations of 0.0009, 0.0052, 0.0109 mg/L are 88.9%, 66.7% and 11.1% of the control. The NOEC for this endpoint is 0.0052 mg/L.
The Guidance on the Application of the CLP Criteria (Version 4.1 – June 2015) in Annex I reports the chronic testing usable for classification, in particular for invertebrates it states (pag. 554): "*Observational endpoints include time to first brood, number of offspring produced per female, growth, and survival. It is recommended that tests consistent with OECD test guidelines 211 and/or 202 Part 2 (Daphnia reproduction) or US-EPA 850.1350 (Mysid chronic) or their equivalents be used in the classification scheme.*"

Long term toxicity to fish:
The study ELS test with *pimephales promelas* is not available to RAC.

Algae and aquatic plants:
RAC is aware that 72 or 96 hours EC50 are requested for algae according to Regulation (EC) 1272/2008. However, according to the TG 201 OECD the test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached, that corresponds to a specific growth rate of 0.92 day^{-1}.
RAC observations: Taking into account the information on the study to effects on algae growth (Hoberg, J.R., 1992) in the Addendum 1 to DAR (October 2006), RAC notes that exponential growth seems guaranteed at 48h.
The declared initial cell density of 3000 cells/mL in each test vessel is slightly lower than the cell density recommended in OECD TG 201 with the freshwater alga *Pseudokirchneriella subcapitata (*$5x10^3$-10^4). Table 9.2.6-3.1 reports the cell density at the time intervals 24, 48, 72 and 120h. In the negative and solvent control, the minimum multiplication factor of 16 is reached and the corresponding specific growth rate of 0.92 day^{-1} is reached at 48h.
RAC highlights that solvent control data were significantly different from control data, therefore it should be guaranteed that the solvent controls rather than the controls without solvents were used for the calculation of percent inhibition. No information on this point is available.

Table 9.2.6-3.1: Cell concentrations of *Selenastrum capricornutum* in 120 h. algae growth inhibition test with fludioxonil (CGA 173506) technical.

Growth function	Time interval (h)	Negative control	Solvent control[c]
Cell density ($x10^4$ cells/mL)	0-24	3	3
		3	2
		5	1
	Mean (SD)	4(1)	2(1)
	0-48	8	9
		9	6
		12	6
	Mean (SD)	10(2)	7(2)
	0-72	25	28
		28	26
		27	23
	Mean (SD)	26(2)	26(2)
	0-96	66	62
		69	51

		77	63
	Mean (SD)	70(6)	59(7)
	0-120	136	121
		141	108
		134	114
	Mean (SD)	137(4)	114(6)

[c] Solvent control data were significantly different ($p<0.05$) from control data based on a t-test.

Conclusion on classification and labelling for environmental hazards:
RAC considers that the lowest reliable NOEC is 0.005 mg/L. Therefore, the chronic M-factor should be changed to 10.

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
30.08.2016	United Kingdom	Lanxess Deutschland GmbH	Company-Downstream user	40
Comment received				
Section 3.1.3 Lanxess Deutschland GmbH agrees with the conclusion for no classification based on the submitted data.				
Dossier Submitter's Response				
Thanks for your evaluation and support regarding the classification proposal				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.09.2016	Switzerland	Syngenta	Company-Manufacturer	41
Comment received				
The physico-chemical properties determined for fludioxonil do no trigger classification. Therefore Syngenta agrees that no classification is necessary. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Fludioxonil - confidential attachments.zip				
Dossier Submitter's Response				
Thanks for your evaluation and support regarding the classification proposal				
RAC's response				
Noted.				

CONFIDENTIAL ATTACHMENTS

1. Fludioxonil - confidential attachments.zip [Please refer to comment No. 6, 9, 13, 16, 18, 20, 22, 25, 27, 29, 31, 38, 41]

ANNEX A: Study summaries (Mutagenicity; *In vitro* gene mutation study in bacteria)

IIIA 8	**TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM**	**Official use only**
IIIA 8.5	**Mutagenicity**	
IIIA 8.5.1	***In vitro* gene mutation study in bacteria**	
IIIA 8.5.1-02	**REFERENCE**	
Reference	Bowles, A. (2009). Technical Fludioxonil – Reverse Mutation Assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli*. Harlan Laboratories Ltd. unpublished report no: 2364/0457, 17.04.2009	
Data protection	Yes	
Data owner	Syngenta	
Companies with letter of access	Lanxess Deutschland GmbH	
Criteria for data protection	Data submitted on a new a.s. for the purpose of entry into the Union List of approved active substances according to Regulation (EU) No 528/2012.	
	GUIDELINES AND QUALITY ASSURANCE	
Guideline study	OECD 471 (1997), EC Method B13/14, US EPA OPPTS 870.5100 (1998) and Japanese Guidelines	
GLP	Yes	
Deviations	None	
	MATERIALS AND METHODS	
Test material	CGA173506 tech. (fludioxonil)	
Lot/Batch number	Batch No SMO5F007	
Specification	Not reported	
Description	Grey solid	
Purity	96.6%	
Stability	Assumed stable for study duration	
Study Type	Bacterial reverse mutation test (Ames test)	
Organism/cell type	*Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100; *Escherichia coli* strains WP2*uvrA*$^-$pKM101 and WP2pKM101	
Deficiencies / Proficiencies	The study is fully compliant with OECD 471 (1997).	
Metabolic activation system	Mammalian microsomal fraction (S9) from the liver of male rats induced with phenobarbitone/ß-naphthoflavone on 3 consecutive days prior to S9 preparation on Day 4. Before use, the S9 fraction was supplemented with co-factors.	
Positive control	Positive controls N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), 4-nitroquinoline-n-oxide (4NQO) and 9-aminoacridine (9AA) were used in the absence of S9; 2-aminoanthracene (2AA) and benzo(a)pyrene (BP) were used in the presence of S9.	
Cytotoxicity testing	A preliminary toxicity test was performed using TA100 and WP2*uvrA*$^-$pKM101, with and without S9 mix at ten concentrations between 0.15-5000 µg/plate. Plates were incubated for 48 hours prior to colony counting. Manual counts were performed at 5000 µg/plate due to excessive test material precipitation. There were no signs of toxicity up to and including the limit dose.	

IIIA 8	TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM	Official use only
Exposure	--	
Concentrations	Concentrations of fludioxonil of 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate were used in the preliminary toxicity assay. For the mutation test; five concentrations (50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate in both Experiment 1 and Experiment 2.	
Method of application	Experiment 1 used the direct plate incorporation method; 0.1 mL of bacterial culture was mixed with 2.0 mL top agar (either trace histidine or tryptophan supplemented), 0.1 mL test material formulation, vehicle (dimethyl sulphoxide) or positive control and 0.5 mL of either S9 mix or phosphate buffer. The mixture was poured onto the surface of Vogel-Bonner Minimal agar plates (plated in triplicate). Plates were incubated for about 48 h at 37°C. The first test was repeated in an independent experiment (Experiment 2) and using fresh cultures. The methodology was the same as for Experiment 1 in the absence of S9 mix. In the presence of S9 mix, 0.1 mL bacterial culture and test material formulation or vehicle (dimethyl sulphoxide) were mixed with 0.5 mL of S9 mix and incubated for 60 minutes at 37°C prior to the addition of 2.0 mL molten top agar (either trace histidine or tryptophan supplemented). The mixture was plated out in triplicate onto the surface of Vogel-Bonner Minimal agar plates.	
Pre-incubation time	Not applicable for Experiment 1 and Experiment 2 without S9 (plate incorporation method) Experiment 2 with S9: 60 minutes	
Evaluation	The laboratory's criteria for a positive response are shown below: In all strains a two-fold increase in the mean number of revertants per plate compared to the mean value of concurrent vehicle control. Increases in revertant numbers for all strains must be dose-related. A positive response in one tester strain either with or without exogenous metabolic activation is sufficient to designate the test material as a bacterial mutagen.	
Examinations	--	
Number of cells evaluated	From each triplicate plate, the number of revertant colonies (mutants) was determined. Means and mutant factors were calculated. Observations such as precipitation of the test substance or reduced background lawn were also recorded. Colony counts were performed using a Domino colony counter. Manual counts were performed at 5000 µg/plate because of excessive test material precipitation.	
	RESULTS AND DISCUSSION	
Genotoxicity	--	
Without metabolic activation	No increases in the frequency of revertant colonies, in excess of two-fold greater than the concurrent solvent controls, were recorded for any of the bacterial strains at any dose level, without metabolic activation. Precipitation of the test material was observed at 1500 and 5000 µg/plate.	
With metabolic activation	No increases in the frequency of revertant colonies, in excess of two-fold greater than the concurrent solvent controls, were recorded for any of the bacterial strains at any dose level, with metabolic activation. Precipitation of the test material was observed at 1500 and 5000 µg/plate.	
Cytotoxicity	The test material was non-toxic to the tested strains (TA100 and WP2*uvrA*$^-$pKM101). The test material formulation and S9 mix were both shown to be sterile. Precipitation of the test material was observed at 1500 and 5000 µg/plate.	

IIIA 8		TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM	Official use only
Discussion		No evidence for a mutagenic effect of fludioxonil was found in this test system either in the absence or presence of a metabolic activation system.	
		APPLICANT'S SUMMARY AND CONCLUSION	
1.1	**Materials and methods**	The mutagenicity of fludioxonil was investigated in an Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli* strains WP2*uvrA*⁻pKM101 and WP2pKM101. Triplicate cultures (plate incorporation method) were exposed to the test substance (dissolved in dimethyl sulphoxide) at five concentrations between 50 and 5000 µg/plate in the absence and presence of an exogenous metabolic activation system (rat liver S9 fraction). The experiment was repeated in an independent assay using the same test concentrations; the plate incorporation method was used without S9; the pre-incubation method was used with S9.	
1.2	**Results and discussion**	No evidence for a mutagenic effect of fludioxonil was seen in this test system, either in the absence or presence of a metabolic activation system. Results were confirmed in an independently repeated assay and are presented below in Table IIIA 8.5.1-01. Appropriate positive control compounds confirmed the sensitivity of the assay.	
1.3	**Conclusion**	Fludioxonil was not found to be mutagenic under the conditions of this study.	
1.3.1	Reliability	1	
1.3.2	Deficiencies	None.	
		Evaluation by Competent Authorities	
		Evaluation by Rapporteur Member State	
Conclusion		*Applicants`s conclusion is supported.*	
Reliability		*1*	
Acceptability		*Study is acceptable.*	
Remarks		*None*	

Table IIIA 8.5.1-1 Mean number of revertant colonies per plate

strains		TA 100		TA 1535		WP2uvrA		WP2pKM101		TA98		TA 1537	
CGA 173506 [µg/plate]	**S9:**	-	+	-	+	-	+	-	+	-	+	-	+
Experiment 1													
Control		98	96	25	15	133	179	99	153	20	24	11	11
50		101	101	24	15	124	172	95	156	18	22	10	12
150		93	98	24	13	126	144	97	157	19	25	11	9
500		97	88	24	13	134	154	99	154	21	20	11	10
1500		98P	92P	26P	12P	115P	150P	95P	151P	20P	23P	10P	13P
5000		98P	86P	26P	14P	108P	132P	90P	135P	20P	25P	11P	11P
Positive controls [µg/plate]													
ENNG:	0.5					3669							
	2			1855				4472					
	3	1361											
	5												
4NQO:	0.2									210			
9AA:	80											1214	
2AA:	1		3356										
	2				140								418
	10						2048		473				
BP:	5										283		
Experiment 2													
Control		129	91	25	24	113	162	114	118	16	20	13	12
50		105	89	23	15	119	152	116	126	18	20	11	5
150		106	88	36	15	125	149	103	115	12	20	11	15
500		120	98	23	21	113	145	91	116	18	23	8	8
1500		126P	89P	20P	15P	116P	130P	93P	102P	16P	23P	9P	5P
5000		123P	106P	22P	13P	113P	158P	88P	108P	15P	17P	11P	8P
Positive controls [µg/plate]													
ENNG:	0.5					2081							
	2							3001					
	3	934											
	5			475									
4NQO:	0.2									230			
9AA:	80											808	

2AA:	1		2651										
	2				172								468
	10						2488		905				
BP:	5										244		

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
BP Benzo(a)pyrene
2AA 2-Aminoanthracene
P Precipitate

IIIA 8	**TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM**	**Official use only**
IIIA 8.5	**Mutagenicity**	
IIIA 8.5.1	***In vitro* gene mutation study in bacteria**	
IIIA 8.5.1-03	**REFERENCE**	
Reference	Chang, S. (2016). Fludioxonil tech. – *Salmonella Typhimurium* and *Escherichia Coli* Reverse Mutation Assay. Envigo CRS GmbH unpublished report no: 1770600, 05.08.2016	
Data protection	Yes	
Data owner	Syngenta	
Companies with letter of access	Lanxess Deutschland GmbH	
Criteria for data protection	Data submitted on a new a.s. for the purpose of entry into the Union List of approved active substances according to Regulation (EU) No 528/2012.	
	GUIDELINES AND QUALITY ASSURANCE	
Guideline study	OECD 471 (1997)	
GLP	Yes	
Deviations	None	
	MATERIALS AND METHODS	
Test material	CGA173506 tech. (fludioxonil)	
Lot/Batch number	Batch No SMO5B1265/FORTIFIED	
Specification	Fludioxonil: 98.4% w/w SYN549410 (fortified by-product): 0.51% w/w CGA278466 (fortified by-product): 0.22% w/w	
Description	Yellowish solid	
Purity	98.4%	
Stability	Assumed stable for study duration	
Study Type	Bacterial reverse mutation test (Ames test)	
Organism/cell type	*Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100; *Escherichia coli* strains WP2*uvrA*pKM101 and WP2pKM101	
Deficiencies / Proficiencies	The study is fully compliant with OECD 471 (1997).	
Metabolic activation system	Mammalian microsomal fraction (S9) from the liver of male rats induced with phenobarbitone/ß-naphthoflavone on 3 consecutive days prior to S9 preparation on Day 4. Before use, the S9 fraction was supplemented with co-factors.	
Positive control	Positive controls, sodium azide (NaN_3), 4-nitro-o-phenylene-diamine (4-NOPD) methyl methane sulfonate (MMS) were used in the absence of S9; 2-aminoanthracene (2-AA) was used in the presence of S9.	
Cytotoxicity testing	A pre-experiment was performed with all strains at eight concentrations between 3-5000 µg/plate (in triplicate). The pre-experiment is reported as Experiment 1.	

IIIA 8	TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM	Official use only
Exposure	--	
Concentrations	Concentrations of fludioxonil of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate were used in the pre-experiment/Experiment 1. Concentrations of fludioxonil of 33, 100, 333, 1000, 2500 and 5000 µg/plate were used in Experiment 2.	
Method of application	Experiment 1 used the plate incorporation method; 0.1 mL of bacterial culture was mixed with 2.0 mL overlay agar (either histidine or tryptophan supplemented), 0.1 mL test material formulation, vehicle (dimethyl sulphoxide) or positive control and either 0.5 mL of S9 mix or phosphate buffer. The mixture was poured onto the selective agar plates (plated in triplicate). Experiment 2 used the pre-incubation method; 0.1 mL vehicle (dimethyl sulphoxide) or positive control, 0.5 mL S9 mix or phosphate buffer and 0.1 mL bacterial culture were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 mL (45°C) overlay agar was added to each tube and the mixture poured onto selective agar plates. Plates were incubated upside down for 72 h at 37°C in the dark.	
Pre-incubation time	Not applicable for Experiment 1 (plate incorporation method). Experiment 2: 60 minutes	
Evaluation	The laboratory's criteria for a positive response are shown below: A biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control. A concentration dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration. An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment. A concentration dependent increase in the number of revertant colonies below the threshold is regarded as an indication of mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls, such an increase is not considered biologically relevant.	
Examinations	--	
Number of cells evaluated	From each triplicate plate, the number of revertant colonies (mutants) was determined. Means and mutant factors were calculated. Observations such as precipitation of the test substance or reduced background lawn were also recorded. Colony counts were performed using a validated computer system; due to precipitation of the test substance the colonies were partly counted manually.	
	RESULTS AND DISCUSSION	
Genotoxicity	--	
Without metabolic activation	No increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level, without metabolic activation..	
With metabolic activation	No increases in the frequency of revertant colonies were recorded for any of the bacterial strains at any dose level, with metabolic activation.	
Cytotoxicity	Cytotoxic effects, evident as a reduction in the number of revertants occurred in Experiment 2 in the presence of S9 mix, in strain TA98 at 2500 and 5000 µg/plate and in strainTA1537 at 5000 µg/plate. No other cytotoxic effects were observed. Normal background growth was reported in all strains at all test concentrations. The test item precipitated in the overlay agar in the test tubes from 333 to 5000 µg/plate. Precipitation of the test item in the overlay agar on the	

IIIA 8		TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM	Official use only
		incubated agar plates was observed from 1000 to 5000 µg/plate in Experiment 1 and from 2500 to 5000 µg/plate in Experiment 2.	
Discussion		No evidence of mutagenicity was seen under the conditions of this study. It can therefore be concluded that fludioxonil containing the impurities SYN549410 (at levels up to 0.51%) and CGA278466 (at levels up to 0.22%) is not mutagenic.	
		APPLICANT'S SUMMARY AND CONCLUSION	
1.4	**Materials and methods**	The study was performed using fludioxonil (98.4% purity), which had been spiked with the impurities SYN549410 (to a level of 0.51%) and CGA278466 (to a level of 0.22%). In an initial (plate incorporation) assay, triplicate cultures of *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E. coli* strains WP2*uvrA* pKM 101 and WP2 pKM 101 were exposed to fludioxonil (dissolved in DMSO) in the absence and presence of an exogenous metabolic activation system (phenobarbital/ß-naphthoflavone induced male Wistar rat liver S9 fraction) at concentrations up to the limit concentration of 5000 µg/plate. In the confirmatory assay, triplicate bacterial cultures were exposed to fludioxonil in the absence and presence of an exogenous metabolic activation system at concentrations of up to the limit concentration of 5000 µg/plate. Cultures were also exposed to DMSO (solvent control), were unexposed (negative control) or were exposed to appropriate positive controls substances in the absence of metabolic activation (sodium azide, methyl methane sulphonate), 4-nitro-o-phenylenediame) or in the presence of metabolic activation (2-aminoanthracene).	*
1.5	**Results and discussion**	Precipitation of the test material was seen at the highest concentrations used. Evidence of cytotoxicity (reduced revertant counts; was seen in some strains at the limit concentration. Exposure to fludioxonil at concentrations of up to the limit concentration of 5000 µg/plate in the absence or presence of metabolic activation did not result in any increase in the numbers of revertant colonies in either the initial or confirmatory assays. Exposure to the positive control substances resulted in marked increases in the numbers of revertant colonies in the absence and presence of metabolic activation, thereby confirming efficacy of the metabolic activation system and the sensitivity of the assay.	*
1.6	**Conclusion**	No evidence of mutagenicity was seen under the conditions of this study. It can therefore be concluded that fludioxonil containing the impurities SYN549410 (at levels up to 0.51%) and CGA278466 (at levels up to 0.22%) is not mutagenic.	
1.6.1	Reliability	1	
1.6.2	Deficiencies	None.	
		Evaluation by Competent Authorities	
		Evaluation by Rapporteur Member State	
Conclusion		*eCA DK agrees with evaluation and conclusion.*	
Reliability		*1*	
Acceptability		*Accepted.*	

IIIA 8	TOXICOLOGICAL PROFILE FOR HUMANS AND ANIMAL INCLUDING METABOLISM	Official use only
Remarks	Additional text proposal in this section "Results and discussion". Sentence two should be changed to "Evidence of cytotoxicity (reduced revertant counts; below the indication factor of 0.5) was seen in some strains at the limit concentration. " Additional text : There was also no tendency of higher mutation rates with increasing concentrations and all mutation rates were within the range of normal biological variability.	

Table IIIA 8.5.1-2 Mean number of revertant colonies per plate

strains		TA 1535		TA 1537		TA98		TA100		WP2pKM101		WP2uvrA	
CGA 173506 [µg/plate]	**S9:**	-	+	-	+	-	+	-	+	-	+	-	+
Experiment 1													
Control		19	14	13	10	26	39	176	165	21	258	360	363
Untreated		12	15	12	16	31	40	204	194	267	296	394	451
3		16	16	14	12	26	47	177	150	237	252	339	392
10		16	16	11	16	25	42	195	177	198	267	333	386
33		11	15	16	10	29	41	183	161	218	265	362	405
100		16	19	10	17	28	40	189	177	227	252	344	370
333		12	13	11	12	32	30	197	168	226	244	349	376
1000		16P	11P	10P	11P	31P	32P	190P	162P	209P	228P	355P	397P
2500		12P	27P	11P	10P	23P	51P	198P	172P	229P	286P	366P	384P
5000		14P	12P	7P	10P	20P	24P	195P	172P	290P	195P	376P	497P
Positive controls [µg/plate]													
NaN3:	10	1219						2268					
4NOPD:	10					368							
	50			73									
MMS:	2.0									5156		4828	
2AA:	2.5		434		170		5732		5456				
	10										1402		2336
Experiment 2													
Control		13	14	13	14	27	42	143	156	195	200	314	374
Untreated		12	14	12	17	22	32	16	194	236	206	341	416
33		15	16	10	14	24	34	135	136	219	224	334	402
100		15	15	10	15	25	30	131	125	195	224	338	353
333		13	18	12	13	27	32	142	131	181	184	319	364
1000		13	15	11	14	27	39	139	135	186	255	325	354
2500		15P	12P	6P	13P	24P	17P	150P	137P	133P	230P	302P	348P
5000		10P	10P	6P	6P	20P	15P	112P	118P	89P	205P	324P	387P
Positive controls [µg/plate]													
NaN3:	10	1111						1557					
4NOPD:	10					295							
	50			70									
MMS:	2.0									3422		2295	
2AA:	2.5		302		207		3183		4617				
	10										1093		2001

NaN3 Sodium azide
2AA 2-Aminoanthracene
4NOPD 4-nitro-o-phenylene-diamine
MMS Methyl methane sulfonate
P Precipitate