Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide

EC number: 251-899-3

CAS number: 34256-82-1

CLH-O-0000001412-86-26/F

Adopted

04 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: **Acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide**

EC number: **251-899-3**

CAS number: **34256-82-1**

Dossier submitter: **Spain**

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2014	France		MemberState	1
Comment received				
FR agrees with the classification proposal for human health and environmental hazards.				
Dossier Submitter's Response				
Thank you for your agreement with our proposal.				
RAC's response				
RAC endorses the comments by France.				

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2014	Belgium	Monsanto Europe	Company-Manufacturer	2
Comment received				
We would like to commend the author(s) of this report for a very thorough, comprehensive and well-written document. Our detailed comments on the scientific evaluation of the data, more specifically on the human health hazard assessment, are summarized in a document uploaded as public attachment. Besides some comments on specific studies or endpoints, we are questioning the classification proposals listed below: • Acute toxicity (oral) : Acute tox. 4; H302 • Skin corrosion/irritation : Skin Irrit. 2 ; H315 • Carcinogenicity : Carc. 2 ; H351 • Specific target organ toxicity – repeated exposure : STOTR RE 2; H373 The results of additional studies have been taken into consideration in our discussion. • [redacted] (2010) Acetochlor sec-methylsulfide : in-vitro metabolism by olfactory turbinate and liver microsomes of male Sprague Dawley rat • [redacted] (1980) CP 55097: 91-day Feeding Study in the Mouse • [redacted] (2013) : MON52706 : Bacterial Reverse Mutation Assay.				

• [redacted] (2014). In vivo Muta™Mouse gene mutation assay with MON 52706

The Tier 2 summaries of these studies are presented in appendix to this document, and the full reports of 3 studies have been uploaded as confidential information. The size of the mouse feeding study didn't allow it to be uploaded. This study is available upon request.

ECHA note: The following attachments were provided:

1. *Attachment 1 Comments on CLH report for Acetochlor, Version 2 (September 2013)*

Study reports (confidential):

1. *Acetochlor sec-methylsulfide: in vitro metabolism by olfactory turbinate and liver microsomes of male Sprague Dawley rats*
2. *Bacterial Reverse Mutation Assay*
3. *In vivo Muta™Mouse gene mutation assay with MON 52706*

Dossier Submitter's Response

Our comments include the response to your statements questioning the acetochlor classification as mentioned above as well as those in the attachment provided.

Major comments:

1. Comparison of rat nasal carcinogenic mode-of-action (MOA) data with CLP criteria for classification:

Quantitative differences in metabolism between rats, mice, monkeys and humans

Although the majority of the DABQI produced from acetochlor is derived from p-hydroxylation of sulfoxide metabolite, the *in vitro* results show that small amounts of EMA can be produced and subsequently can be para-hydroxylated to DABQI in rats, monkeys and/or humans. In this regard, we want to add some information included in the EPA document "Acetochlor Report of the Cancer Assessment Review committee (Fourth Evaluation), August 31, 2004" (US EPA, 2004a), that we did not assess in the CLH report and now it seems important to the relevance of nasal tumours to humans.

In the CLH Report we stated that the para-hydroxylation rate of EMA in primate nasal tissue was substantially less (~24-fold) than in rats and only a very low rate of para-hydroxylation (approximately 4% of the rate in rats) was observed when monkey nasal tissue was incubated with EMA (Green, 1998a). We also said that both monkey and human nasal tissues were unable to para-hydroxylate the sulfoxide metabolite of acetochlor, which is the primary precursor to DABQI in the rat. However, after a more in depth review of the US EPA (2004a) document, we have found that there is a refinement of these values.

a. EMA hydroxylation

In vitro metabolism of acetochlor to p-OH-EMA was compared in liver and nasal tissues from rats, mice and primates (Green 1998a). Quantitative differences were studied in the rate between rats, mice, and squirrel monkeys of three reactions. The first reaction is the conjugation of acetochlor with GSH, the second reaction is the cleavage of secondary sulfide to EMA, and the third one is the p-hydroxylation of EMA to p-hydroxy-EMA (a precursor of the quinone imine). These reactions were studied using cytosolic and microsomal fractions from liver and nasal epithelium of the tested species. Olfactory and respiratory epitheliums were separated for rats and mice, but not for the squirrel monkey. All three reaction rates were significantly higher in the nasal olfactory tissue of the rat vs. monkey (26.2-fold, GSH conjugation of acetochlor, 86-fold, hydrolysis of secondary sulphide metabolite of acetochlor and 23.7-fold, hydroxylation of EMA), suggesting that the rate of formation of precursors to reactive metabolites (quinone imines) that are implicated in nasal tumor formation are greater in the rat than in monkeys or other primates.

However, a review of the data by EPA indicated that **there was uncertainty** in the rat-to-monkey ratios of activities because olfactory and respiratory epithelium were not separately analyzed for the monkey and the respiratory epithelium in the monkey might be acting as an enzymatically inert diluent for this *in vitro* assay. To evaluate the impact of this uncertainty, the Acetochlor Reregistration Partnership (ARP) provided calculations (Dybowski, 2003b) of the estimated rates (Vi) for p-hydroxylation of EMA assuming (1) a "conservative" estimate of monkey nasal tissue samples containing 10% olfactory tissue and (2) a "worst-case" estimate of samples containing 1% olfactory tissue. Based on these estimations, the presence of 10% olfactory tissue would give a primate Volfactory for p-hydroxylation of EMA that is 8.7-fold lower (not 23.7-fold lower, as reported initially) than that of rat olfactory tissue and 2.6-fold lower than rat respiratory tissues. If the samples contained only 1% olfactory tissue, the study author calculated that the primate Volfactory would be approximately 7.2-fold less than the rat olfactory tissue and 2.1-fold less than the respiratory tissues. These results are summarized in the following table I. Examination of this table indicates that although hydroxylation of EMA in the monkey is slower than in the rat, the rate is not so slow as to negate totally the possibility of oxidation of EMA in the monkey's olfactory epithelium, if EMA is available.

Table I: Rat-to-monkey ratios of rate of **p-hydroxylation of EMA to p-hydroxy-EMA** using microsomal suspensions of rat olfactory epithelium and mixed monkey olfactory/respiratory epithelium (in unknown proportion). [From Green, 1998a and Dybowski, 2003b]

Experimental ratio with no correction	Corrected ratio assuming 10% contamination with respiratory epithelium	Corrected ratio assuming 1% contamination with respiratory epithelium
23.7	8.7	7.2

Therefore, *in vitro* studies of p-hydroxylation of EMA using olfactory epithelium enzymes indicate that rat-to-monkey ratios of activities (Green, 1998a and Dybowski, 2003b) are not as large as 23.7 and could be as small as 7 or 8 (rate of para-hydroxylation in monkey is **14%** of the rate of rats).

b. Hydroxylation of Acetochlor sulfoxide metabolite

Acetochlor sulfoxide is the major circulating acetochlor metabolite in plasma of rats, which requires hydroxylation in the formation of DABQI. The rate of hydroxylation of radiolabeled acetochlor sulfoxide metabolite to p-hydroxy-acetochlor sulfoxide was compared using microsomal fractions derived from Sprague-Dawley rat and CD-1 mouse separated nasal olfactory and respiratory tissues and from squirrel monkeys and 33 human morphologically normal nasal tissue surgical explants (olfactory and respiratory tissues from primates and humans were combined and not separated) (Green 2000).

Reported results indicated that the highest rates of hydroxylation of acetochlor sulfoxide were observed in the olfactory tissue of the rat and the mouse with comparable activities in these species (6 to 7-fold higher than the activity in rat respiratory tissue, respectively). There was no detectable activity in the primate or human samples. The investigators concluded, therefore, that acetochlor-induced nasal tumors in the rat are not relevant to humans, because the quinone-imine metabolite derived from acetochlor sulfoxide and, believed to be responsible for olfactory tumorigenesis would not be produced at sufficient levels in the human. However, a review of the study by EPA indicated that **there was uncertainty** in the data because olfactory and respiratory epithelium were not separately analyzed and the respiratory epithelium in the human and primate might be acting as an enzymatically inert diluent for this *in vitro* assay.

Following Agency review of the data, because of **concerns raised regarding the possible dilution effect of inactive respiratory epithelium**, the Registrant provided estimations of the activity that would be present if the sample contained only 10% or 1% olfactory

tissue (Dybowski, 2003a). Based on these estimates, hypothetical rates of hydroxylation of acetochlor sulfoxide in human or primate olfactory tissue would yield rates that were 132-fold or 88-fold lower than the activity in the rat olfactory tissue. These values for primates, although much smaller than those for rats, are still consistent with a finite probability of bioactivation of acetochlor metabolites in the nasal mucosa. These ratios of activities are summarized in the following table II.

Table II: Rat-to-monkey ratios of rate of **p-hydroxylation of acetochlor sulfoxide** metabolite using microsomal suspensions of rat olfactory epithelium and monkey or human unseparated olfactory/respiratory epithelium (in unknown proportion). (From Green, 2000 and Dybowski, 2003a).

Experimental ratio with no correction	Corrected ratio assuming 10% contamination with respiratory epithelium	Corrected ratio assuming 1% contamination with respiratory epithelium
Extremely high (no activity for primates)	132	88

Therefore, *in vitro* studies of p-hydroxylation of Acetochlor sulfoxide metabolite using olfactory epithelium enzymes indicate that rat-to-monkey ratios of activities (Green, 2000 and Dybowski, 2003a) could be of 88 (rate of para-hydroxylation in monkey is **1%** of the rate of rats).

Comparison of rat nasal carcinogenic mode-of-action (MOA) data with CLP criteria for classification:

The CLP guidance version 4.0 (November 2013) states, in section 3.6.2.3.4 (k), that the IPCS Framework for Analyzing the Relevance of a Cancer Mode of Action for humans (2007) can be a useful way to construct and present a robust and transparent assessment of such data. The IPCS human relevance framework (HRF) incorporates a weight-of-evidence approach to evaluate whether there are sufficient data to exclude the human relevance of the MOA on the basis of qualitative or quantitative species differences in toxicokinetics and/or toxicodynamics.

Data suggest that, even though it's a minor pathway, the formation of reactive metabolites could occur in humans. Based on the *in vitro* data, we cannot exclude the possibility that EMA/ sulphoxide may be produced by humans. The Agency review of the data (Dybowski, 2003a) shows that the quantitative differences in toxicokinetics/toxicodynamics between animal species and humans are not so large as initially thought.

Therefore, the answer to third question of the IPCS HRF, can the human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetics or dynamics between animals and humans? In our understanding the answer is **No**.

This conclusion is supported by:

- The realization that production of a metabolite (EMA) with the capacity of undergoing transformation to a quinone imine is possible for humans (Coleman et al., 2000). Although nasal tissue was not included in the Coleman et al. (2000) study, the data indicate that human liver has the potential to produce EMA, which would then be available to all organs via the circulatory system.
- Finding that, the human liver microsomes metabolize EMA at a 2-fold higher rate than the liver microsomes from rat.
- In *in vitro* studies of p-hydroxylation of EMA using olfactory epithelium enzymes indicate that rat-to-monkey ratios of activities (Green, 1998a and Dybowski, 2003b) are not as large as 23.7 but could be as small as 7 or 8.
- *In vitro* studies, the ratio of rat to monkey for p-hydroxylation of the sulfoxide metabolite of acetochlor may be not astronomically large, as initially postulated, but as small as 88 (Dybowski, 2003a).
- The cytochrome P450 isoforms responsible for human metabolism of acetochlor are

CYP3A4 and CYP2B6.

Besides, although EMA was not identified in rat plasma, this could be justified on the absence of an adequate profile of metabolites in rat plasma. Then, based on *in vitro* data, we cannot exclude the possibility that higher amounts of EMA that initially thought may be formed ***in vivo*** in rats and/or humans, and subsequently be para-hydroxylated to a DABQI.

Therefore, we believe that based on the *in vitro* data, we cannot exclude that the MOA responsible for the rat nasal tumours could be relevant to humans and thus these tumours should trigger a Category 2 (CLP) cancer classification.

2. Toxicological significance of marginal increases in mouse lung tumours and histiocytic sarcomas:

A treatment-related effect cannot be excluded for the lung and uterus tumours, although the association is considered to be weak or/ equivocal, and in our opinion they contribute to the total tumour profile of acetochlor.

We consider that the US EPA HED "health effect division" analysis of PWG data (5th USEPA Report of Cancer Assessment Review Committee, 2007b) which use the censored incidence adjusted for mortality, even in the absence of censored historical control data to compare with, are relevant for the assessment of the carcinogenic potential of acetochlor. Survival adjusted methods are strongly advocated for comparison of tumour incidences among groups (OECD Guidance Document 116, of 13 April 2012, on the design and conduct of chronic toxicity and carcinogenicity studies). See additional considerations in chapter 4.10.4.2.: Carcinogenesis in mice.

Comments on specific endpoints

4.1. Toxicokinetics

4.1.1. Non-human information

We coincide with Monsanto comments that, *in vitro* studies conducted without an NADPH-regenerating system could favour EMA formation in cellular fractions from liver or nasal epithelia. However, this could be the most realistic situation *in vivo*. See comment on 4.1.2.

Monsanto further supported his position in deemphasizing p-OH EMA as a metabolic pathway of quinone, based on the result of a new *in vitro* study in rat liver and nasal microsomes in the presence of NADPH and glutathione (GSH) ([redacted] 2010). At this regards, we think that the results of this study should be interpreted with extreme care. *In vivo* levels of GSH and NADPH-regenerating systems in critical macromolecular targets, as the liver or the nasal tissue, could be lower than *in vitro*, rendering them more susceptible to the toxic action of acetochlor or other types of reactive intermediates.

Glutathione seems to have a dual role in preventing the cytotoxicity of acetochlor, through detoxification and through the reduction of oxidized groups in critical macromolecular targets. Chloroacetanilides preferentially react with glutathione (GSH) and therefore cause depletion of the protective nucleophile. Such depletion could be of particular concern for tissues with relative low level of endogenous GSH, rendering them more susceptible to the toxic action of this or other types of reactive intermediates. Regarding the NADPH-regenerating systems, see comment on 4.1.2

Therefore, we don't agree in removing the metabolic pathway resulting in the formation of p-OH EMA in rat. Instead, the headline of figure 1 of the CLH report could be changed as follows: "Metabolic pathways of acetochlor in rat" and consider adding the following remark at the end of the last paragraph before figure 2 of the CLH report "this seems to be a minor pathway".

4.1.2 Human information

In response to the following Monsanto comment "Quantitative species differences in formation of EMA *in vivo* as well as its subsequent conversion to DABQI should be considered in the assessment, as indicated by the IPCS human relevance framework" see response in major comment 1 on rat nasal carcinogenic mode of action.

Regarding Monsanto comments to the publication Coleman et al. (2000), we also think that the results from all *in vitro* experiments conducted in the absence of an NADPH-regenerating system should be interpreted with extreme care, however for a different reason. Incubation of acetochlor or metabolites as CMEPA with rat or human liver microsomes in presence of an NADPH-regenerating system could not be a realistic condition. The toxicological response could be attenuated if not prevented when a NADPH-regenerating system is added *in vitro* due to the reduction of oxidized groups in liver microsomes. On the other hand, the *in vivo* levels of NADPH-regenerating systems in critical macromolecular targets, as the liver or the nasal tissue, could be lower than *in vitro*, rendering them more susceptible to the toxic action of acetochlor or other types of reactive intermediates.

Metabolites with potential to exceed 0.1 µg/L only in exceptional circumstance (t-NCA)

For this information see comment 5.

4.2 Acute Toxicity

4.2.1 Non-human information

4.2.1.1 Acute Toxicity: oral

Acute oral LD_{50} for males in the Cummins study should be 4238 mg/kg, as reported in Table 14, not 2389 mg/kg as indicated in text of page 33. We apologize for the mistake.

4.2.4 Comparison with the criteria

We disagree with your comment questioning the classification for acute oral toxicity. The LD_{50} obtained in Branch (1982a) study of 1929 mg/kg in females supports the proposal for acute oral classification as Acute Tox. 4; H302.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.4 Comparison with criteria

Results obtained in Barlow (1989) skin irritation study are sufficient to support a classification for acetochlor as skin irritant and even lesions indicating a more severe classification were observed in this study and in 21-days dermal repeated exposure studies. However, the weight of the evidence from all the available data leads to a classification as skin irritant.

4.7 Repeated dose toxicity

4.7.1.1.1 Short term oral toxicity studies

We are along with your comments on the NOAEL from Broadmeadow (1989) study. Granuloma in one male and one female at 2 mg/kg bw/day in liver was not observed at higher dose levels. This lesion was neither accompanied by other correlated effects at higher dosage. Only reduced glycogen in one male at 2 mg/kg bw/day seems therefore not to be sufficiently relevant for LOAEL.

Oral short term toxicity studies in dogs

With respect to testicular effects in dogs, we meant in the CLH Report that the testicular lesions observed at 50 mg/kg bw/day were not associated with lymphoid orchitis. We apologize if the description of data in the CLH Report was not sufficiently clear.

In paragraphs below, we answer to comments on lesions that were regarded relevant for classification after repeated exposure.

4.7.1.10 Conclusions on classification for repeat dose toxicity

With respect to the comment on the effects of chronic vasculitis and interstitial nephritis reported from one 12-month dog study (Broadmeadow, 1989), we consider these findings sufficient to trigger classification after repeated exposure. These lesions in male kidneys at the mid dose level of 10 mg/kg bw/day are dose-related with a marked increase in the incidence and the severity.

Tubular basophilia was observed in a dose-related manner in 18-month study in mice (Amyes, 1989) with an incidence above the historical controls which lead to think that acetochlor can exacerbate this lesion. This finding is common in the initial stages of chronic progressive nephropathy (CNP), a spontaneous renal disease reported in rodents with absence of renal counterpart in humans. The lesion was not observed in other 23-month mice study.

Regarding comments on liver fatty infiltration observed in one male dog at 12 mg/kg bw/day in [redacted] (1981) study, it should be pointed out that this lesion was accompanied by a relative increase of liver weight at this dose level of 13.7% in males not statistically significant ($p \leq 0.05$). At the highest dose level of 40 mg/kg bw/day, the increment of liver weight in males was statistically significant (18%). In the comments you stated that fatty infiltration was scored at high dose of "mild". It has to be noted that fatty infiltration observed in one male dog at 12 mg/kg bw/day was regarded as "moderate diffuse infiltration" and fatty infiltration in one male dog at 40 mg/kg bw/day was considered "mild multifocal periportal fatty infiltration". Thus, the effects at the highest dose were apparently more important than at mid dose level showing an increase in the severity with the dose level. We regard that fatty infiltration should be considered in the evaluation for STOT.

Conclusion of MSCA

Comparison of data with the criteria for classification indicates that this is borderline and the criteria leave a margin for different interpretations. However, after a detailed and careful review of all available information and taking into account the importance of lesions in kidney and liver below cut-off values, we are of the opinion that a classification for specific target organ toxicity after repeated exposure is justified.

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1.2 Oral carcinogenesis in mice

Non-neoplastic kidney findings in 78-wk mouse study (Amyes, S.J. (1989))

We apologize for not being sufficiently clear about the incidence of tubular hyperplasia observed in males of this study. For clarity, we agree to add the following data for hypertrophy and hyperplasia which are relevant to Table 45.8 of the CLH report.

Selected Renal Tubular Lesions in 18-Month Mouse Oncogenicity Study (Amyes, 1989)

	Males				Females			
Dose (ppm)	**0**	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**

# animals examined	50	50	50	50	50	50	50	50
hypertrophy	1	1	0	3	0	1	1	1
hyperplasia	0	0	1	4	0	0	0	0

** p≤ 0.05
** p ≤ 0.01

4.10.3 Other relevant information

Alachlor

We agree that the sentence "It was concluded to classify alachlor with R40 based on the same tumours found in rats and mice after administration of acetochlor (ECB, 2002)" is misleading. It should be corrected as follows: "It was concluded to classify alachlor with R40 based on tumours found in rats and mice (ECB, 2002)"

4.10.4 Summary and discussion of carcinogenicity

4.10.4.1 Carcinogenesis in rats

Mode of action for nasal tumours

Monsanto stated that sulfoxide metabolite is the key precursor to DABQI formation following acetochlor administration and that EMA plays only a very minor role and it is not reflected in the CLH report. However, headline of figure 3 (page 136) indicates that DABQI derived from p-hydroxylation of sulfoxide metabolite represents the predominant pathway for acetochlor. Besides, in the paragraph at top of page 142 of CLH report it is also stated that: "Although it is likely that in the rat the quinoneimine is formed from the sulfoxide metabolite, the possibility that EMA might also be a precursor cannot be ruled out". We apologize if it is not sufficiently clear in the CLH report.

We agree to replace "EMIQ" with "DABQI in the text in page 139, 4th and 5th paragraphs and bottom of page 141. We also agree to remove, from the paragraph at top of page 142, the following statement: "Furthermore, the analysis of adducts cannot distinguish whether it is originated from the sulfide or EMA".

Concerning discussions on the importance of the EMA pathway:

The rat *in vitro* data results show that small amounts of EMA can be produced with acetochlor and subsequently can be para-hydroxylated to DABQI.

According to Monsanto, EMA was not identified in rat plasma, while DEA phenol suphate, an alachlor metabolite corresponding of a precursor of DEA (analogous to EMA), was confirmed in rat plasma. However, several aspects should be noted. Firstly, DEA phenol sulphate is only present in rat urine and faeces, according to the information of the Appendix EFSA Journal (2004) of alachlor (Figure 1), but not in plasma. Secondly, comparative of metabolism of acetochlor in rats and mice performed by Green (1998c) showed that the only identified metabolite in rat plasma was sec-methylsulfoxide. Other peaks were seen in this study, corresponding to metabolites not assigned. In absence of a more detailed profile about these minor metabolites, the presence of EMA in rat plasma can't be ruled out.

See also major comment 1 on rat nasal carcinogenic mode of action and comments in previous 4.1. Toxicokinetics section.

4.10.4.2 Carcinogenesis in mice

Lung tumours in CD-1 mice

Reported table 49 of CLH report should be replaced by the following. We apologize for the mistakes.

Source	Approx Time Period	Study Duration	No. Studies	Incidence (%)					
				Adenoma		Carcinoma		Total	
				Range	Mean	Range	Mean	Range	Mean
Tee *et al*. (1988)	1978 - 1983	24 months	11	na	14.5	na	12.1	17.5-38.8	26.6
Charles River (1995)	1981 - 1991	18 months	12	0-15.4	6.5	0-9.6	4.0	3.3-20.0	10.5
		21 months	6	0-10.0	6.2	0-10.0	3.1	6.0-14.0	9.4
		24 months	11	4.0-18.4	9.8	0-13.5	6.6	12.0-23.1	16.4
Inveresk Research (1996)	1990 – 1993	18 months	5	0-16.0	7.6	2-10.0	4.0	6.0-18.0	11.6
		24 months	8	0-14.0	8.5	2-10.0	6.5	12.0-20.0	15.0
Life Sciences Research (1989) *	1985 - 1988	18 months	13	0-9.6	5.4	0-9.6	4.4	3.8-19.2	9.8

The CLP guidance version 4.0 (November 2013) states, in section 3.6.2.3.2 (a), that "the historical control data set should be matched as closely as possible to the study being evaluated. The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. Therefore, the use of the in-house LSR historical data (13 studies of 18-month of duration) is the most appropriate to be matched to the 18-month mice study (Amyes, 1989). Regarding 23-month study ([redacted] 1983b), data were compared with historical control incidence data reported in 24 months contemporary studies included in table 49 of CLH Report.

Monsanto argues against classification based on the fact that mouse lung tumours and histiocytic sarcomas are very common spontaneous tumours in aged CD-1 mice. However, the CLP guidance version 4.0 (November 2013) considers to classify for carcinogenicity even when they are spontaneous tumours. In section 3.6.2.3.2 (a), it is stated that "Even when a particular tumour type may be discounted, expert judgment must be used in assessing the total tumour profile in any animal. However, appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification. Expert judgment is required to evaluate the relevance of the results".

In our opinion, although mouse lung tumours and histiocytic sarcomas are very common spontaneous tumours and the evidence of carcinogenic potencial is weak, we consider that they contribute to the total tumour profile of acetochlor.

In your document you draw our attention to the fact that US EPA compared censored data from chronic studies with uncensored historical control data. This comparison was reflected in the CLH report (p. 148) indicating that "It is noted that the historical control values have been calculated to include all of the control animals on the study and were not adjusted for mortality".

OECD Guidance Document 116, of 13 April 2012, on the design and conduct of chronic toxicity and carcinogenicity studies states that "survival adjusted methods are strongly advocated for comparison of tumour incidences among groups". Therefore, we consider that HED analysis of PWG reevaluation using censored data, even in the absence of censored

historical control data to compare with, are relevant for the assessment of the carcinogenic potential of acetochlor.

According to HED analysis of the 23-month mice study, there was a statistically significant increase of benign lung tumours in all groups of female mice accompanied of a clear dose-dependent relationship with a positive trend. The OECD guideline also points out that "A trend is more powerful than a pairwise test", which reinforces the weight of HED analysis data.

In the 18 month mice study (Amyes, 1989), benign tumours in male were observed out of the range of historical controls according to PWG reevaluation. Besides, HED analysis of data indicated a pair wise increase with positive trend of these benign tumours.

4.10.5 Comparison with criteria:

See major comment 1 on rat nasal carcinogenic mode of action and major comment 2 on mouse lung tumours and histiocytic sarcoma.

4.10.6. Conclusions on classification and labelling

In view of the considerations reflected in this document and based on findings of rare nasal olfactory epithelial tumours in the male and female rat (relevance to humans could not be ruled out) and on weak evidence for benign lung tumours in male and female mice and histiocytic sarcomas in female mice (mode of actions not identified), acetochlor fulfils the criteria for classification as Carc. 2; H351 (CLP) and Carc. Cat. 3; R40 (DSD).

4.12 Other effects

Neurotoxicity

Your approach linking brain lesions with severe renal toxicity in the 52 weeks oral study in dogs (Broadmeadow, 1989) could be correct.

RAC's response
These are good and detailed responses by the DS. The RAC supports the DS opinions.

Date	Country	Organisation	Type of Organisation	Comment number
14.01.2014	Germany		MemberState	3
Comment received				
The DE CA supports the proposal of the ES CA for harmonized classification and labelling for Acetochlor.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
RAC endorses the comments by Germany.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2014	Belgium	Monsanto Europe	Company-Manufacturer	4
Comment received				
We have two major comments on this section: (1) the failure to consider quantitative species differences in toxicokinetics and/or toxicodynamics, as recommended by the 2007				

IPCS Human Relevance Framework, when assessing the human relevance of the rat nasal tumours and (2) failure to utilize all of the available historical control data when evaluating the toxicological significance of equivocal increases in the incidences of mouse lung tumours and histiocytic sarcomas.
The rat nasal tumours have been shown to be produced by a non-genotoxic, threshold-mediated mode of action. The key event is formation of a reactive quinoneimine (DABQI) metabolite that forms adducts with nasal proteins and induces cytotoxicity, prolonged cell proliferation, and eventual development of nasal olfactory tumours. We cannot exclude the possibility that very small amounts of DABQI could be produced in humans. However, because of substantial qualitative and quantitative species differences in toxicokinetics and/or toxicodynamics, the amount of DABQI that might theoretically be produced in humans would be at least several orders of magnitude lower than in mice, which did not develop nasal tumours following long-term administration of acetochlor. Therefore, we believe that there are sufficient mode of action data to "reasonably conclude" that the rat nasal tumours produced by acetochlor are not relevant to humans and should not trigger classification.
Mouse lung tumours and histiocytic sarcomas are very common spontaneous tumours in aged CD-1 mice. Thus, increases in the incidences of these tumours should be evaluated very carefully, and should utilize all appropriate historical control information. An independent Pathology Working Group, the ECB Group of Specialised Experts, and an independent panel of European pathologists and toxicologists have reviewed the data and available historical control data and concluded that these tumours were not treatment related. The USEPA has also recently concluded that these tumours were equivocal and/or only possibly related to treatment, represented no greater than a negligible risk of cancer, and that they were not a "tumor of concern" for humans. Thus, we believes that these tumours should not be considered relevant for the purposes of classification.
A more detailed discussion can be found in the document uploaded as public attachment.

Dossier Submitter's Response
See response to comment 2.
RAC's response
The RAC endorses the views of the DS but has some concern over the relevance of the histiocytic sarcomas. The RAC considers these to be borderline but potentially relevant, see discussion about other tumour types in the ODD, Carc Panel, section 6, part g, CD-1 Mouse: Histiocytic Sarcomas.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2014	Belgium	Monsanto Europe	Company-Manufacturer	5
Comment received				
We agree that acetochlor may be genotoxic in vitro but not in vivo and thus should not be classified for mutagenicity. However, we believe that there are several errors in the genotoxicity section of the CLH report which should be corrected and are submitting detailed comments concerning these statements in our attachment. More information can be found in the document uploaded as public attachment. 4.9. Germ cell mutagenicity 4.9.1. Non-human information <u>*In vitro* gene mutation studies</u> • The text in the 3rd paragraph (page 65) states that "Nevertheless, the concentrations which induce[d] a mutagenic response in the 1st study were not tested in the 2nd." We believe this is erroneous as the 2nd study included concentrations that were higher than the				

1st study (e.g., no mutagenic response at 150 or 200 µg/ml in the 2nd study compared to weakly positive responses at 125 and/or 150 µg/ml in the 1st study).
• Although the 3rd paragraph states that purity was unspecified, the purity of the test material used in the mouse lymphoma assay (Mitchell et al., 1982) was specified as 95.6% in the appendix of the study report. A similar statement about lack of purity information was also mentioned on page 79, section 4.9.1.1.2.

In vivo genotoxicity: Chromosomal aberrations
Page 68 of the CLH report (related to in-vivo studies) indicates that two of the three studies evaluating chromosomal aberrations "were not considered acceptable, either by the absence of toxicity or cytotoxicity at the highest dose tested (Farrow and Cortina, 1983) or because no certainty that the bone marrow has been exposed to the test substance (Cavagnaro and Cortina, 1985)." Similar statements were made elsewhere (Table 24, pages 85 and 87). Although a third study was considered acceptable, we believe that the rationales cited for rejection of the first two studies are incorrect for the following reasons:
• Farrow and Cortina (1983): The highest dose tested (500 mg/kg) in this rat bone marrow chromosomal aberration study caused a statistically significant decrease in body weight gain in both males and females over the 48-hr test period. In addition, this dose level was one half of the dose (1000 mg/kg) that caused 75% mortality in the range-finding study. Thus, the highest dose level tested should be considered acceptable.
• Cavagnaro and Cortina (1985): The highest dose tested in this mouse bone marrow micronucleus study (2000 mg/kg) is the limit dose according to the current OECD guideline, and caused mortality in 43% (23/54) of the animals dosed, with an apparent decrease in the PCE/NCE ratio in surviving high-dose animals. In addition, clinical signs of toxicity were noted at both 2000 and 660 mg/kg. Furthermore, previous ADME studies have demonstrated that acetochlor is well-absorbed after oral administration to rodents and is found at significant levels in the blood and bone marrow. Since bone marrow is a highly perfused tissue, the levels of acetochlor and/or its metabolites in bone marrow should be similar to that observed in the blood (Guidance for Industry, S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use, US FDA, ICH, June 2012).
Therefore, based on the toxicity and ADME data, there should be high confidence that the bone marrow was indeed sufficiently exposed to the test material.

In-vivo genotoxicity: Comet assay
The CLH report indicates that the rat nasal comet assay was not considered acceptable due to an inappropriate rationale for the dose level used (1750 ppm). We disagree with this criticism for the following reasons:
• This study was intended to determine if there was any evidence of chromosomal or DNA damage in nasal turbinates at a dose level (1750 ppm) that slightly exceeded the chronic MTD and produced nasal tumors in rats. This would help determine if there was a genotoxic or non-genotoxic mode of action for the formation of these tumours.
• Although body weight data were not reported for this study, clear evidence of toxicity was noted at the same or similar dose levels in other studies. Decreased food consumption, decreased body weights, increased organ weights and/or nasal pathology (including tumors) were observed after a similar duration of exposure at 1750 ppm in two 2-generation rat reproduction studies (Milburn, 2001; Willoughby, 1989). In addition, significantly decreased food consumption and body weight gain and increased relative liver and kidney weights were noted at 2000 ppm in a previous 90-day rat study (Broadmeadow, 1986).
In vivo genotoxicity - Dominant lethal assays
The text and Table 25 indicates that no toxicity was observed in 3 of the dominant lethal studies.
However, we believe this is inaccurate for 2 of the 3 studies. Decreases in body weight (~7.5%) and cumulative weight gain (~27%) were noted in the high-dose (2000 ppm)

male rats after 9 weeks of dosing in the Naylor (1987) study. Initial weight loss followed by decreases (generally ~5%) in body weight were observed in high-dose (3500 ppm) male mice in the Milburn (1986b) study. In addition, although a minor detail, the rats used in the Naylor study were Charles River Sprague Dawley rats, not Alderly Park (Alpk AP (SD)) rats, and the high-dose level in this study was 2000 ppm, not 1500 ppm as reported near the top of page 102 (section 4.9.1.2.3).

ECHA note: The text above was provided in:

1. *Attachment 1 Comments on CLH report for Acetochlor, Version 2 (September 2013)*

Dossier Submitter's Response

4.9 Germ cell mutagenicity

4.9.1 Non-human information

In vitro gene mutation studies

We agree that the following text on 3rd paragraph (page 65) could be deleted: "Nevertheless, the concentrations which induce[d] a mutagenic response in the 1st study were not tested in the 2nd."

With respect to the mouse lymphoma assay (Mitchell et al., 1982), the appendix of the study report does not specify the purity of the test material since it is referred to equations for calculations of L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay data. In order to accept that the purity is 95.6% we need documentary evidence.

In vivo genotoxicity: Chromosomal aberrations

With respect to acceptability of the Farrow and Cortina (1983) rat bone marrow chromosomal aberration study, the study report indicates at the section of "Methods and Material" that body weights were recorded once, prior to compound administration, for the 6 and 12 hour sacrifices, and twice, prior to compound administration and prior to colchicine administration, for the 24 and 48 hour sacrifices. However, at the section of "Results" only appear the data for mean pretreatment body weights with standard deviations. On the other hand, the appendix for the dose range finding study includes body weights mean with standard deviations for pre- and post-treatment with doses of 100, 300 and 1000 mg/kg, and the only dose which caused a statistically significant decrease in body weight gain was that of 1000 mg/kg. Therefore, we have no data to support the statement that the dose of 500 mg/kg caused a statistically significant decrease in body weight gain in both male and female rats over the 48-hr test period. Since there were neither overt toxic effects in the clinical observations nor significant differences between the negative control and the test groups when comparing modal number and mitotic indices, we need you to submit data showing a statistically significant decrease in body weight gain at 500 mg/kg. In order to accept the study there must be some indication of toxicity to the highest dose level tested.

We agree with your comment about the acceptability of the Cavagnaro and Cortina (1985) mouse bone marrow micronucleus study.

Therefore, the CLH report may be modified in two different ways depending on provided information in Farrow and Cortina (1983) study:

a) If the rat study data showing a statistically significant decrease in body weight gain at 500 mg/kg are not submitted, the CLH report should be modified as follows:

   - Page 68, 1st paragraph after title, 2nd and 3rd sentences: "*The chromosome study in rats is not considered acceptable because there are not data supporting that the highest dose tested can be regarded as an indicator of toxicity (Farrow and Cortina, 1983).* However, according to results from the *two* mouse micronucleus test *(Cavagnaro and Cortina, 1985;* Randall, 1989*)* it can be said that Technical Acetochlor does not cause neither structural nor numerical chromosome

aberrations"
- Table 24, 2nd line after title, 1st column: "Acceptability: *Yes*"
- Page 87, 1st paragraph, delete the last sentence.
- Page 87, 3rd paragraph: "*The study is considered acceptable"*

b) If the rat study data showing a statistically significant decrease in body weight gain at 500 mg/kg are submitted, the CLH report should be modified as follows:

- Page 68, 2nd and 3rd sentences of 1st paragraph after title: "*According to results from the chromosome study in rats (Farrow and Cortina, 1983) and the two mouse micronucleus test (Cavagnaro and Cortina, 1985; Randall, 1989) it can be said that Technical Acetochlor does not cause neither structural nor numerical chromosome aberrations"*
- Table 24, 1st line after title, 1st column of "Acceptability: *Yes*"; 6st colum: "*Toxicity at 500 mg/Kg*. No cytotoxicity."
- Table 24, 2nd line after title, 1st column: "Acceptability: *Yes"*
- Page 85, "Deviations from OECD TG 475 (1984): *None*" and "*The study is considered acceptable".*
- Page 86, in paragraph 6th it should be included a mention to the statistically significant decrease in body weight gain at 500 mg/kg.
- Page 87, 1st paragraph, delete the last sentence.
- Page 87, 3rd paragraph:"*The study is considered acceptable"*

<u>*In-vivo genotoxicity: Comet assay*</u>

In your comment you were in disagreement with the indication that the rat nasal comet assay was not considered acceptable due to an inappropriate rationale for the dose level used (1750 ppm). We accept reasons given. Consequently, the CLH report should be modified as follows:

a) Page 68, last sentence of 3rd paragraph after title: "*This study is considered acceptable for the proposal for which it was designed, i.e. provide information on if mode of action for the formation of nasal tumors in rats is genotoxic or not"*
b) Table 24, 4th line after title, 1st column: "Acceptability: *Yes, as information on mode of action for the formation of nasal tumors in rats*"; 6th column: "*Data on toxicity not reported. Choice of dose justified by the results of other toxicity tests"*
c) Page 90, 4th paragraph "*The study is considered acceptable for the proposal for which it was designed, i.e. provide information on if mode of action for the formation of nasal tumors in rats is genotoxic or not. While not toxicity indicative data are provided, the dose level used (≈ 175 mg/Kg/day) is justified by results obtained in other toxicity studies."*

<u>*In vivo genotoxicity - Dominant lethal assays*</u>

We agree with these comments. Consequently, the CLH report should be modified as follows:

a) Page 70, 3rd and 4th sentences of 1st paragraph: "*One study in rats is not considered acceptable because there are not data supporting that the highest dose tested can be regarded as an indicator of toxicity (Milburn, 1996a). However, according to results from the other studies, two in rats (Hodge, 1991; Naylor, 1987) and one in mouse (Milburn, 1996b) it can be said that Acetochlor is not mutagenic for germ cells."*
b) Table 25, 2nd line after title, 1st column:"Acceptability: *Yes";* 3rd column: "male and female *Sprague-Dawley* rats"; 6th column: "*Toxicity at 2000 ppm*"
c) Table 25, 4th line after title, 1st column: "Acceptability: *Yes";* 6th column: "*Toxicity at 3500 ppm*"

d) Page 102, 1st paragraph: "Deviations from OECD TG 478 (1984): *None*"
e) Page 102, 3rd paragraph:"*The study is considered acceptable"*
f) Page 102, last paragraph, last sentence: "*Decreases in body weight (≈7.5%) and accumulative weight gain (≈27%) were noted in the high-dose (2000 ppm) male rats after 9 weeks of dosing*".
g) Page 105, "Deviations from OECD TG 478 (1984): *None*" and "*The study is considered acceptable".*

Metabolites with potential to exceed 0.1 µg/L only in exceptional circumstance (t-NCA)

Germ cell mutagenicity of *t*-Norchloroacetochlor

According to CLH report, *t*-Norchloroacetochlor was considered to be negative in the Ames test. However it did induce mutations in the mouse lymphoma test. The increases in mutant frequency, although small (less than 3 times control values), were statistically significant, showed a general dose relationship, were reproducible and were associated with increases in the number of both large and small mutant colonies. In the presence of S9-mix, the dose relationship was less clear than in the absence of S9-mix and the maximum increase was within the historical control range of values.

In the mouse lymphoma test, positive results associated mainly with increases in the number of small mutant colonies are generally considered to be indicative of chromosomal damage. Nevertheless, this clastogenic activity for *t*-Norchloroacetochlor was not confirmed in either the *in vitro* chromosomal aberration assay with human lymphocytes using comparable concentrations or in an *in vivo* mouse micronucleous assay up to the limit dose of 2000 mg/kg. The relevance, if any, of this positive finding is unclear but is of doubtful significance since the parent compound, Acetochlor, is clastogenic in cultured human lymphocytes *in vitro* and the response was not expressed in whole animal models. The conclusion in CLH report was that *t*-Norchloroacetochlor is not clastogenic either *in vitro* or *in vivo.*

In the mouse lymphoma test, positive results associated mainly with increases in the number of large mutant colonies are generally considered to be indicative of gene mutation. Therefore, to address the potential for gene mutation, Monsanto has provided two additional mutagenicity studies: the Muta™Mouse *in vivo* gene mutation assay, which is required to satisfy EU regulatory requirements, and another Ames test. A summary of these studies is provided below.

No evidence of mutagenicity was noted in either assay, which further supports the overall weight of evidence that *t*-Norchloroacetochlor does not have any significant genotoxic potential. Besides, *t*-Norchloroacetochlor will not be frequent in groundwater at levels > 0.1 µg/l when Acetochlor is applied under GAP.

A summary of the two studies carried out with this metabolite is shown below.

MON 52706: Bacterial Reverse Mutation Assay

[redacted] **(2013)**

Date of experimental work: 22 March 2013 to 16 April 2013.

Date of report: 4 September 2013.

Objective: To evaluate the mutagenic potential of *t*-Norchloroacetochlor by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvr*A in the presence and absence of Arochlor-induced rat liver S9.

Guidelines: OECD TG 471 (1997).

Deviations from OECD TG 471 (1997): None.

GLP: Yes, except that analysis to determine the uniformity, concentration and/or stability of the test substance in the formulation were not performed.

The study is considered acceptable.

Material and methods together with findings

The test substance was *t*-Norchloroacetochlor with purity > 99.9%, batch: GLP-1212-22321-T. The bacterial reverse mutation assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test substance. *S. typhimurium* strains (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* strain WP2 *uvr*A were used. The S9 preparations were from livers of male Sprague-Dawley rats previously induced with Aroclor 1254 (Lot Nos. 3017 and 3062 from Moltox, Boone, NC). The metabolic activation ability of the S9 was characterized using varying S9 and positive control concentrations. The composition of the S9 mix is shown below:

Component	**Final Concentration**
β-nicotinamide-adenine dinucleotide phosphate	4 mM
Glucose-6-phosphate	5 mM
Potassium chloride	33 mM
Magnesium chloride	8 mM
Phosphate Buffer (pH 7.4)	100 mM
S9	10% (v/v)

Negative (untreated) and solvent control (DMSO) as well as appropriate positive controls such as 2-nitrofluorene (2NF) for TA98, sodium azide (SA) for TA100 and TA1535, 9-Aminoacridine (9AAD) for TA1537, and methyl methanesulfonate (MMS) for WP2 *uvr*A, without S9, as well as 2-aminoanthracene (2AA) for all strains, with S9, were used concurrently.

The condition of the bacterial background lawn was evaluated for evidence of test substance toxicity by using a dissecting microscope. Precipitate was evaluated after the incubation period by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the solvent control plate using the codes shown in the following table.

Code	**Description**	**Characteristics**
1 or no code	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the solvent control plate
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the solvent control plate
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the solvent control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies

5	Absent	Distinguished by a complete lack of any microcolony lawn over greater than or equal to 90% of the plate.
6	Obscured by particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test substance particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., greater than 3 particles on a plate with 30 revertants). These plates are counted manually.

The mutagenicity assays were considered valid if the following criteria are met. All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the solvent controls as follows: TA98, 10-50; TA100, 80-240; TA1535, 5-45; TA1537, 3-21; WP2 *uvr*A, 10-60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titres must be greater than or equal to $0.3x10^9$ cells/mL. The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective solvent control. A minimum of four non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) a >50 % reduction in the mean number of revertants per plate as compared to the mean solvent control value; this reduction must be accompanied by an abrupt dose-dependent drop in the revertant count; and (2) at least a moderate reduction in the background lawn (background code 3, 4 or 5).

For the test substance to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test substance. Data sets for strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 3.0-times the mean solvent control value. Data sets for strains TA98, TA100 and WP2 *uvr*A were judged positive if the increase in mean revertants at the peak of the dose response was greater than or equal to 2.0-times the mean solvent control value. An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

In the initial toxicity-mutation assay, *t*-Norchloroacetochlor was tested at eight dose levels (1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 µg per plate). All dose levels of test substance, solvent control and positive controls were plated in duplicate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Precipitate was observed at 5000 µg per plate. No toxicity was observed.

In the confirmatory mutagenicity assay, *t*-Norchloroacetochlor was tested at six dose levels

(15, 50, 150, 500, 1500 and 5000 µg per plate). All dose levels of test substance, solvent control and positive controls were plated in triplicate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Precipitate was observed at 5000 µg per plate. No toxicity was observed.

The solvent controls and positive controls in the initial toxicity-mutation assay and confirmatory mutagenicity assay were within the acceptable historical ranges.

Conclusion

t-Norchloroacetochlor was not mutagenic for any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9, under the conditions of this study.

***In vivo* Muta™Mouse gene mutation assay with MON 52706**

[redacted] **(2014)**

Date of experimental work: 6 April 2013 to 10 October 2013.

Date of report: 16 January 2014.

Objective: To evaluate the potential of *t*-Norchloroacetochlor to induce gene mutations in the *lacZ* transgene within lung and bone marrow from treated Muta™Mice.

Guidelines: OECD TG 488 (2013)

Deviations from OECD TG 488 (2013): None.

GLP: Yes

The study is considered acceptable.

Material and methods together with findings

The test substance was *t*-Norchloroacetochlor with purity > 99.9%, batch GLP-1212-22321-T. The vehicle was the ground diet. The positive control was Ethylnitrosurea (ENU). The study was conducted using female Muta™Mice (CD_2-lacZ80HazfBR strain).

The Muta™Mouse (*lacZ/GalE*) assay is capable of detecting point mutations and small deletions in different tissues from treated animals. The genome of the Muta™Mouse strain contains 40 copies of a transgenic lambda gt10 vector, each of which contains a bacterial *lacZ* gene, within every DNA bearing cell of the animal. The *lacZ* gene encodes for the β-galactosidase enzyme, which is not present in the normal mouse. Treatments are performed *in vivo*, with sufficient treatment and expression time to permit any mutations to be expressed.

A 14-day dose range-finding study was performed to assess palatability and overt toxicity following dietary administration. Three female mice were fed a diet containing 4500 ppm *t*-Norchloroacetochlor for seven days followed by additional seven days of dosing with diet containing 6000 ppm *t*-Norchloroacetochlor. A control group of three female mice were fed normal diet for 14 days. Transient slight decreases in food consumption were noted in the *t*-Norchloroacetochlor-treated animals. However, there were no test substance related findings on survival, clinical observations or bodyweight.

In the mutation experiment, *t*-Norchloroacetochlor was administered for 28 days to 4 groups of seven female mice at dietary concentrations of 0, 600, 1800 and 6000 ppm (intended to achieve dose levels of 0, 100, 300 and 1000 mg/kg/day, respectively). A fifth group of seven female mice was administered ENU, the positive control.

All animals were observed for ill health and overt toxicity (twice daily). Vehicle and test substance animals were weighed prior to dosing on Day 1 and twice weekly thereafter. Food consumption and food efficiency were also determined weekly. Blood samples for clinical pathology evaluations (hematology and serum chemistry) were collected at the scheduled necropsy from vehicle control and test substance treated animals. All animals were

sacrificed on Day 31, three days after last dosing, and subjected to a complete gross necropsy. Selected organs were weighed and examined microscopically.

DNA was isolated from the lungs and bone marrow. These two tissues were selected for evaluation since equivocal increases in the incidence of lung tumors and histiocytic sarcomas were observed in female mice in chronic feeding studies with parent Acetochlor.

The isolated DNA was packaged into lambda bacteriophage, which was then transfected into *E. coli* C *lac- galE*$^-$ Kan^r (*galE*$^-$ Amp^r). Only bacteriophage units containing a *lacZ* gene from the transgenic vector in the mouse DNA are viable and capable of transfecting a bacterial cell. Successful transfection enables replication of the bacteriophage and transfection of neighboring bacterial cells. This results in the formation of a visible plaque, *i.e.* an area of bacterial cell lysis, in a lawn of uninfected viable bacterial cells. Furthermore, due to the *galE*$^-$ status of the host bacterial strain cells, only cells infected with a non-functional (i.e. mutated) *lacZ* gene can form plaques on the positive selection plates.

Mutant frequency (MF) is calculated by dividing the number of bacteriophages, which contain mutated *lacZ* transgenes (i.e. plaques on positive selection plates) by the total number of viable bacteriophage (i.e. phages that have been successfully packaged with the *lacZ* transgene and visible as plaques on titre plates).

Statistical analysis of toxicology parameters was performed as follows: Food consumption, feed efficiency, body weight gains, terminal body weights, absolute organ weights, organ weight to body weight ratios, haematology and clinical chemistry variables were analysed using one-way analysis of variance (ANOVA). Levene's test for equality of variances among the groups was performed. Where this showed no evidence of heterogeneity ($P>0.05$), and the group effect from the ANOVA was significant ($P\leq0.05$), pair wise comparisons with control were made using Dunnett's test. Where Levene's test was significant ($P\leq0.05$), a rank-transformation was applied to the data prior to analysis. No analysis was performed for variables with values above or below the limit of the assay. Some variables were not analyzed due to there being too few values for meaningful analysis, or there being at least one group with less than three values.

A statistically significant decrease in overall weight gain was seen at the 1000 mg/kg bw/day dose. A statistically significant decrease in food consumption was observed in week 1 solely in the high dose (1000 mg/kg bw/day target dose); the effect was transient and was attributed to reduced palatability in the first 48 hours of dosing. No other clinical signs of toxicity and no treatment-related changes in organ weight, haematology or histopathology

Statistical analysis of MF per tissue was performed as follows: The positive control group was compared to the vehicle control using a two-sample t-test. The test was interpreted with one-sided risk for increased response with increasing dose. The vehicle control group and the three test substance groups were analysed using one-way analysis of variance (ANOVA). An overall dose response test was performed along with Dunnett's test for pair wise comparisons of each treated group with the vehicle control. All tests were performed with a one-sided risk for increasing response. Levene's test for equality of variances between the groups was also performed and, in all cases, this showed no evidence of heterogeneity ($P>0.01$).

There were no statistically significant increases in MF in the lung or bone marrow of animals treated with *t*-Norchloroacetochlor. Vehicle control data were within historical control range and the positive control induced a statistically significant increase in MF.

Conclusion

Under the conditions of this study, *t*-Norchloroacetochlor did not induce mutation in the *lacZ* transgene in the lung and bone marrow of Muta™Mice, following dietary administration for 28 days at doses up to 1000 mg/kg bw/day.

RAC's response
The RAC supports the DS in its responses noted above. However, concerning the DS response to "In vivo genotoxicity: Chromosomal aberrations" (above) RAC would like to make the following comment: In terms of % body weight gain there is no toxicologically significant decrease for males or females at the 48 hour time point in the highest dose group. Males: control group (+0.9 ± 0.5)%; 500 mg/kg bw group (-2.0 ± 0.6)%. Females: control group (+0.4 ± 1.2)%; 500 mg/kg bw group (-3.2 ± 0.7)%. In terms of actual grams lost all animals in the high dose groups show a small decrease in body weight. However, as a indication of toxicity in the high dose group, the reduction in mitotic index (25%) from 2.4 in controls to 1.8 in the high dose groups at 24 hours might provide sufficient justification (although current guidance in OECD 475 1997 suggests a reduction of 50% as a significant indicator of toxicity while a slightly greater reduction in the current draft OECD 475 2013 of (55±5)% is being proposed).

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
19.01.2014	Belgium	Monsanto Europe	Company-Manufacturer	6
Comment received				
We believe that the weight of evidence indicates that acetochlor should not be classified for acute oral toxicity. There were no significant differences between the sexes in either of the two acute oral toxicity studies and the combined sex LD50 value of 2148 mg/kg from the Branch (1982) study as well as the separate male (4238 mg/kg) and female (4015) LD50 values from the Cummins (1986) study do not meet the criteria for classification.				
Dossier Submitter's Response				
We disagree with your comment. The LD_{50} obtained in Branch (1982a) study of 1929 mg/kg in females supports the proposal for acute oral classification as Acute Tox. 4; H302.				
RAC's response				
The results from the female subgroup in the Branch (1982) study support classification. The results from the remaining three subgroups do not support classification. The two studies used different substrains of rat. RAC concludes that the data are not sufficient to warrant classification as Acute Tox. 4 - H302.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
14.01.2014	Germany		MemberState	7
Comment received				
Pages 5, 10-11: 1.2 "Harmonised classification and labelling proposal" It was noted that in Table 2 (page 5) in the box "Current proposal for consideration by RAC", the classification for skin irritation (H315) is lacking although it is mentioned in the "History" subsection on page 10 and under 2.3.1 on page 11. The reason for the different approach is not clear.				
Dossier Submitter's Response				
As we agree with the current Annex VI entry for skin irritation (H315) according to CLP Regulation, it is not necessary to include it in the box "Current proposal for consideration by RAC" of Table 2.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment

				number
17.01.2014	Belgium		MemberState	8
Comment received				
Based on the results of the aquatic toxicity test on the most sensitive species (algae : Pseudokirchnierella subcapitata with 72hErC50 = 0.00052mg/l and 72hNOErC=0.00013mg/l) the fact that the substance is considered as not rapidly degradable it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic Acute 1, H400 and Aquatic chronic 1,H410. Furthermore, the substance shows low potential to bioaccumulate (BCF =20). In view of the proposed classification and toxicity band for acute toxicity between 0.0001mg/l and 0.001 mg/l, an M-factor for acute toxicity of 1000 could be assigned, and an M-factor for chronic toxicity of 100 (not rapidly degradable substance and toxicity band between 0.0001mg/l and 0.001 mg/l). Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, Acetochlor should be classified as N, R50/53 with SCL : N, R50/53: C≥0.025% N, R51/53: 0.0025%≤C<0.025% R52/53: 0.00025%≤C<0.0025% In conclusion : we agree with the proposed environmental classification				
Dossier Submitter's Response				
Thank for agreeing.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
14.01.2014	Germany		MemberState	9
Comment received				
Page 201 Table 75: "Algae and aquatic plants toxicity for Acetochlor": There are some minor mistakse in the results of toxicity to the following species: Selenastrum cacpricornutum (Hoberg, J.R. 2003): NOECb (72h) = 0.059 µg a.i./L instead of 0.052 µg a.i./L. Anabaena flos-aquae (Smyth et al 1992): NOECb (120 h) = 7.5 µg a.i./L and NOECr (120 h) = 1.9 µg a.i./L instead of NOECb (120 h) = 1.9 µg a.i./L and NOECr (120 h) = 7.5 µg a.i./L. Lemna gibba (Putt, A.E. 2003): all NOECf/r/dw values (4 d) and (7 d) should be < 0.85 µg a.i./L instead of < 85 µg a.i./L.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

ATTACHMENTS RECEIVED

1. **Attachment 1** - Comments on CLH report for Acetochlor, Version 2 (September 2013), submitted by Monsanto Europe on 19 January 2014 *[Please refer to comments 2, 4, 5, 6]*

STUDY REPORTS (confidential):

1. [redacted] (2010) Acetochlor sec-methylsulfide: in-vitro metabolism by olfactory turbinate and liver microsomes of male Sprague Dawley rat

2. [redacted] (2013): MON52706: Bacterial Reverse Mutation Assay
3. [redacted] (2014). In vivo Muta™Mouse gene mutation assay with MON 52706