Committee for Risk Assessment

RAC

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-0000001412-86-29/F

Adopted

04 December 2014

04 December 2014

CLH-O-0000001412-86-29/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonized classification and labelling (CLH) of:

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

EC number: 251-899-3
CAS number: 34256-82-1

The proposal was submitted by **Spain** and received by RAC on **27 September 2013.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **05 December 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **20 January 2014.**

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2. The RAC opinion on the proposed harmonized classification and labelling was reached on **04 December 2014** and was adopted by **consensus.**

OPINION OF RAC

The RAC adopted the opinion that **Acetochlor (ISO)** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-037-00-6	acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	251-899-3	34256-82-1	Acute Tox. 4 * STOT SE 3 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H335 H315 H317 H400 H410	GHS07 GHS09 Wng	H332 H335 H315 H317 H410			
Dossier submitters proposal	616-037-00-6	acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	251-899-3	34256-82-1	**Retain** STOT SE 3 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 **Add** Carc. 2 Acute Tox. 4 STOT RE 2 **Modify** Acute Tox. 4 (inhalation) Skin Sens. 1B	**Retain** H335 H315 H400 H410 **Add** H351 H302 H373 (liver, kidney)	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H335 H315 H410 **Add** H351 H302 H373 (liver, kidney)		**Add** M=1000 (acute) M=100 (chronic)	
RAC opinion	616-037-00-6	acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	251-899-3	34256-82-1	Carc. 2 Repr. 2 Acute Tox. 4 STOT SE 3 STOT RE 2 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H335 H373 (kidney) H315 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H351 H361f H332 H335 H373 (kidney) H315 H317 H410		M=1000 (acute) M=100 (chronic)	

Resulting Annex VI entry if agreed by COM	616-037-00-6	acetochlor (ISO); 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide	251-899-3	34256-82-1	Carc. 2 Repr. 2 Acute Tox.4 STOT SE 3 STOT RE 2 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H361f H332 H335 H373 (kidney) H315 H317 H400 H410	GHS07 GHS08 GHS09 Wng	H351 H361f H332 H335 H373 (kidney) H315 H317 H410		M=1000 (acute) M=100 (chronic)	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

(1) Acute oral toxicity of acetochlor in rats:

The results of two acute oral toxicity studies were presented by the dossier submitter (DS). The first study was a pre-guideline, but acceptable, GLP study, conducted in 1982 by Branch on male and female Sprague-Dawley (SD) rats. Acute oral LD_{50} values for acetochlor (purity 96.3%) were calculated to be 2389 mg/kg bw for males and 1929 mg/kg bw for females.

The second study was a US EPA-81-1 guideline compliant, acceptable, GLP study, reported in 1986 by Cummins, on male and female CD rats. Acute oral LD_{50} values for acetochlor (89.9 – 91.5%) were calculated to be 4238 mg/kg bw for males and 4015 mg/kg bw for females.

The DS proposed Acute Tox. 4; H302 on the basis of the LD_{50} results for female SD rats in the study by Branch (1982).

(2) Acute inhalation toxicity of acetochlor in rats:

The results of three guideline (US EPA 81-3) compliant, GLP, acute inhalation toxicity studies were presented by the DS. All exposures were for 4 hours.

In the first study, by Bechtel (1988), whole body exposure of SD male and female rats were performed with no deaths at the highest attainable concentration of 3 mg/L/4h. The LC_{50} was > 3 mg/L/4h.

The second study, by Brammer (1989), was a nose-only exposure of Alpk:APfSD male and female rats. Groups of five male and female rats were exposed to aerosols of the test substance at analytical concentrations of 1.81, 3.57 and 4.46 mg/L in air. At 4.46 mg/L, four females were found dead on day 3, the remaining female and one male were killed in extremis on days 3 and 8, respectively, due to severity of clinical effects observed. The LC_{50} was 3.99 mg/L/4h for females and > 4.46 mg/L/4h for males.

The third study, by Duerden & Lewis (1990), was a nose-only exposure of APfSD male and female rats. Groups of five male and/or female rats were exposed to aerosols of test substance at the highest attainable analytical concentration of 2.07 mg/L/4h in air. No deaths were observed. The LC_{50} was > 2.07 mg/L/4h.

The DS proposed Acute Tox. 4; H332 on the basis of the LD_{50} results for female SD rats in the study by Brammer (1989).

(3) Acute dermal toxicity of acetochlor:

The results of two, pre-guideline, GLP but acceptable acute dermal toxicity studies were presented by the DS. The New Zealand White (NZW) rabbit study by Branch (1982) showed mortality in the 3536 and 5000 mg/kg groups. The lowest LD50 was for females at 3856 mg/kg.

The second study by Cummins (1986) was conducted using male and female CD rats. There were no deaths and the dermal LD50 was determined to be > 2060 mg/kg bw.

The DS did not propose classification for the dermal route.

Comments received during public consultation

Two Member States (MSCA) commented during the public consultation. Both supported the classification proposals for human health submitted by the DS.

Industry also commented, disagreeing with the interpretation of the acute oral toxicity results. The DS supported classification based on the results from the female subgroup of the Branch (1982) study.

Assessment and comparison with the classification criteria

(1) Acute Oral Toxicity:

RAC noted that the acute oral toxicity classification proposal was based on the conflicting results of the two acute oral rat studies. Both studies (Branch, 1982 and Cummins, 1986) are pre-guideline, GLP, and were evaluated as acceptable for regulatory purposes by the original rapporteur Member State and EFSA. The purity of acetochlor differed slightly between the two studies: Branch (1982) used acetochlor with 96.3% purity and Cummins (1986) used a batch with 90.5% purity.

Dose level (mg/kg bw)	Number of deaths	
	Male	Female
1600	0 / 5	2 / 5
2172	2 / 5	2 / 5
2947	4 / 5	5 / 5
4000	5 / 5	5 / 5
LD_{50} both sexes: 2148 mg/kg (1795-2496mg/kg) LD_{50} for males: 2389 mg/kg (1873-3032mg/kg) LD_{50} for females: 1929mg/kg (966-2489mg/kg)		

In the Branch (1982) study, acetochlor was administered undiluted by gavage at single dose levels of 1600, 2171, 2947 and 4000 mg/kg to four groups of five SD rats by sex and group (see table above). The acute oral LD_{50} for each sex and for the combined sexes was originally calculated using probit analysis according to the method of Finney (1971). The lowest reported LD_{50} was in females and reported to be 1929 mg/kg bw.

The increased incidences of death at lower doses may indicate that the rat strain used in this study was more sensitive than the strain used in the Cummins (1986) study, but there are no further data to corroborate this. RAC notes, however, that these two strains of rats are in fact considered to be substrains and are often viewed as biologically comparable since the derivation of the CD stock from the original SD stock in the 1950's. However, differences between the two stocks in lifespan and morbidity have been documented (Pettersen *et al*., 1996).

The Cummins (1986) study was conducted using male and female CD strain rats. Acetochlor was administered at four single dose levels by gavage to groups of five animals by sex. The dose levels of test substance administered were 2324, 3000, 3873 and 5000 mg/kg bw.

Dose level (mg/kg bw)	Number of deaths	
	Male	Female
2324	0 / 5	0 / 5

Dose level (mg/kg bw)	Number of deaths	
	Male	Female
3000	1 / 5	1 / 5
3873	1 / 5	2 / 5
5000	4 / 5	4 / 5
LD_{50} combined: 4124 mg/kg (3557 - 4691mg/kg) LD_{50} for males: 4238 mg/kg (3384 - 5092mg/kg) LD_{50} for females: 4015 mg/kg (3258 - 4772mg/kg)		
LD_{50} combined: 4124 mg/kg (3557 - 4691mg/kg) LD_{50} for males: 4238 mg/kg (3384 - 5092mg/kg) LD_{50} for females: 4015 mg/kg (3258 - 4772mg/kg)		

In general, the lowest LD_{50} value from a study associated with one sex is used to determine if that study supports classification or not. The lowest reported acute oral LD_{50} value from the Branch (1982) study is 1929 mg/kg in female SD albino rats. The lowest reported LD_{50} value from the Cummins (1986) study is 4015 mg/kg in female CD rats. There are two substrains of rat but no corroborating evidence to suggest one strain maybe more susceptible than the other except that LD_{50} values are lower for both sexes in the Branch (1982) study. This complicates breaking down the results by sex and study as an unknown factor (substrain) makes the interpretation of the LD_{50} across studies difficult.

A brief evaluation of the repeated dose short term studies conducted with acetochlor did not find any evidence for lethality at early time points and therefore does not lend support for acute toxicity by the oral route. In a 119-day dog oral study (12 animals per dose), 3 animals dosed with 200 mg/kg bw/d were found dead at days 43 – 44 with a further 8 animals sacrificed from day 35 – 84. In a 21-day rabbit dermal toxicity study, 15/20 high dose (1200 mg/kg bw/day) animals were found dead at days 7 – 19. In a rat 21-day dermal study no deaths were recorded, highest dose was 100 mg/kg bw/d.

According to CLP, for acute oral toxicity, Category 4, the LD_{50} values should be between 300 and 2000 mg/kg bw. The conservative approach to classification takes the lowest LD_{50} value as was concluded by the original rapporteur Member State and EFSA in their 2007 technical experts meeting. The RAC considers the DS conclusion as not sufficiently convincing that classification with Acute Tox. 4 is appropriate. The LD_{50} is above the threshold for classification in three subgroups of data: males in the Branch (1982) study and both females and males in the Cummins (1986) study.

Prism 6	Female	Male	Both
$LogEC_{50}$	3.328	3.362	3.347

HillSlope	52.23	5.131	6.055
Top	= 5	= 5	= 10
Bottom	= 0	= 0	= 0
EC_{50}	**2127**	**2302**	**2223**

Prism, version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Nonlinear curve: asymmetric sigmoidal, 5PL, X is log (concentration) – also known as Richard's five parameter dose-response curve.

Small variations in the numerical result for the LD_{50} can be expected depending on how the statistical procedure is performed. A re-evaluation of the Branch (1982) data using Prism 6 (windows) from GraphPad Software gave an LD_{50} of 2127 mg/kg bw for female rats as shown in the table above.

Overall, RAC concludes that the data are not sufficient to warrant classification as Acute Tox. 4 - H302 (Harmful if swallowed) and hence no classification is proposed.

(2) Acute Inhalation Toxicity:
The lowest acute inhalation LC_{50} was 3.99 mg/L/4h for female Alpk:APfSD rats.

According to CLP, for Acute inhalation, Category 4, the LC_{50} values should be between 1.0 and 5.0 mg/L for dust/mist. The RAC is in agreement with the DS that Acute Tox. 4 - H332 (Harmful if inhaled) is warranted.

(3) Acute Dermal Toxicity:
The LD_{50} values for dermal toxicity are above the threshold value of 2000 mg/kg bw for triggering classification and hence no classification is required.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

The DS described in the CLH report numerous and significant clinical signs from two of the three acute inhalation studies that support the current classification for respiratory tract irritation (RTI). In the first of these studies (Brammer, 1989), salivation and lachrymation were observed in all test groups. Deep breathing and reduced rate breathing was seen during exposure. Abnormal respiratory noise was present in some animals after exposure and during the maintenance period in all test groups. Respiratory noises did not abate until 3 – 4 days after exposure to the highest dose of 4.46 mg/L/4h. Mucoid nasal discharge was observed at 4.46 mg/L/4h in 5 males and one female and persisted until day 4 and 2 respectively. Hyperaemia was observed in 5 males on day 3 (persisted in one animal until day 4) at 4.46 mg/L/4h.

In the second study by Duerden & Lewis (1990), salivation and lachrymation were observed during the first day of the study. Mucus secretion from the nose was seen in 4 males on day 1 and persisted in two of them until day 2. Abnormal respiratory noise was also observed in 5 males and 3 females on day 1 which did not subside completely until days 6 and 3 in males and females, respectively.

All these effects are transitory in nature, limited to the upper airways and are indicative of respiratory tract irritation.

The DS proposes to retain the current classification for acetochlor as STOT SE 3; H335 on the basis of the effects described in the two studies above.

Comments received during public consultation
No comments were received during public consultation.

Assessment and comparison with the classification criteria
In Section 3.8.2.2.1(d), Annex I, CLP, under "Criteria for respiratory tract irritation" it is stated that "*…useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc.) and histopathology (e.g. hyperaemia, oedema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation"*.

Findings observed in the Duerden and Lewis (1990) and Brammer (1989) acute inhalation studies (lachrymation, salivation, mucoid nasal discharge, abnormal respiratory noise and hyperaemia) are considered signs of reversible respiratory tract irritation.

Therefore, RAC concludes, in agreement with the DS that acetochlor meets the criteria for classification for specific target organ toxicity after single exposure as STOT SE 3; H335 (May cause respiratory irritation).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal
In the CLH report, the DS provided convincing evidence from several studies (including a rabbit 21-day repeated dose dermal toxicity study by Johnson, 1981, and a rat 21-day repeated dose dermal toxicity study by Leah, 1989) in addition to the two specific skin irritation studies from Branch (1982) and Barlow & Ishmael (1989) that acetochlor is highly irritating to the skin. The Branch (1982) study is considered unacceptable but the Barlow & Ishmael (1989) study (guideline and GLP -compliant), conducted using 6 male NZW rabbits, showed extensive irritation over a 20 – 30 day period following a 4-hour exposure and persisting beyond the normal observation period of 14 days.

In addition, the DS also considered whether acetochlor was corrosive based on observations from the Barlow & Ishmael (1989) study which included necrosis in one animal. Having evaluated the data in this and the 21-day dermal toxicity studies, and confirmed the lack of any corrosive reaction in other relevant studies (acute dermal toxicity studies, skin sensitisation studies or eye irritation studies), the DS concluded that the effects did not fit the criteria for classification for skin corrosion (section 3.2.2.6.1, Annex I CLP).

The DS proposes to retain the current classification, Skin Irrit. 2; H315 on the basis of the erythema and oedema scores in the study by Barlow & Ishmael (1989).

Comments received during public consultation
Industry questioned the current classification for skin irritation. The test material used in the Barlow & Ishmael (1989) study is no longer produced by ICI and the material used in the Branch (1982) study is more representative of the current technical material. The DS replied in the RCOM document that using a weight of evidence approach, the current classification is justified.

Assessment and comparison with the classification criteria
In the Barlow and Ishmael (1989) skin irritation study, necrosis was present in one animal after 4 days. There are doubts concerning the interpretation of this effect as there is no information related to the nature or extent of the necrosis available. Subsequent examination revealed a thickened area of grey/brown coloured skin which was described as eschar. Cracking of the area was observed with intact skin beneath. Other significant dermal effects were still present at the end of the study; however, they don't fully fit the skin corrosion criteria in 3.2.2.6.1, Annex I, CLP.

Although severe dermal effects may have been observed in the Barlow and Ishmael (1989) skin irritation study and in the 21-days dermal toxicity studies, the weight of evidence presented in the studies leads the DS to conclude that acetochlor is not a corrosive substance, especially given the lack of any corrosive reaction in other more relevant studies (acute dermal toxicity studies, skin sensitisation studies or eye irritation studies). The overall weight of evidence does not appear sufficient for classifying acetochlor for skin corrosion.

In the Barlow and Ishmael (1989) skin irritation study, the overall mean index score was greater than 2.3 for erythema and for oedema, and inflammation persisted until day 29 in at least two animals. These data are consistent with the criteria for skin irritation Category 2 when using the results of animal testing (Section 3.2.2.7.1, Annex I, CLP).

Acetochlor meets the criteria in the CLP Regulation as an irritant to the skin. In support of the DS, RAC agrees that acetochlor should retain the current classification as Skin Irrit. 2 - H315 (Causes skin irritation).

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The results of two rabbit eye irritation studies were presented by the DS. The first was a pre-guideline but acceptable, GLP study, conducted in 1982 by Branch on male and female NZW rabbits. Acetochlor caused very slight conjunctivitis (overall mean score: 0.66) that resolved within 3 days.

The second study (guideline and GLP -compliant) by Pemberton & Ishmael (1989) showed no corneal or iridial effects. A slight conjunctivitis was present but resolved within 2 days.

The DS did not propose classification for eye irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC notes that the individual and group mean eye irritation scores do not meet the criteria for classification as irritating to the eyes according to CLP. Therefore, RAC supports the DS conclusion that no classification is required for this hazard class for acetochlor.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The results of two dermal sensitisation studies were presented by the DS. The first was a pre-guideline, but acceptable, non-GLP, modified Buehler test, conducted by Auletta (1983). All test animals (5 male, 5 female) showed positive test substance responses at 24 and 48 hours after challenge (undiluted test material in induction and challenge phases). Adequate controls were present and the undiluted test substance was confirmed as the highest non-irritating dose.

The second study by Botham & Ishmael (1989) was a guideline compliant (US EPA 81-6), GLP, GPMT test using female Alpk Dunkin Hartley Guinea pigs (20 test animals, 10 controls). A sensitisation response was seen in 18/19 animals (94.7%) following challenge with an undiluted sample of acetochlor, and in 13/19 animals (68%) following challenge with 30% acetochlor in corn oil. The intradermal induction concentration was 10%.

There are no data with respect to respiratory sensitisation.

The DS proposes to classify acetochlor as Skin Sens. 1B - H317 on the basis of positive results from both a modified Buehler test and an Magnusson and Kligman (M&K) Guinea Pig Maximisation Test (GPMT).

Comments received during public consultation

Two Member States commented during the public consultation. Both supported the classification proposals for human health submitted by the DS.

Assessment and comparison with the classification criteria

Acetochlor is currently classified as Skin Sens. 1 – H317 in Annex VI to the CLP regulation, without sub-categorisation. Under DSD it was classified as R43 by the TC C&L in November 1997.

According to 3.4.2.2.1.1, Annex I, CLP, skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. However, according to 3.4.2.2.1.2, Annex I, CLP, where data are sufficient, a refined evaluation on the basis of the occurrence or potency of the sensitising effect allows the allocation of skin sensitisers into sub-category 1A (high frequency of occurrence, strong sensitisers), or sub-category 1B (a low to moderate frequency of occurrence, low to moderate potency).

(1) Criteria for potency of skin sensitisation on the basis of the Buehler Assay:
If a test substance is present at > 20% for topical induction and the incidence of sensitisation is ≥ 15% then potency is judged to be moderate and the predicted sub-category shall be 1B.

In the Auletta (1983) study, a 100% response rate was obtained with topical induction using undiluted acetochlor. The classification criteria were satisfied for Skin Sens. 1B – H317.

(2) Criteria for potency of skin sensitisation on the basis of the M&K GPMT:
If a test substance is present at > 1% for intradermal induction and the incidence of sensitisation is ≥ 30% then potency is judged to be moderate and the predicted sub-category shall be 1B.

In the Botham & Ishmael (1989) study, a 68% response rate was obtained with an intradermal induction using 10% acetochlor. The classification criteria seem to be satisfied for Skin Sens. 1B – H317.

However, the strength of the response in the two sensitisation studies suggests that acetochlor might be a more potent sensitiser than that considered for sub-category 1B. Additional points should also be considered, i.e. the minimum criteria for sub-category 1A. Acetochlor is further discussed in relation to classification for skin sensitisation (Category 1A) below.

(1) Criteria for potency of skin sensitisation on the basis of the Buehler Assay:
If a test substance is present at > 0.2% to ≤ 20% for topical induction and the incidence of sensitisation is ≥ 60% then potency is judged to be high and the predicted sub-category shall be 1A.

Auletta (1983) tested technical acetochlor (purity 96.3%) undiluted and observed a 100% response rate. The data are *not sufficient* to decide if the criteria for Skin Sens. 1A are met. It is possible that a lower concentration of acetochlor (i.e. ≤ 20%) would still have a high response rate (i.e. ≥ 60%), but this presumption has not been tested.

(2) Criteria for potency of skin sensitisation on the basis of the M&K GPMT:
If a test substance is present at > 0.1% to ≤ 1% for intradermal induction and the incidence of sensitisation is ≥ 60% then potency is judged to be high and the predicted sub category shall be 1A.

Botham & Ishmael (1989) tested technical acetochlor (purity 89.4%) with an intradermal induction using 10% acetochlor (actual value 8.9% taking into account the technical purity), and observed a 68% response rate. As explained for the study of Auletta (1983), the data are *not*

sufficient to decide if the criteria for Skin Sens. 1A are met. It is possible that a lower concentration of acetochlor in the GPMT (i.e. ≤ 1%) would still have a high response rate in excess of the trigger value of 60%, but this presumption has not been tested.

RAC noted that Category 1 and 1A should be considered in addition to Category 1B. A clear case can be made for Category 1B based on the available data from two independent studies. However, the response in both the GPMT and Buehler assays was > 60% and acetochlor was not tested at ≤ 1% intradermal induction in the case of the GPMT assay nor was it tested at ≤ 20% topical induction in the Buehler assay. According to the classification criteria, classification in sub-category 1A cannot be excluded even though the criteria for classification in 1B are clearly fulfilled. Therefore, there is insufficient information for a complete evaluation into sub-categories and RAC recommends retaining classification as Skin Sens. 1 – H317.

In conclusion, an in-depth evaluation of the sensitising response observed for acetochlor does not allow classification into sub-categories. According to the criteria in Section 3.4.2.2.1.1, Annex I, CLP, "skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation" is appropriate for acetochlor.

RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

The DS evaluated a variety of sub-chronic and chronic studies from rats, dogs and mice including two short term (21-day) repeated dose dermal toxicity studies in rabbits and rats. The DS applied the guidance values for rats to other species. Toxicologically significant effects were observed after acetochlor administration in several repeated dose toxicity studies with doses below the guidance values for oral STOT RE 2 (see table below, cells shaded grey). The DS is of the opinion that effects on liver (mainly fatty infiltration, organ weight and enzymes) and kidney (interstitial nephritis, chronic vasculitis, fatty infiltration and tubular basophilia) are deemed toxicologically relevant since they indicate a histological disturbance in these organs with the potential to affect normal function. The thyroid was also a target organ, secondary to liver induction of UDP glucuronyltransferase (UDPGT), as was the nervous system, testes, thymus and erythrocytes.

Dermal Toxicity Studies.

The only acceptable 21-day dermal toxicity study was that performed by Johnson (1981) in rabbits, at dose levels of 100, 400 and 1200 mg/kg bw/d. The highest dose level produced about 75% mortality with severe clinical effects. There were no signs of systemic toxicity observed up to 400 mg/kg bw/d. The NOAEL was set at 400 mg/kg bw/d and the DS did not propose classification for the dermal route. The second study by Leah (1989) using rats was considered unacceptable because the controls also showed significant effects.

Sub-chronic and Chronic Oral Toxicity Studies:

The DS summarised the repeated dose toxicity studies outlining the main effect observed with acetochlor treatment. Liver enlargement at higher doses was sometimes accompanied by increased plasma levels of GGT or other liver enzymes, and microscopic alterations.

Acute liver toxicity studies in the rat (Ashby and Lefevre, 1993, 1994; Section 4.9.1.2.2 of the CLH report) have shown that acetochlor at high dose levels causes depletion of hepatocellular glutathione reserves and cytotoxicity.

Measurement of tissue distribution of radioactivity in rat metabolism studies shows that significant amounts of acetochlor metabolites are found in whole blood bound to erythrocytes.

Nephrotoxicity was seen in mice, rats and dogs. The occurrence of tubular basophilia at the lowest dose, above historical control data and accompanied by an increased kidney weight in the 18-month mouse study (Amyes, 1989) was considered to be toxicologically relevant, a finding endorsed by EFSA in its conclusion on the Pesticide Peer Review on acetochlor (2011).

Clinical signs of neurotoxicity were observed in several studies. Brain lesions with associated clinical effects were reported in one chronic dog study (Broadmeadow, 1989). During public consultation, industry suggested that the brain lesions may be secondary to severe renal toxicity and in their response the DS agrees with this position.

Summary of toxicologically relevant effects and comparison to cut-off values (adapted from Table 21, CLH report, with *additional information (italics) including % incidence, severity and historical control data (HCD)*)

Effect / Dose		Study duration	Cut-off value for STOT RE 2 [mg/kg bw/d]	Reference
Mortality	**50 mg/kg bw/d**: 60% of mortality (2/5 males and 4/5 females).	52-week dog study	**25**	(Broadmeadow, 1989
Neurotoxicity	**50 mg/kg bw/d**: • Dehydration, pallor and emaciation, salivation, swaying or shaking of the head and body, stiffness and rigidity of the hind limbs resulting in incoordination, ataxia and/or abnormal gait, hunched posture, tremor, hopping and flexor reflexes and exaggerated tonic neck reflex. • Histopathological changes in brain as degeneration of granular layer in the deeper parts of the vermis of cerebellum (4/5 males and 3/5 females, *slight to moderate*), depletion of Purkinje cells (4/5 males and 2/5 females*, slight to minimal*) and demyelination and degeneration of granule cell axons (1/5 males, *moderate*).	52-week dog study	**25**	(Broadmeadow, 1989)
	10 mg/kg bw/day: • Increase of salivation in males.	52-week dog study	**25**	(Broadmeadow, 1989)
	60 mg/kg bw/day: • Diarrhoea, mucus in the faeces, salivation, emesis and vocalisation during defecation.	13-week dog study	**100**	(Broadmeadow, 1986)
Haematology toxicology	**60 mg/kg bw/day**: • "Middle" anaemia with a decrease of RBC (13.51%), haemoglobin concentration (9.2%) and haematocrit (12.76%) in females.	13-week dog study	**100**	(Broadmeadow, 1986b)
	75 mg/kg bw/day: • Decrease of RBC (9.71%), haemoglobin concentration (10.06%) and haematocrit (8.46%).	119-day dog study	**75.6**	(Ahmed, 1980b)
Renal toxicity	**50 mg/kg bw/day**: • High urinary volume associated with low specific gravity. • Increase in the plasma concentrations of urea and creatinine in both sexes. • Cysts in kidneys (2/5 males and 2/5 females). • Interstitial nephritis (5/5 males and 4/5 females). • Collecting duct and transitional cell hyperplasia (5/5 males and 5/5 females). • Chronic vasculitis (4/5 males and 5/5 females) • Cortical fibrosis (4/5 males and 5/5 females). • Dilatation of Bowman's space (4/5 males and 4/5 females). • Cortical atrophy (4/5 males and 4/5 females) • Transitional cell hyperplasia (5/5 males and 5/5 females). • Lipofuchsin pigment in cortical tubules (4/5 males and 3/5 females). • One female showed papillary necrosis and another female focal necrosis.	52-week dog study	**25**	(Broadmeadow, 1989)

	10 mg/kg bw/day: • Interstitial nephritis (2/5 males, *minimal*) • Chronic vasculitis (3/5 males, *minimal*). • Granuloma (1/5 males, *just marked as present, parasitic origin*).	52-week dog study	**25**	(Broadmeadow, 1989)
	2 mg/kg bw/day: • Lipofuchsin pigment in cortical tubules (1/5 males) (*slight*).	52-week dog study	**25**	(Broadmeadow, 1989)
	75 mg/kg bw/day: • Fatty infiltration of the kidney (1/6 males, *mild*). • *Statistically significant increase in BUN (males +58%) relative to concurrent controls.*	119-day capsule dog study	**75.6**	(Ahmed, 1980b)
	25 mg/kg bw/day: • *Non statistical significant increase in BUN (males +15%).*	*119-day capsule dog study*	***75.6***	(Ahmed, 1980b)
	2 mg/kg bw/day: • Slight, not statistically significant increase in the incidence of chronic nephritis (*62/70 vs. 57/70*) and its secondary effects (tubular cast/cyst/dilation*, 59/70 vs. 50/70*) in males.	24-month rat study	**12.5**	(Naylor, 1986)
	1.1 mg/kg bw/day: • Statistically significant increase *(+8%*) of absolute kidney weight in males. • Statistically significant increase incidence of renal tubular basophilia in males (26% vs 6% in concurrent controls) over the maximum percentage in HCD (HCD max 17.31%). *Minimal or slight*. • *HCD % incidence of renal tubule basophilia in males between 1985 and 1988 (13 studies): 0, 0, 0, 0, 0, 0, 1.9, 17.3, 5.8, 0, 0, 0, 0.*	18-month mice study	**16.7**	(Amyes, 1989)
	11.2 mg/kg bw/day: • Statistically significant increase in relative kidney weight in males *(+16%*). • *Statistically significant increase incidence of renal tubular basophilia in males (22% vs 6% in concurrent controls) over the maximum percentage in historical control data (17.31%). Minimal or slight.* • *HCD data presented above.*	18-month mice study	**16.7**	(Amyes, 1989)
	116 mg/kg bw/day: • *Tubular basophilia (38% vs. 6% in concurrent controls), interstitial fibrosis (52%), cortical mineralization and hyaline casts in males and dilatation of cortical tubules in females (24% vs. 4% in concurrent controls).*	18-month mice study	**16.7**	(Amyes, 1989)
Testicular toxicity	**50 mg/kg bw/day:** • Small testes. • Decrease in absolute (*-47.7%*) and relative (*-33.9%*) testes weights. • Degeneration of seminiferous tubules (5/5 males). • Maturation arrest (5/5 males). • Spermatic giant cells (4/5 males). • Hypospermia in epididymides (5/5 males). • *The histopathology at this dose was not contradicted by the re-evaluation by Creasy (2003).*	52-week dog study	**25**	(Broadmeadow, 1989). *OECD guideline 409 (1981) FIFRA 82-2 (1983), GLP, acceptable*

	40 mg/kg bw/day: • *Diffuse testicular atrophy in 6/6 males in the high dose group only (no further detail).* • *Statistically significant decrease in absolute (-51%) and relative (-44%) weights of testes compared to controls.*	12-month capsule dog study	**25**	(Ahmed, 1981). *Pre-guideline, GLP, acceptable, DAR section B.6.3.6*
	10 mg/kg bw/day: • *Seminiferous tubule degeneration in 4/5 animals (0/5 controls). Severity minimal to moderate.* • *Hypospermia in the epididymides in 2/5 animals (slight, severe).* • *Contradicted by re-evaluation by Creasy (2003)*	52-week dog study	**25**	Broadmeadow, 1989). *OECD guideline 409 (1981) FIFRA 82-2 (1983), GLP, acceptable*
Liver toxicity	**75 mg/kg bw/day:** • Statistically significant increase of SGPT that exceeded the upper limit of historical controls in both sexes and LDH in males. • Increase in relative liver weight. • Atrophy in liver (1/6 males). • Fatty infiltration in liver (2/6 males). • *Statistically significant increase in BUN (males +58%) relative to concurrent controls.*	119-day capsule dog study	**75.6**	(Ahmed, 1980b)
	25 mg/kg bw/day: • Statistically significant increase of SGPT that exceeded the upper limit of historical controls. • *Increase in relative liver weight in females (+27%).*	119-day capsule dog study	**75.6**	(Ahmed, 1980b)
	60 mg/kg bw/day: • Statistically significant increase of SGPT. • Increase in relative liver weight.	13-week dog study	**100**	(Broadmeadow, 1986b)
	40 mg/kg bw/day: • Liver fatty infiltration (1/6 males). • Statistically significant increase of SGOT, LDH and cholesterol. • Increase in relative liver weight.	12-month capsule dog study	**25**	(Ahmed, 1981)
	12 mg/kg bw/day: • Liver fatty infiltration (1/6 males, *incidence only, no grade*). • Statistically significant increases in SGOT (*+113% males*), LDH (*+14% males*) and cholesterol (*+11% males*).	12-month capsule dog study	**25**	(Ahmed, 1981)
	4 mg/kg bw/day: • Statistically significant increases in SGOT and cholesterol in males.	12-month capsule dog study	**25**	(Ahmed, 1981)
	50 mg/kg bw/day: • High levels of transaminases (SGPT, ornithine carbamyl transferase, γ-GT, alkaline phosphatase and/or SGOT). • Statistically significant increase in cholesterol. • Pigment in hepatocytes (1/5 each, males and females). • Reduced glycogen in the liver (4/5 males and 4/5 females). • Increase in relative liver weight in males.	52-week dog study	**25**	(Broadmeadow, 1989)
	10 mg/kg bw/day: • Reduced glycogen (2/5 males and 1/5 females). • *Increase in blood triglycerides (+34% in females).*	52-week dog study	**25**	(Broadmeadow, 1989)
	2 mg/kg bw/day:	52-week	**25**	(Broadmeadow,

	• Reduced glycogen (1/5 males). • Granuloma in the liver (1/5 males and 1/5 females).	dog study		1989)
Thymus toxicity	**75 mg/kg bw/day:** • Thymus atrophy (1/6 males).	119-day capsule dog study	**75.6**	(Ahmed, 1980b)
Abbreviations: RBC = red blood cells; SGPT = serum glutamic-pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; LDH = lactate dehydrogenase				

Classification for STOT RE 2; H373 via the oral route is proposed by the DS – the liver and kidney are considered to be the main target organs for acetochlor.

Comments received during public consultation

Industry questioned the proposal for classification with STOT RE 2; H373. They agreed that the kidney and liver are target organs following high doses of acetochlor but disagreed with the interpretation of the severity of the effects by the DS. Industry outlined three points as a rebuttal to the severity of the effects in the kidney and liver:

> 1. "*The interstitial nephritis and/or chronic vasculitis reported in the kidneys from a few male dogs at 10 mg/kg/day from one 1-year dog study (Broadmeadow, 1989) were reported to be of "minimal" severity (individual histopathology data). Minimal severity is generally considered to be a histologic change that barely exceeds the normal limits and that is unlikely to produce any functional impairment. As such, these findings should not trigger classification.*"
>
> 2. "*An increased incidence of renal tubular basophilia was reported in male mice at 1.1 mg/kg/day in the 18-month mouse study (Amyes, 1989). However, the severity of the lesion in all animals at this level was minimal, there was no clear dose-response, and no such findings were reported even at ~500-fold higher dose levels in a 23-month mouse study (Ahmed, 1981). These findings were not considered evidence of a treatment-related adverse effect by either the USEPA or Japan FSC. Therefore, we believe that these findings were of questionable toxicological significance and should not trigger classification. This is further supported by the lack of renal effects in the mouse 90-day study (Ahmed, 1980).*"
>
> 3. "*Moderate diffuse fatty infiltration of the liver was reported in one male dog at 12 mg/kg/day in the other 1-year dog study (Ahmed, 1981) but was not accompanied by any treatment related changes in liver weight or clinical pathology. The few statistically significant differences in LDH, SGOT or cholesterol at this dose level were sporadic (only seen occasionally despite being evaluated monthly throughout the study), were similar to the values seen in control animals at other time points in this study, and were well within the normal historical control ranges for beagle dogs of this age. In addition, the fatty infiltration observed in 1/6 animals at the higher dose was scored as "mild".*"

The DS responded in the Response to Comments (RCOM) document under comment number 2, quoting section 4.7.1.10 of the CLH report indicating its view that the effects were toxicologically significant, substance related, and showed increasing incidence and severity with dose.

Industry in their "Comments on CLH Report for acetochlor, Version 2 (September 2013)" under section 4.12.2 appear to endorse renal toxicity (at a dose above the threshold value for STOT RE 2) indicating that it is responsible for the brain lesions and clinical signs of neurotoxicity noted in one of the 1-year dog studies (Broadmeadow, 1989). They state, "The neurological effects were noted only at 50 mg/kg/day, a level which produced significant renal pathology (nephritis, hyperplasia of the collecting ducts and pelvic epithelium, cortical atrophy and /or fibrosis, vasculitis, and sometimes necrosis) in all animals. Surviving high-dose animals exhibited significantly increased levels of urea, creatinine and BUN. No evidence of brain or kidney pathology was noted at 40 mg/kg/day in the other 1-yr dog study. Thus, the brain lesions may have been the result of uremic encephalopathy. The renal toxicity also would have resulted in less efficient renal clearance and thus substantially higher blood levels of acetochlor and/or its metabolites, which could have contributed to the neurological effects observed."

Two Member States commented during the public consultation. Both supported the classification proposals for human health submitted by the DS.

Assessment and comparison with the classification criteria

Within the CLP Regulation, the rat is the species on which the oral cut-off values for repeated exposure are based. Additionally, Haber´s rule is used to adjust the standard guidance values (which are typically for studies of 90 days duration), for studies of longer or shorter duration. Currently, there is no EU agreed position on how to apply the guidance values for classification of tested species other than rats. In the current assessment, the DS has applied the guidance values for rats to other species.

The guidance cut-off values for a classification for STOT RE in category 2 under CLP are: ≤ 300 mg/kg bw/d from subacute studies on rat (28 days), ≤ 100 mg/kg bw/day from subchronic studies on rat (90 days), ≤ 25 mg/kg bw/day from 1-year studies and ≤ 12.5 mg/kg bw/day from long term studies.

A substance is classified with STOT RE under CLP when it has produced, or has been shown to have the potential to produce, significant toxicity following repeated exposure by the oral, dermal or inhalation routes at or below given guidance values. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed, are included under this classification.

Critically evaluating the data from the 2005 Draft assessment report (DAR) in conjunction with the data presented in the table above, confirms the variety of effects following repeated dosing. There is ample evidence for the toxicity of acetochlor at doses above the criteria for STOT RE 2. Effects that are toxicologically relevant at or below the guidance values for STOT RE are seen for haematology (RBCs, haemoglobin and haematocrit) and renal toxicity (tubular basophilia and interstitial nephritis). The liver effects tend to be borderline, but increases in liver weight, triglycerides and decreases in glycogen confirm it as a target for acetochlor. As the DS rightly points out, the data are extensive, sometimes borderline and somewhat complex. It requires a weight of evidence approach to provide a balanced assessment.

Based on the available data from several studies across different species (see table above), the overall weight of evidence is sufficient for classification of acetochlor for Specific Target Organ Toxicity - repeated exposure. Comparison of repeated dose toxicity data with CLP guidance levels indicates that effective doses for toxicological effects are frequently below the CLP guidance cut-off limits and fulfil the CLP criteria for STOT RE 2; H373 (kidney).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

(1) *In Vitro* Studies:

Acetochlor was tested in a wide variety of genetic toxicology assay systems. A brief summary is presented below along with a more detailed table of the individual studies further down.

Summary of genotoxicity studies

Mutagenicity Study	Result		Comment
	Negative	***Positive***	
***(a) in vitro* studies**			
5 × Bacterial gene mutation	**3 (±S9)**	**2 (mixed)**	4 × studies from before the 1997 update of TG 471 1 × study from after the 1997 update of TG 471 (negative) (Yong Xu, 2006). Mixed results amongst different bacterial strains.
3 × Mammalian gene mutation	**1 (±S9)** **1 (-S9)**	**1 (+S9)** **1 (±S9)**	
2 × Chromosomal aberration		**2 (±S9)**	Human lymphocytes (whole blood). S9 from livers of rats induced with Aroclor 1254.
1 × UDS	**1**		UDS assay in rat hepatocytes, negative results (Naismith & Matthews, 1983)
***(b) in vivo* studies**			
3 × somatic Chromosome aberration	**3**		Including 2 Micronucleus tests.
1 × somatic Comet assay	**1**		Nasal cells (olfactory and respiratory) from male rats (Alpk: APfSD)
1 × somatic UDS		**1**	Trueman (1989), positive. UDS response observed secondary to severe liver toxicity.
2 × complementary UDS			Ashby & Lefevre (1993, 1994) investigated UDS, non-protein sulphydryl groups, enzymes and histopathology.
4 × germ cell Dominant lethal assay	**4**		No evidence of treatment related embryonic or foetal death in rats or mice.

In vitro studies consisted of bacterial gene mutation (4 studies, but conducted before the update of TG 471 in 1997, where they did not include strains capable of detecting certain oxidising mutagens, crosslinking agents and hydrazines), mammalian gene mutation (3 studies), chromosome aberration (2 studies) and effects on DNA synthesis in mammalian cells (1 study). A combination of negative and positive results was obtained. These are tabulated in Table 23 of the CLH report. In addition, there is also a more recent GLP, guideline compliant (OECD 471, 1997) study available from the Acetochlor Registration Partnership and supplied by Monsanto (Yong Xu, 2006, study 6103-507) that was negative for mutagenicity. This was not assessed by the DS.

Summary of *in vitro* genotoxicity studies (Table 23, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
Bacterial mutation (spot test). Pre-guideline. Outline from OECD TG 471. GLP: No Acceptability: No	Technical acetochlor (92.5 % purity)	*S. typhimurium* TA1535, TA1537, TA98 and TA100 S9 from livers of rats and mice.	50 µl /plate (±S9).	Negative with all strains (±S9)		Kulik, F.A. and Ross, W.D, 1978 (IIA, 5.4.1a/01)
Bacterial mutation (plate incorporation test). Pre-guideline. Outline from OECD TG 471. GLP: No Acceptability: No	Technical acetochlor (92.5 % purity)	*S. typhimurium* TA1535, TA1537, TA98 and TA100 S9 from livers of rats.	1st exp (all strains): 0.001, 0.004, 0.02, 0.1, 0.3, 1 µl/plate (±S9). 2nd exp (TA100): 0.001, 0.004, 0.02 µl/plate (-S9)	Negative with all strains (±S9)	Significant increases in revertants with TA100 (-S9) in the 1st exp. were not reproduced in the 2nd exp. No cytotoxicity.	
Bacterial plate incorporation mutation assay. OECD TG 471 GLP: Yes Acceptability: Yes	Technical acetochlor (89.9% purity)	*S. typhimurium* TA1535, TA1537, TA1538, TA98, and TA100 S9 from livers of rats induced with Araclor 1254.	1st and 2nd exp. (all strains): 1.6, 8, 40, 200, 1000, 5000 µg/plate (±S9) 3rd exp. (TA1538): 100, 200, 500, 1000, 2500, 5000 µg/plate, (±S9)	Weak positive with TA1538 (+S9). Negative with TA1538 (-S9). Negative with TA1535, TA1537, TA98 and TA100 (±S9).	Slight significant increases in revertants with TA1538 (+S9) were observed in 2 of 3 exp. Cytotoxicity at 5000 µg/plate (±S9)	Callander, R.D., and Priestley, K.P., 1989 (IIA, 5.4.1a/03)
Bacterial plate incorporation and pre-incubation mutation assay. OECD TG 471(1983). UK Department of Health Guidelines (1989). GLP: No Acceptability: Yes	Analytical acetochlor (99.6% purity)	*S. typhimurium* TA1538. S9 from livers of rats induced with Araclor 1254.	1 exp. (-S9) and 3 exp.(+S9): 500, 875, 1250, 2500, 3750, 5000 µg/plate	Negative with TA1538 (± S9).	No reproducible significant increases in revertants. Cytotoxicity at 5000 µg/plate (+S9) and at 3750 µg/plate (-S9).	Callander, R.D., 1992 (IIA, 5.4.1a/02)
	Technical acetochlor (89.9% purity)	*S. typhimurium* TA1538. S9 from livers of rats induced with Araclor 1254.	1 exp. (-S9) and 3 exp.(+S9): 500, 875, 1250, 2500, 3750, 5000 µg/plate	Weak positive with TA1538 (+S9) Negative with TA1538 (-S9).	Slight significant increases in revertants with TA1538 (+S9) were observed in 2 of 3 exp. Cytotoxicity at 5000 µg/plate (+S9) and at 3750 µg/plate (-S9).	
Bacterial mutation (pre-incubation) test) OECD TG 471	Analytical acetochlor (99.6% purity)	*S. typhimurium* TA1538. S9 from livers of rats induced with Araclor 1254.	1 exp. (+S9): 500, 875, 1250, 2500, 3750, 5000 µg/plate	Negative with TA1538 (+S9)	Cytotoxicity at 5000 µg/plate (+S9)	

Summary of *in vitro* genotoxicity studies (Table 23, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
(1983). UK Department of Health Guidelines (1989). GLP: No Acceptability: Yes	Technical acetochlor (89.9% purity)	*S. typhimurium* TA1538. S9 from livers of rats induced with Araclor 1254.	1 exp. (+S9): 500, 875, 1250, 2500, 3750, 5000 μg/plate	Negative with TA1538 (+S9).	Cytotoxicity from 2500μg/plate (+S9)	
Bacterial plate incorporation mutation assay. Followed the Ames protocol (1983) modified in accordance with the recommendations of the UK Environmental sub-committee on guidelines for mutagenicity testing. GLP: Yes Acceptability: Yes	Technical acetochlor (89.9% purity, batch A)	*S. typhimurium* TA1538 S9 from livers of rats induced with Phenobarbital and β-naphthoflavone	2 exp. (± S9) 100, 200, 500, 1000, 2500, 5000 μg/plate	Negative with TA1538 (± S9).	Cytotoxicity at 2500 and 5000 μg/plate (± S9)	Callander, R.D., 1998 (IIA, 5.4.1a,b//04)
	Analytical acetochlor (99.6%, purity, batch B)	*S. typhimurium* TA1538 S9 from livers of rats induced with phenobarbital and β-naphthoflavone	2 exp. (+S9): 100, 200, 500, 1000, 2500, 5000 μg/plate	Negative with TA1538 (+S9).	Cytotoxicity at 2500 and 5000 μg/plate (+S9)	
	Technical acetochlor (94.4% purity, batch C)	*S. typhimurium* TA1538 S9 from livers of rats induced with Phenobarbital and β-naphthoflavone	2 exp (± S9) and 1 exp (+S9) 100, 200, 500, 1000, 2500, 5000 μg/plate	Negative with TA1538 (± S9).	Cytotoxicity at 2500 and 5000 μg/plate (± S9)	
	Technical acetochlor (94.4% purity, batch C)	*S. typhimurium* TA1538 S9 from livers of rats induced with Araclor 1254	1 exp (± S9) 100, 200, 500, 1000, 2500, 5000 μg/plate	Negative with TA1538 (± S9).	Cytotoxicity at 5000 μg/plate (-S9)	
In vitro mammalian gene mutation assay The study is pre-guideline. GLP: Yes Acceptability: Yes	Acetochlor (96.3%purity)	Chinese hamster ovary cells (CHO-K_1-BH_4) S9 from livers of rats induced with Arochlor 1254	1st exp (± 1, 2, 5 and 10% S9) 25, 100, 175 μg/mL 2nd exp (± 10% S9) 25, 75, 100, 125, 150 μg/mL (-S9) 25, 50, 75, 100, 125 μg/mL (+S9)	1st exp Negative 2nd exp Positive (± S9). Significant increases in mutation frequency from 125 μg/mL (±S9)	1st exp Cytotoxicity at 175 μg/mL (± S9). 2nd exp Cytotoxicity at 125 μg/mL (+S9).	Li, A.P., 1983 (IIA, 5.4.1b/04)
In vitro mammalian gene mutation assay EPA 84-2 GLP: Yes Acceptability: Yes	Acetochlor (91.4% purity)	Chinese hamster ovary cells (CHO-K_1-BH_4) S9 from livers of rats induced with Arochlor 1254	1st exp (± 1, 2, 5 and 10% S9) 50, 100, 200 μg/mL 2nd exp (± 10% S9) 50, 75, 100, 150, 200 μg/mL	Negative (± S9) in both exp.	1st exp Cytotoxicity at 200 μg/mL (- S9). 2nd exp Cytotoxicity at 200 μg/mL (± S9).	Li, A.P., Myers, C.A., 1989 (IIA, 5.4.1c/01)
In vitro mammalian gene mutation assay The study is	Acetochlor (unspecified purity)	L5178Y mouse lymphoma cells. S9 from livers of rats induced with Arochlor 1254.	-S9: 20, 30, 45, 60, 76, 100, 400 μL/L +S9:	Negative (-S9) Positive (+S9)	-S9: Cytotoxicity from 76 μL/L +S9 Cytotoxicity from	Mitchell, A.D., Rudd, C.J. and Coleman, R.L., 1982 (IIA,

Summary of *in vitro* genotoxicity studies (Table 23, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULTS	COMMENTS	REFERENCE
pre-guideline. GLP: Yes Acceptability: Yes (with reservations because unspecified purity of acetochlor)			5, 15, 20, 30, 40, 50, 100, 250 µL/L		40 µL/□	5.4.1c/02)
In vitro mammalian chromosome aberration assay OECD TG 473 GLP: Yes Acceptability: Yes	Acetochlor (89.4% purity)	Human lymphocytes (whole blood). S9 from livers of rats induced with Aroclor 1254.	10, 50 and 100 µg/mL (±S9)	Positive (±S9)	There was a reduction in MI between 36 and 69 %) at 100 µg/mL (±S9)	Howard, C.A., 1989 (IIA, 5.4.1b/02)
In vitro mammalian chromosome aberration assay No guideline cited GLP: No Acceptability: Yes	Analytical acetochlor, (99.6% purity)	Human lymphocytes (whole blood). S9 from livers of rats induced with Aroclor 1254.	10, 75 and 150 µg/mL (±S9)	Positive (±S9)	Concentrations selected based on reduction in MI and suitability of preparations. Results with related materials establish the chloro substituent in acetochlor as the clastogenic entity and suggest a protective cellular effect of the SH group of glutathione.	Fox, V., 1998 (IIA, 5.4.1b/03)
	Technical acetochlor, (94.4% purity)	Human lymphocytes (whole blood and separated lymphocytes). Without S9.	Whole blood: 100 µg/mL Separated lymphocytes: 75 µg/mL	Positive		
In vitro UDS assay The study is pre-guideline. GLP: Yes Acceptability: Yes (with reservations because a low number of cells was scored, and results were not confirmed)	Acetochlor (99.7% purity)	Rat hepatocytes	0.016, 0.053, 0.16, 0.53, 1.6, 5.3, 16.0, 53.3 and 160 µg/mL	Negative	Cytotoxicity from 5.3 µg/mL. UDS determination at the five lower non-toxic concentrations.	Naismith, R.W. and Matthews, R.J., 1983 (IIA, 5.4.1b/01)
exp = experiment; MI = mitotic index						

(2) *In Vivo* Studies

In vivo studies included somatic (see Table 24 in the CLH report) and germ cell (see Table 25 in the CLH report) genotoxicity testing. In somatic cells, studies consisted of chromosome aberration (3 studies including 2 micronucleus tests), and effects on DNA (a single *in vivo* comet assay and a UDS assay) with a complementary study to the UDS assay. In germ cells, four dominant lethal assay studies were evaluated.

The UDS assay by Trueman (1989) was positive, acetochlor induced DNA repair. Two subsequent studies by Ashby & Lefevre (1993, 1994) investigated UDS, non-protein sulphydryl groups, enzymes and histopathology. The DS concluded that the UDS response observed was secondary to severe liver toxicity.

The DS described a temporal sequence of events leading to UDS in mammalian hepatocytes upon exposure to acetochlor:

(1) There is a rapid and dose-related depletion of liver glutathione (GSH). This depletion is probably enzyme mediated rather than a direct reaction of the chlorine group of acetochlor with GSH.
(2) When this depletion reaches a critical level ($\approx$ 40% of control group values), single cell necrosis becomes evident and increases rapidly in a dose related manner (1000-2000 mg/Kg).
(3) These pathological changes lead to a leakage of hepatic enzymes into the peripheral blood.
(4) As the liver attempts to repair the damage an increase in DNA synthesis is observed which accounts for the positive UDS assay.

Summary of *in vivo* somatic cell genotoxicity studies (based on Table 24, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULT	COMMENTS	REFERENCE
In vivo mammalian chromosome aberration assay The study is pre-guideline. GLP: Yes Acceptability: Yes (on revision of comments received from industry by DS in the RCOM). DS still has a question over the acceptability of the study pending data from industry to prove a statistically significant reduction in body weight gain.	Acetochlor, (96.3% purity)	Bone marrow cells from Sprague-Dawley rats.	40, 150, 500 mg/kg by intraperitoneal injection. Sampling at 6, 12, 24 hours.	Negative.	Slight effects on body weight gain. Reduction in MI of 25% in high dose animals at 24 hours may indicate toxicity. No cytoxicity.	Farrow, M.G. and Cortina, T., 1983 (IIA, 5.4.2a/01)
In vivo mammalian micronucleus test Guideline: not cited GLP: Yes Acceptability: Yes (on revision of comments received from industry by DS in the RCOM).	Acetochlor (96.7% purity)	Bone marrow cells from CD-mice.	200, 660 and 2000 mg/kg by oral gavage. Sampling at 24, 48 and 72h.	Negative	Mortality at 2000 mg/kg (43%). Clinical signs of systemic toxicity at ≥ 660 mg/kg. No cytotoxicity	Cavagnaro, J. and Cortina T., 1985 (IIA, 5.4.2a/02)
In vivo mammalian micronucleus test Guideline: OECD TG 474 GLP: Yes Acceptability: Yes	Acetochlor (89.4% purity)	Bone marrow cells from male and female CD- mice.	898 and 1436 mg/kg (males) 1075 and 1719 mg/kg (females) Sampling at 24, 48 and 72h.	Negative	Cytotoxicity at both doses tested for females, and at the highest dose tested for males (24 and 72h sampling	Randall, V., 1989 (IIA, 5.4.2a/03)

Summary of *in vivo* somatic cell genotoxicity studies (based on Table 24, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULT	COMMENTS	REFERENCE
					time)	
In vivo comet assay Guideline: not available GLP: No Acceptability: Yes (on revision of comments received from industry by DS in the RCOM).	Acetochlor (96.6% purity)	Nasal cells (olfactory and respiratory) from male rats (Alpk: APfSD)	1750 ppm (≈ 175 mg/kg) for 1 and 18 weeks.	Negative	Data on toxicity not reported. Choice of dose justified by the results of other toxicity tests.	Ashby, J., Clapp, M.J,L,, Tinwell, H. et al., 1996 (IIA, 5.4.2b/01)
In vivo UDS assay The study is pre-guideline. GLP: Yes Acceptability: Yes	Acetochlor (89.4% purity)	Hepatocytes from male rats (Alpk AP_fSD)	500, 1000 and 2000 mg/kg, by gavage. Liver samples at 4 and 12h.	Positive at 2000 mg/kg (12h time point)	Toxicity at 2000 mg/kg (12h time point).	Trueman, R.W., 1989 (IIA, 5.4.2b/02)
Complementary study to *In vivo* UDS assay (liver UDS, GSH levels and histopathology; blood ALT, AST, ALP and ALB levels). The study is pre-guideline. GLP: No Acceptability: Yes	Acetochlor, (89.9% purity)	Hepatocytes and liver and blood samples from male rats (Alpk AP_fSD)	1st exp: 2000 mg/kg, by gavage (for UDS, GHS levels and necrosis in liver) Liver samples at 12h. 2nd and 3rd exp: 500, 1000 and 2000 mg/kg, by gavage (for GHS levels and necrosis in liver; and for blood ALT, AST, ALP and ALB levels). Liver and blood samples at 12h.	Positive at 2000 mg/kg (12h time point)	Dose-related decrease in GSH levels. Necrosis at 1000 and 2000 mg/kg. Elevated ALT and AST levels at 2000 mg/kg. No variability inter-animal for GSH depletion. Inter-animal variability for liver necrosis and for blood ALT and AST levels	Ashby, J., Lefevre, P.A., 1993 (IIA, 5.4.2b/03)

Summary of *in vivo* somatic cell genotoxicity studies (based on Table 24, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULT	COMMENTS	REFERENCE
Complementary study to *In vivo* UDS assay (liver GSH levels and histopathology; blood ALT, AST, ALP and ALB levels). Guideline: not a genotoxicity study. GLP: No Acceptability: Yes (as additional information)	Acetochlor, (89.9% purity)	Liver and blood samples from male rats (Alpk AP_fSD)	1st exp: 500, 1000 and 2000 mg/kg, by gavage (for GHS levels and necrosis in liver; and for blood ALT, AST, ALP and ALB levels) Liver and blood samples at 12, 24, 48h. 2nd exp: 500, 1000 and 2000 mg/kg, by gavage (for blood ALT, AST, ALP and ALB levels). Blood samples at 3 and 6h.		Dose-related decrease in GSH levels between 3-12h. Maximum depression at 6h. By 24h GSH levels were recovering and exceeded control levels by 48h. The major pathological changes were in the 2000 mg/kg group at 12h time point. There were elevated ALT and AST levels in the 2000 mg/kg group at 12h time point.	Ashby, J., Lefevre, P.A., 1994 (IIA, 5.4.2b/04)

The DS has summarised all the *in vivo* germ cell genotoxicity studies in the CLH report and while there are some discrepancies between the studies with respect to toxicity at the highest dose tested, there is no evidence for acetochlor related embryonic or foetal death in rats or mice. All four dominant lethal studies summarised in Table 25 of the CLH report were negative.

Summary of *in vivo* germ cells genotoxicity studies (based on Table 25, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULT	COMMENTS
Dominant lethal assay Guideline: Not cited GLP: Yes with some deviations. Acceptability: Yes	Acetochlor (90.4% purity)	Alpk AP_fSD rats.	200, 1000 and 2000 mg/kg by single oral gavage for 4 days	Negative	2000 mg/kg caused three deaths, clinical signs of toxicity and 10% bodyweight reduction. 1000 and 2000 mg/kg caused a reversible reduction in fertility at weeks 2 and 3.
Dominant lethal assay Guideline: Not cited GLP: Yes Acceptability: Yes (on revision of comments received from industry by DS in the RCOM).	Acetochlor, (94.3% purity)	Male and female Charles River Sprague Dawley rats.	100, 200 and 2000 ppm in the diet for 65 days (5.33, 52.80 and 106.40 mg/kg)	Negative	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.
Dominant lethal assay Guideline: OECD TG 478 GLP: Yes Acceptability: No	Acetochlor (94.4% purity)	Male and female Alderley Park (Alpk AP_fSD) rats.	200, 1000 and 1500 ppm in the diet for 10 weeks (13, 62 and 88.5 mg/kg)	Negative	No toxicity.
Dominant lethal	Acetochlor	Male and	200, 1000 and	Negative	Initial weight loss

Summary of *in vivo* germ cells genotoxicity studies (based on Table 25, CLH report)

TEST	TEST SUBSTANCE	SYSTEM	DOSAGE	RESULT	COMMENTS
assay OECD TG 478 GLP: Yes Acceptability: Yes (on revision of comments received from industry by DS in the RCOM).	(94.4% purity)	female CD-1 mice	3500 ppm in the diet for 8 weeks (38, 186 and 812 mg/kg)		followed by decreases (generally ~5%) in body weight were observed in high-dose (3500 ppm) male mice.

The DS has provided an extensive and thorough evaluation of all the studies concerned with determining the genotoxic potential of acetochlor. Acetochlor, according to the DS, is mutagenic *in vitro* but importantly, it is not genotoxic according to the *in vivo* test results.

Comments received during public consultation

Numerous comments were received from industry and two new studies on the metabolite t-OXA were submitted. There was no disagreement with respect to the DS conclusion on acetochlor for no genotoxic potential. Instead many of the points were minor in nature and were concerned with the interpretation of toxicity at the highest dose tested in the different assays. In those cases where the DS thought a study was not acceptable, industry presented its arguments to illustrate why those studies should be considered as acceptable. The DS in its response to industry in the RCOM has agreed with these points.

Two Member States commented during the public consultation. Both supported the classification proposals for human health submitted by the DS.

Assessment and comparison with the classification criteria

According to the CLP Regulation classification in *Category 1A* is based on positive evidence from human epidemiological studies. No such evidence exists, therefore classification in Category 1A is not supported.

Classification into *Category 1B* is not supported. There is no evidence for positive effects in the *in vivo* heritable germ cell mutagenicity tests. The DS has evaluated an extensive toxicological dataset on acetochlor and the main metabolites of acetochlor and has found no evidence of genotoxicity. The DS has also evaluated two new studies submitted by industry and summarised their evaluation in the RCOM document. These evaluations are hence not present in the CLH report.

Classification into Category 2 is not proposed by the DS though this is the only category that can be further considered. A few *in vitro* mammalian cell studies are positive for clastogenicity but this may be associated with the redox environment, e.g. glutathione levels, within the cells tested. The des-chloro analogue of acetochlor was confirmed as non-clastogenic using whole blood in the presence and absence of S9-mix suggesting that the chloro substituent in acetochlor is a potential clastogenic entity. The only *in vivo* finding suggestive of genetic toxicity for acetochlor is in the severely compromised livers of rats exposed to 2000 mg/kg (Trueman, 1989). The DS is of the opinion that this UDS assay cannot be interpreted in the context originally envisaged for this assay and that the UDS activity observed in the livers of rats exposed to acetochlor at 2000 mg/kg is of limited relevance to the chronic toxicology of this material. These effects, according to the DS, do not justify classification of acetochlor as Muta. 2. The RAC endorses this view.

In summary, the RAC endorses the DS opinion that acetochlor is not mutagenic nor is it genotoxic *in vivo* and thus requires no classification with respect to germ cell mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

(1) Introduction

The DS has presented an in-depth evaluation of the long term studies and discussed at length the carcinogenic potential of acetochlor in both rats and mice. The data are complex and open to interpretation but can be distilled down into a simple fact: there are statistically significant increases in tumour incidences for several types of tumours in two different species (rat and mouse).

Acetochlor is currently listed in Annex VI of the CLP Regulation and it is not classified for carcinogenicity. Acetochlor was reviewed by the ECBI Specialized Group of Experts in carcinogenicity in 1997 (ECBI SE) and it was recommended not to classify acetochlor for carcinogenicity on the basis of insufficient evidence and lack of relevance to humans (Doc. ECBI/28/97, see Annex 8.1 of the CLH report).

The available genotoxicity data on acetochlor does not support a genotoxic mode of action (MoA) for tumour induction. The evidence suggests the clastogenicity of acetochlor is due to cytotoxicity from oxidative damage. The extensive mechanistic data suggests that acetochlor is carcinogenic in nasal tissues by a secondary mechanism with a practical threshold. However, RAC notes there are publications in the public domain that suggest that precursor dialkylanilines and quinone imines may damage DNA through indirect mechanisms and reactive oxygen species generation (Chao *et al.*, 2012; Te *et al.*, 2012).

The primary evidence for carcinogenicity is a treatment-related increased incidence of nasal olfactory tumours in all three rat studies, at dose levels ≥ 1000 ppm (~ 54 mg/kg bw/d; maximum tolerated dose (MTD)). The main question for RAC is if there is convincing data that this carcinogenic effect is not relevant to man.

The mechanism for the formation of nasal olfactory epithelial tumours was determined to be local cytotoxicity secondary to quinone imine formation. Although rats appear to be more sensitive than humans in the formation of nasal tumours, the available data do not preclude their relevance in man.

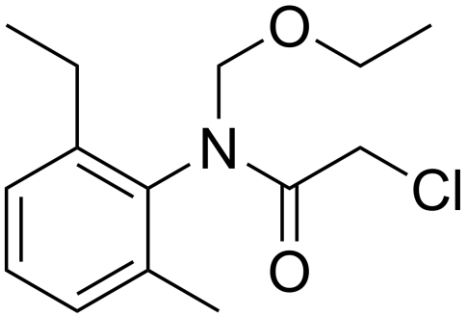
Several other neoplasms were noted. Carcinogenic effects in mice associated with the lung and uterus are considered to provide supporting evidence but the data is weak in affirming a treatment-relationship with acetochlor. Tumours in the lung are complicated because of a high background spontaneous incidence in aged CD-1 mice. A treatment-related effect cannot be excluded for the lung tumours and histiocytic uterine sarcomas in mice, although the association is considered to be quite weak. In the absence of mechanistic studies, there is insufficient data available to determine the MoA for these tumours. Consequently their relevance for humans cannot be ruled out.

Femur and stomach tumours were also initially described in rats. Chondroma of the femur (rat) was a result of misdiagnosis in the initial pathological evaluation. A subsequent re-evaluation (Hardisty, 2001b), revealed that these lesions were actually not tumours but cartilaginous hyperplasia, which was also present in one control animal. The lesions in the forestomach were squamous cell carcinomas, and there were no pre-neoplastic lesions present which are a hallmark of chemical induction of neoplasia in the rodent forestomach. These tumours are considered spontaneous neoplasms unrelated to acetochlor administration.

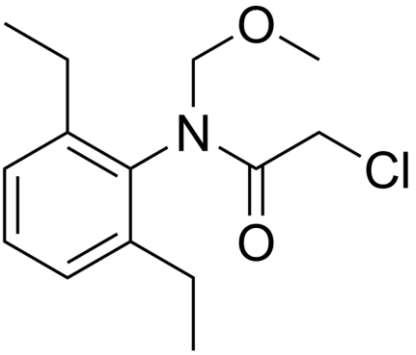
A slight increase in the incidence of rat thyroid follicular tumours was also noted but these tumours were a result of induction of hepatic UDPGT and subsequent disturbance of thyroid hormone homeostasis. This is a well-known, threshold-mediated, species-specific MoA that is generally not considered to be relevant to human hazard assessment.

Acetochlor is structurally related to other chloroacetanilide herbicides, including alachlor, propachlor, butachlor and metolachlor. These chemicals have overlapping, but not identical,

tumour profiles. Butachlor also induces nasal epithelial tumours and thyroid follicular cell tumours. Of particular note is that alachlor is also currently listed in Annex VI of the CLP Regulation. The general MoA for the rat nasal tumours produced by acetochlor is essentially the same as that for alachlor, a close structural analogue. However, unlike acetochlor, alachlor was classified for carcinogenicity. Interestingly, alachlor, also produces a variety of tumours in the rat: nasal olfactory epithelium, thyroid and stomach tumours along with lung tumours in mice.



Acetochlor



Alachlor

(2) Relevant Core Studies – Carcinogenicity / Chronic toxicity

The main relevant studies include three chronic rat and two chronic mouse studies that were conducted with acetochlor, at dietary concentrations up to 5000 ppm (approximately 297 mg/kg bw/d for rats and 973 mg/kg bw/d for mice). All five studies are considered valid for an assessment of carcinogenicity, although the high-dose level in the first chronic rat and mouse studies greatly exceed the MTD based on a number of signs of toxicity and increased mortality. Nasal neoplastic findings also occurred in a rat dietary 2-generation reproductive toxicity study. The following table illustrates key data from Tables 43 and 45 of the CLH report.

Reference/ Method	Main Results and Remarks
2-year study SD rats Ahmed, F.E. (1983a) OECD TG 453. Dose: 0, 500, 1500 and 5000 ppm (0, 22, 69 and 250 mg/kg bw/d in males, 0, 30, 93 and 343 mg/kg bw/d in females).	**<u>Neoplastic findings:</u>** - Hepatocellular adenomas and carcinomas combined (significant in males and females at 5000 ppm only). Incidence in males: 5%, 7%, 7% and 18% (0, 2, 2, and 8% in females). - Thyroid follicular adenomas (males at 1500 and 5000 ppm). The overall incidence of follicular cell adenomas in the males was as follows: control 0%, low-dose 0%, mid-dose 4% and high-dose 7%. - Testicular Interstitial cell tumours exhibited a dose-related increase in the treated males compared to the male control group. The overall percentages of interstitial cell tumours were: control (2/70), low-dose (4/70), mid-dose (4/70) and high-dose (7/70). There was no statistically significant positive trend (Peto analysis). - Nasal papillary adenomas (males at 1500 and 5000 ppm). The overall incidence in males was as follows: control 0%, low-dose 1%, mid-dose 9% and high-dose 26%. There was in addition 2/69 positive for adenocarcinoma only at the high dose.
2-year study SD rats Naylor, M. W., (1986) OECD TG 453 Dose: 0, 40, 200 and 1000 ppm (0, 1.9, 9.4, 47.5 mg/kg bw/d in males, 0, 2.4, 11.8, 60.0 mg/kg bw/d in	**<u>Neoplastic findings:</u>** - Incidence of papillary adenomas of the nasal mucosa in males was 2%, 0%, 0% and 20%. In females the incidence was 0%, 0%, 0% and 28%. - Hepatic neoplastic nodules (benign) were increased in female at 1000 ppm (8%, 5/60). The occurrence of malignant liver tumours was not increased in any dietary level in either sex. - The incidence of thyroid adenomas/cystadenomas was slightly higher in females treated with 1000 ppm. This difference was not statistically significant. - The incidence of <u>adenocarcinomas in pituitary</u> in the high dose group females (1000 ppm, 60mg/kg bw/d) was higher than in controls but without statistical significance.

females).

2 year study

CD rats

Broadmeadow, A., (1988)

OECD TG 453

Dose: 0, 18, 175 and 1750 ppm (0, 0.67, 6.37 and 66.9 mg/kg bw/d in males; 0, 0.88, 8.53 and 92.1 mg/kg bw/d in females).

Neoplastic findings:

- Significantly increased incidence of polypoid adenomas in the nasal mucosa at the high dose in both sexes (males 35/70 (50%) and females 36/65 (55%)). There were three cases (2 males and 1 female) of carcinoma of the nasal epithelium at the high dose. Examination of HCD showed a zero incidence of nasal epithelial tumours out of 300 control animals.
- Increased incidence in thyroid follicular cell adenoma (females, 5%) at the high dose. For both sexes, HCD of follicular adenoma ranged up to 6%.
- Rare tumours: 2 animals in the high dose group - chondroma of the femur. A subsequent evaluation (Hardisty, 2001b), revealed that these lesions were actually not tumours but cartilaginous hyperplasia, which was also present in one control animal.
- Rare tumours: 2 animals in the high dose group - basal cell tumours were found in the forestomach (non-glandular region of the stomach) of one male and one female at 1750 ppm. A subsequent independent Pathology Working group (PWG) evaluation concluded that these lesions were actually squamous cell carcinomas, not basal cell tumours (Hardisty, 2001b).

2-generation Reproductive toxicity study

Rat CD(SD) IGS BR (Sprague-Dawley)

Milburn, G.M., (2001)

OECD TG 416

Dose: 0, 200, 600 and 1750 ppm (0, 20, 61 and 181 mg/kg bw/d in males; 0, 22, 68 and 207 mg/kg bw/d in females).

This two-generation reproductive toxicity study was considered part of the carcinogenicity assessment, because nasal tumours were observed.

Neoplastic findings:

- Polypoid adenomas were observed in F0 and F1 adults of both sexes receiving 1750 ppm acetochlor. The incidence was higher in F1 than in F0 animals by about 2-fold (at 1750 ppm, males 27% vs. 12% and females 54% vs. 27%). In both generations the incidence of hyperplasia was greater in females.

Table 43.12: Incidence of nasal proliferative lesions in F0 and F1 adults

	Findings	Dietary concentration of acetochlor (ppm)							
		Males				Females			
		0	200	600	1750	0	200	600	1750
F0	# tissues examined	26	26	26	26	26	26	26	26
	Olfactory epithelial Hyperplasia	0†	0	0	3 12%	0††	0	0	7** 27%
	Respiratory epithelial Hyperplasia	0	0	0	2 8%	0	0	0	2 8%
	Papillary adenoma	0††	0	0	4 15%	0††	0	0	6* 21%
F1	# tissues examined	26	26	26	26	26	26	26	26
	Olfactory epithelial Hyperplasia	0††	0	0	7** 27%	0††	0	4* 15%	14** 54%
	Respiratory epithelial Hyperplasia	0	0	0	1 4%	0	0	0	0
	Papillary adenoma	0††	0	3 12%	8** 31%	0††	0	1 4%	17** 65%

* $p \leq 0.05$, Fisher's exact test
** $p \leq 0.01$, Fisher's exact test
† $p \leq 0.05$, Peto trend test
†† $p \leq 0.01$, Peto trend test

1-year study

SD (male) rats

Mainwaring, G., (2004)

Acetochlor sulphoxide 52-week feeding study.

Control: 0 ppm

Acet: 1750 ppm (99.6 mg/kg bw/d)

Acet.Sulfox: 300 ppm

This study was designed to compare nasal tumorigenicity of acetochlor sulphoxide with that of acetochlor and to demonstrate that acetochlor sulphoxide is a key metabolite in the development of the rat nasal tumours.

Table 43.13: Histopathological findings in nasal cavities of rats treated with acetochlor and sec-amide methyl sulphoxide (acetochlor sulphoxide)

Week	Finding	Dose level (ppm)		
		Control	Sulphoxide	acetochlor
		0	300	1750
26	*# tissues examined*	32	32	32
	Polypoid adenoma	0	7** (22%)	21** (66%)

(14.6 mg/kg bw/d)

Non-GLP and non-guideline but acceptable.

Study: 3 groups of 96 rats.

	Hyperplasia, total	0	2	6*
52	*# tissues examined*	31	27	26
	Rhinitis, total	14	12	10
	Polypoid adenoma	0	8** (30%)	17** (65%)
	Hyperplasia, total	0	11** (41%)	23** (88%)

** $p \leq 0.01$

The findings demonstrate that acetochlor sulphoxide, a major acetochlor plasma metabolite in the rat, was a nasal carcinogen. The morphology, location (in the olfactory epithelium) and development with time of the tumours were identical to those seen with acetochlor. Therefore, this metabolite can be directly associated with the mechanism of formation of nasal tumours.

24-month study

CD-1 mice

Ahmed (1983)

OECD TG 451

Dose: 0, 500, 1500 and 5000 ppm (0, 75, 227 and 862 mg/kg bw/d in males; 0, 95, 280 and 1084 mg/kg bw/d in females).

Neoplastic findings:

- Hepatocellular adenoma (38%) and hepatocellular adenoma/carcinoma combined (52%) were statistically increased in males from the high dose group.
- Statistically significant higher incidence of alveolar/bronchiolar adenomas (control 2%, 14%, 18%, 14%) and combined adenomas/carcinomas (control 2%, 20%, 20%, 22%) of the lung were noted in female low, mid and high-dose groups. The incidence of carcinomas was significantly increased in females only at 5000 ppm (12%). **No clear dose response in any of the lung tumours.** High background in males obscures any effect.
- A higher incidence of histiocytic sarcomas of the uterus was observed in females at all dose levels tested (0%, 6%, 14%, 12%). The increase was statistically significant and slightly above the historical control data for the 1500 and 5000 ppm dose groups.
- Renal tumours (2/50, 4%) were observed in females at the high dose only.
- Ovarian tumours were observed in females in the 1500 and 5000 ppm dose groups, no apparent dose response.

18 month Mouse

CD-1 mice

Amyes (1989)

OECD TG 451

Dose: 0, 10, 100 and 1000 ppm (0, 1.1, 11.21 and 115.9 mg/kg bw/d in males; 0, 1.4, 13.0 and 134.9 mg/kg bw/d in females).

Neoplastic findings:

No increase in the incidence of pulmonary carcinomas was seen.

In the PWG re-evaluation, these incidences were not statistically significant. The incidence of tumours in these groups was outside the historical control range from Life Science Research Ltd, the testing laboratory.

Table 45.10: Neoplastic histopathology lesions in the lung[a] of mice

Observation	Dose (ppm)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Dose (mg/kg bw/d)	0	1.11	11.2	116	0	1.4	13	134.9
# tissues examined	50	50	50	50	50	50	50	50
Alveolar/bronchiolar adenoma	9 18%	5 10%	11 22%	16 32%	4 8%	4 8%	5 10%	9 18%
Alveolar/bronchiolar carcinoma	3 6%	3 6%	3 6%	4 8%	1 2%	0	2 4%	2 4%
Adenoma/carcinoma combined	11 22%	8 16%	13 26%	18 36%	5 10%	4 8%	7 14%	11 22%

[a] Data derived from the PWG re-evaluation (Hardisty, 1997b).

(3) Mechanistic Studies

Several mechanistic studies were evaluated to address the mechanism of nasal and thyroid tumourigenesis such as *in vitro* metabolism studies, characterisation of protein binding and localisation in nasal tissues and cellular proliferation studies. Studies have also been performed to address the toxicity of acetochlor on rat liver and hepatocellular proliferation in mice. A study investigating the association of acetochlor and/or its metabolites with rat, mouse and human blood was also included in the CLH report. Of particular interest is the occurrence of nasal tumours in rats but not in mice.

The main points of note from these studies are given below:

(1) Green (1998c); Comparative metabolism between rat and mouse: Rats form glutathione conjugates, sulphoxide and sulphone derivatives of acetochlor. Mice form a series of glucuronides which are excreted rapidly in urine.

(2) Hotz & Wilson (1996a); Rat nasal cell proliferation:
Cell proliferation in nasal turbinate olfactory epithelium was significantly increased at 1750 and 5000 ppm acetochlor (1.3- to 1.5-fold and 1.5- to 2-fold, respectively), but not in respiratory epithelium.

(3) Hotz & Wilson (1996b); Mouse nasal cell proliferation:
acetochlor did not cause increased nasal olfactory or respiratory epithelial cell proliferation in mice.

(4) Lau et al., (1998); Quinone imine-protein binding, autoradiography in rat:
In rat nasal turbinate tissue, a dose-dependent formation of 3-ethyl, 5-methyl-benzoquinone imine-cysteine (EMIQ-cysteine) adducts was observed. Whole body autoradiography showed localization of radioactivity in gut, stomach contents, urinary bladder, highly perfused organs and in the nasal turbinates, adrenal and preputial glands.

(5) Lau & Wilson (1998a); Quinone imine-protein binding, autoradiography in mouse:
EMIQ-cysteine adduct formation was not observed in mice.

(6) Lau et al., (1998); Quinone imine protein binding and autoradiography acetochlor secondary sulphide in rat:
EMIQ-cysteine adducts were observed in nasal turbinate tissue. Autoradiography showed localization in nasal turbinates.

(7) Lau & Wilson (1998b); Quinone imine protein binding and autoradiography in Rhesus monkey:
EMIQ-cysteine adducts were not detected in nasal turbinate tissues.

(8) Morgan (1997); Nasal tumour mapping in rat:
Hyperplastic and preneoplastic/neoplastic lesions were located primarily in the nasal ethmoid turbinates in animals exposed to acetochlor, butachlor and alachlor.

(9) Green (1998b); *In vitro* metabolism (rat/mouse/ human):
acetochlor sulfoxide was rapidly hydroxylated to the para-hydroxy metabolite of acetochlor sulfoxide in rat and mouse olfactory microsomal fractions, but not in respiratory epithelial or liver fractions. Hydroxylation of acetochlor sulfoxide was not detected with human nasal tissue microsomes.

(10) Green (1998a); *In vitro* metabolism (rat/mouse/squirrel monkey):
The study evaluated metabolic rates of acetochlor to p-hydroxy-2-ethyl-6-methylaniline (pOH-EMA), a precursor to quinone imine formation. The overall conversion of acetochlor to pOH-EMA was slower in mice than rats, lowering potential to form reactive intermediates. Rates of all reactions were much lower in monkey nasal tissue than rat nasal or liver tissue, suggesting lower potential to form reactive intermediates.

(11) Green (2001a); *In vivo* protein adduct formation in the rat:
There were higher levels of acetochlor sulfoxide binding in the olfactory mucosa compared with respiratory epithelium. Sites coincide with the cellular location of xenobiotic metabolizing enzymes in the nasal passages.

(12) Green (2000); *In vitro* metabolism of acetochlor sulphoxide (rat/mouse/squirrel monkey/human):
The *in vitro* metabolism of acetochlor sulphoxide (major metabolite circulating in the plasma of rats) was highest in the rat and mouse nasal olfactory tissue microsomes. The hydroxylation of acetochlor sulphoxide could not be detected in human nasal tissue samples or in primate nasal samples.

(13) Hotz & Wilson (1996c); Characterization of thyroid toxicity and liver effects-time course (rats):

Effects on liver and thyroid weights, thyroid hormones and liver UDPGT activity were observed at 1750 and 5000 ppm, consistent with perturbation of thyroid-pituitary homeostasis via UDPGT-mediated clearance of T4. There was increased hepatic UDPGT activity.

(14) Ashby & Lefevre (1993); Acute liver toxicity (rats):
Dose-dependent depletion of hepatocellular glutathione leading to mild to marked necrosis at > 500 mg/kg, with slight stimulation of UDS at 2000 mg/kg. Increased serum AST and ALT were observed at 2000 mg/kg.

(15) Ashby & Lefevre (1994); Acute liver toxicity (rats):
Dose-dependent depletion of hepatocellular glutathione observed at >500 mg/kg, peaking 6-12 h post-dosing.

(16) Hotz & Wilson (1999); Hepatocellular proliferation (mice):
Incorporation of BrdU in mice treated with acetochlor was approximately doubled (0.15, 0.35, 0.38 at 0, 1000 and 5000 ppm, respectively).

(17) Macpherson & Jones (1991); acetochlor: blood binding study:
The binding of several ^{14}C acetochlor metabolites to erythrocytes from control rat blood was demonstrated to be a rat specific phenomenon. Rat metabolites, which bound readily to rat erythrocytes, had no affinity for erythrocytes from mouse and human.

(18) Zhang *et al*., (2010); acetochlor *sec*-methylsulfide: *in vitro* metabolism by olfactory turbinate and liver microsomes of male Sprague-Dawley rats:
New study submitted by industry – showed that rat nasal turbinate microsomes are much better than liver microsomes at producing the methyl sulphoxide quinone imine precursors. The report also showed that the production of quinone imine precursors via 2-ethyl-6-methylaniline (EMA) is a minor pathway.

(4) Proposed Mechanism of Action

The primary evidence for carcinogenicity is a treatment-related increased incidence of nasal olfactory tumours in all three chronic rat studies. The target site in the rat is the well vascularised mucosal tissue lining the nasal turbinates. The nasal turbinates are bony structures that project into the airway lumen in the main chamber of the nose. They increase the inner surface area of the nose, which is important for filtering, humidification and warming of inspired air. The mechanism for the formation of nasal olfactory epithelial tumours was determined to be local cytotoxicity secondary to quinone imine formation.

The postulated MoA proposes that:

(1) Acetochlor in the rat preferentially conjugates with glutathione (GSH) in the liver and is excreted in the bile. In mice, glucuronidation appears to be the favoured metabolic route with excretion via the kidneys.

(2) In the rat there is subsequent biotransformation by gut microflora acting on the conjugate.

(3) A series of sulphur-containing moieties including acetochlor *sec* methyl sulphide (ASMS) are produced, followed by absorption via enterohepatic circulation.

(4) Hepatic ASMS is S-oxidised to acetochlor *sec* methyl sulphoxide (ASMSO; acetochlor sulphoxide) and enters the systemic circulation (it is the major acetochlor metabolite in rat plasma). In contrast, very little ASMSO is found in mouse plasma.

(5) ASMSO is delivered to the nose, where it undergoes further biotransformation in the nasal microsomes.

(6) Metabolism *in situ* by the nasal olfactory epithelium results in formation of p-OH-ASMSO which undergoes oxidation to produce the highly reactive

dialkylbenzoquinone imine (DABQI) derivative. The hydroxylation process in human and primate nasal microsomes is considered by the Green *et al*. studies to be deficient or at very low activity.

(7) The reactive quinone imine readily reacts with cysteine residues of essential cell proteins in the olfactory epithelium, producing covalent adducts, causing oxidative stress and cytotoxicity. Note that this assumes exhaustion of cellular GSH since the presence of GSH would be protective against this process.

(8) Regenerative cell proliferation appears in response to cellular necrosis/injury in the olfactory region.

(9) A sustained cytotoxicity and cell proliferation results in metaplasia of the olfactory epithelium (indicative of death of olfactory cells and replacement of these cells).

(10) Sustained stimulation of cellular proliferation eventually leads to fixation of spontaneous mutations and tumour formation.

Important points:

- The polypoid adenomas in the nasal mucosa are only observed in rats, not mice.
- Rats appear to favour glutathione conjugation and biliary excretion of acetochlor metabolites.
- Mice appear to favour glucuronidation of acetochlor and excretion via the kidneys.
- Gut microflora are assumed to further act on acetochlor glutathione conjugates and produce acetochlor *sec* methyl sulphide (ASMS).
- Enterohepathic circulation ensures significant ASMS is produced which is then metabolised in the liver to give the sulphoxide derivative (acetochlor *sec* methyl sulphoxide; ASMSO).
- ASMSO is the major plasma metabolite of acetochlor in rats, much smaller quantities are found in mice.
- The generation of reactive quinone imines from EMA is much less than those generated from ASMSO.
- Major questions remain as to the metabolic potential of the nasal epithelium from different species, e.g. the rate of para-hydroxylation in monkey was 4% of the rate in rats (Green, 1998a).
- The hydroxylation of ASMSO on the para position of the phenyl ring is key to the ultimate formation of reactive quinone imines within the nasal epithelium.
- This process is facilitated by cytochrome P450 enzymes, but is it specific to the nasal epithelium of rodents? Cyp 2E1 is known to produce reactive quinone imines in the liver of man in cases of paracetamol overdosing.
- The nasal epithelium is particularly efficient (more so than liver) at metabolising ASMSO.
- Whole-body autoradiography studies established that sulphoxide residues accumulate and persist in the olfactory epithelium of rats. The sulphoxide side-chain is retained.
- Alachlor metabolism is probably similar to that for acetochlor; the older studies could not distinguish if the reactive quinone imines arose from EMA or ASMSO. The end result is the same.

The Importance of Glutathione

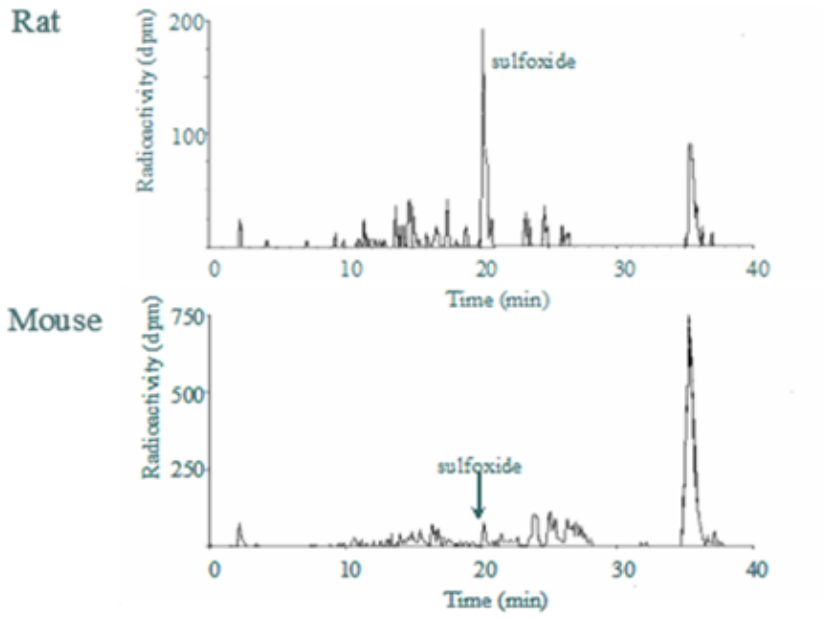
Glutathione is pivotal in this process. It seems to have a dual role in preventing the cytotoxicity of acetochlor, through detoxification and the reduction of oxidized groups in critical macromolecular targets. Chloroacetanilides preferentially react with glutathione (GSH) and therefore cause depletion of this protective nucleophile. Such depletion may be of particular concern for tissues with relatively low levels of endogenous GSH, rendering them more susceptible to the toxic action of acetochlor metabolites or other types of reactive intermediates. The metabolism of acetochlor in the rat changes with increasing dose-levels. At low dose levels of acetochlor, endogenous levels of glutathione will attenuate, if not prevent, the toxicological response. This detoxification pathway would remove low levels of any DABQI precursors by conjugation with glutathione, minimizing the formation of toxic quinone imine and/or removing the quinone imine. Exposure to acetochlor at a sufficiently high dose to saturate this detoxification pathway, results in a depletion

of target organ glutathione levels and sufficient quantities of quinone imine accumulate to the extent that toxicity becomes evident.

Why are there no Nasal Tumours in Mice?

In mice, acetochlor is metabolised primarily to a series of glucuronides which are excreted directly into the urine and thus bypass to a large extent the key steps (e.g., glutathione conjugation and enterohepatic circulation) that lead to the formation of the sulphide / sulphoxide metabolites. In contrast to the results with rats, only very low levels of the sulphoxide metabolite were detected in the plasma of mice after administration of acetochlor. Thus the acetochlor *sec* methyl sulphide (ASMS) may not be available to the mouse liver in sufficient quantity because the major route of acetochlor metabolism in the mouse is through glucuronide conjugation and renal elimination.

Figure: Plasma metabolites in rats and mice 17 hours after a single oral dose of ^{14}C acetochlor (200 mg/kg). Green *et al*., (1998), The comparative metabolism of acetochlor in rats and mice (DAR B.6.1.12)

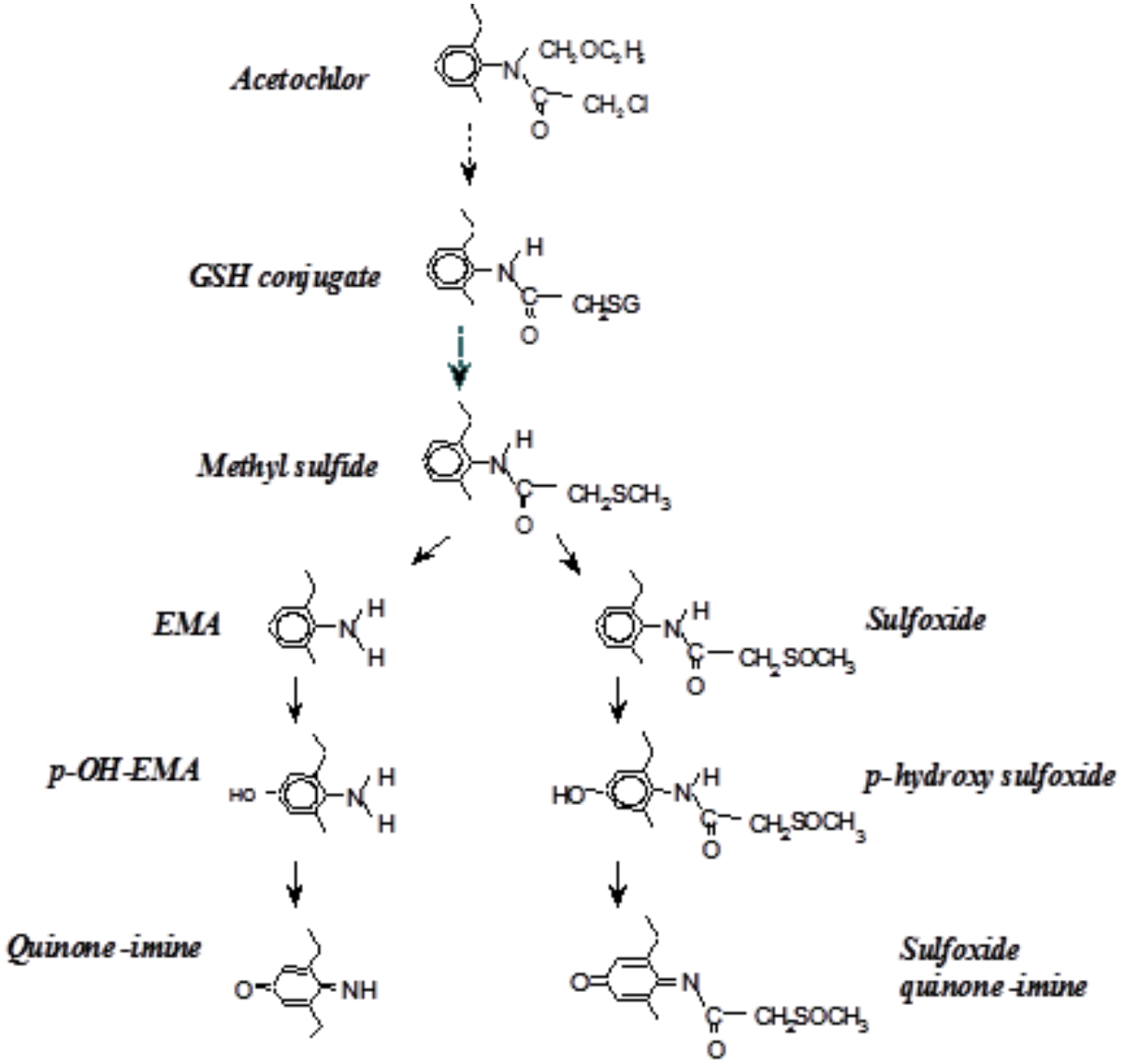


The enzymatic studies on nasal epithelia suggest that mice would be susceptible to nasal tumours if a sufficient quantity of ASMSO was available to the olfactory epithelium microsomal system. Mice have a similar complement of nasal cytochromes responsible for hydroxylase activity and the rate of para-hydroxylation of ASMSO in olfactory microsomes is similar in rats and mice, *the key substrate (ASMSO), is not produced in significant quantities in the mouse liver to allow conversion to quinone imine derivatives in the nasal epithelium*. The absence of quinone imine binding is also consistent with the negative autoradiography studies with the mouse and the negative nasal tumour results seen in the mouse chronic studies.

Overview of the metabolic fate of acetochlor

Two pathways have been reported (see Figure, next page) for production of reactive species, the original pathway proposed for the structural analogue alachlor (left branch of diagram) which involve formation of EMA, hydroxylation of EMA to the para-hydroxy aniline derivative pOH-EMA and the subsequent production of the reactive quinone imine species. Further investigations with acetochlor suggest an activation pathway in which the sulphoxide metabolite plays the major role (right branch of figure) in rats.

Figure: The metabolism of acetochlor to reactive DABQI. The left branch represents the originally proposed pathway based on alachlor data while the right branch represents the predominant pathway for acetochlor (adapted from Green *et al*., 2000)



Could there be sufficient substrate in monkeys and humans to be of concern?

In monkeys, the plasma metabolic profile for acetochlor was not determined. However, analysis of urine from studies of acetochlor metabolism in monkeys indicates that monkeys produce high levels of metabolites derived from glutathione conjugation and metabolites via the mercapturic acid pathway (Purdum and Livingstone, 1983; Kurtzweil, 2003). However, these metabolites are excreted primarily via the urine rather than in the bile, due to the higher molecular weight threshold for biliary excretion in primates compared to rats (Millburn, 1975; Williams, 1971) and thus would not be subjected to the extensive enterohepatic circulation and metabolism by gut flora that occurs in the rat.

Conclusions:

The DS has presented a very thorough and extensive explanation of the metabolic fate of acetochlor in the CLH report. They have discussed the data consistent with the MoA for the induction of nasal tumours in rats and outlined the important events in the process leading to quinone imine adduct formation. The DS has also made many comparisons with the structurally related molecule alachlor, particularly with regard to the postulated cytotoxicity MoA in rats (generation of ROS → DNA damage → tissue damage → cell proliferation → olfactory nasal tumours). The DS also has discussed a paper by Coleman *et al*. (2000) investigating both rat and human liver microsomal activities which outlines the possibility that EMA is also a relevant

precursor. The DS noted that the analysis of quinone imine precursors in this study cannot distinguish whether they originated from the sulphoxide metabolite or EMA. This issue was highlighted by the DS because the *in vitro* work of Coleman *et al.* (2000) indicated that human liver microsomes are as effective, if not more so, than the rat in forming EMA from acetochlor.

The sequence of events leading to the formation of nasal olfactory tumours appears to be specific to the rat. Mechanistic studies indicate that the reactive agents, quinone imine derivatives, have higher rates of formation in the nasal tissue of rats than in mice, primates or humans.

The potential for acetochlor to cause nasal tumours in humans cannot, however, be ruled out for several reasons:

- Production of the metabolite EMA with the capacity to undergo transformation to a quinone imine is possible for humans (Coleman *et al.,* 2000).
- Human liver microsomes oxidatively dealkylate acetochlor (to CMEPA) at a similar rate to that of rat liver microsomes and the subsequent metabolic rates of CMEPA to EMA with human liver microsomes exceed those of rat liver microsomes.
- This is a minor pathway though, but it does suggest the formation of reactive metabolites could occur in humans.
- 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.
- What quantity of EMA might be produced or if it could be available to the systemic circulation is unknown.
- How well the nasal epithelium could metabolise EMA in humans is unknown. In rats the preferred substrate is ASMS and very little EMA appears to be involved in quinone imine production.

The weight of evidence indicates that rats are more sensitive than humans and although it is unlikely that sufficient concentrations of active metabolite would be achieved to initiate the chain of events terminating in nasal tumours, it cannot be ruled out that the MoA is relevant to humans.

(5) Comparisons with Alachlor and other Chloroacetanilides

A comparison with other chloroacetanilides is useful in reaffirming the effects seen with acetochlor. Both alachlor and butachlor confirm the susceptibility of the rat nasal turbinates to chemical induced tumours via reactive quinone imines.

Alachlor is also currently listed in Annex VI of the CLP Regulation. The general MoA for the rat nasal tumours produced by acetochlor is essentially the same as that for alachlor, a close structural analogue. However, unlike acetochlor, alachlor is classified for carcinogenicity (ECBI, 2002). It also displays a variety of tumours in the rat, involving nasal epithelium, thyroid and stomach. In addition, lung tumours are also observed in mice.

The chloroacetanilide herbicides, including acetochlor, alachlor, propachlor, butachlor and metolachlor have overlapping, but not identical, tumour profiles. Butachlor also induces nasal epithelial tumours and thyroid follicular cell tumours.

Summary of tumour findings for related chloroacetanilides (Table 46, CLH report)

TUMOUR TYPES	**EPA CANCER CLASSIFICATION**
Rat nasal epithelial, thyroid follicular, rare mixed gastric tumours.	Likely to be a human carcinogen at high doses but not low doses. Margin of exposure (MOE) approach. (EPA, 1997)

Rat nasal epithelial cell, thyroid follicular cell, rare stomach, and renal cortical tumours.	Likely to be a human carcinogen. MOE approach for all tumours except renal-use linear low-dose approach for renal tumours. (EPA, 1999)
Rat thyroid c-cell, ovarian granulosa/theca cell tumours. Mouse hepatocellular tumours.	Likely to be a human carcinogen. Linear low-dose extrapolation for ovarian tumours. (EPA, 1997)
Rat hepatocellular tumours.	Group C (probable human carcinogen). MOE approach for liver
Rat hepatocellular tumours (males).	Group C (possible human carcinogen). Linear low-dose extrapolation.

Acetochlor, alachlor and butachlor may be grouped together based on a common end-point (nasal turbinate tumours in rats) and a known mechanism of toxicity for this endpoint. All three compounds produce tumours of the nasal olfactory epithelium in rats by way of a non-linear, non-genotoxic MoA that includes cytotoxicity of the olfactory epithelium, followed by regenerative cell proliferation of the nasal epithelium that can then lead to neoplasia if cytotoxicity and proliferation is sustained.

(6) Relevance of different tumour types

(a) SD Rat: Testicular Leydig Cell tumours.
These tumours exhibited a non-statistical dose-related increase in treated males over the control group in one rat study (Ahmed, 1983a): control (2/70), low-dose (4/70), mid-dose (4/70) and high-dose (7/70). No increase in incidence was observed in mice. The background incidence of Leydig cell tumours (LCT) in Sprague Dawley rat was reported to be in a range from 5% to 10% (Cook *et al*., 1999 and Mati *et al*., 2002). It appears from the text that the DS considers these to be ***not relevant*** to man. The RAC concurs with this view.

(b) SD Rat: Hepatic tumours.
The DS explains how these tumours were interpreted by several other international regulatory bodies who all recognised the increased incidence at the highest dose in the presence of excessive liver toxicity. Taking into account historical control data and mechanistic studies as well as the evaluations of other professional groups, the DS is of the opinion that liver tumours in rats, while showing a treatment-related increase in both sexes, were only observed at 5000 ppm, a dose that exceeded the MTD and are ***not relevant*** to man in this case. The RAC concurs with this view.

(c) SD Rat: Thyroid Follicular tumours.
The thyroid follicular cell tumours are considered to be related to treatment. They are ***not considered to be relevant*** to human health, based on relatively low incidences and evidence for disruption of thyroid and pituitary homeostasis secondary to increased clearance of thyroid hormones by increased hepatic UDPGT activity. The RAC concurs with this view.

(d) SD Rat: Pituitary tumours.
The DS compared the incidences of pituitary tumours (carcinomas) from the Naylor (1986) study with the other rat studies (Ahmed 1983a; Broadmeadow, 1988). The control groups from the other studies had a higher incidence than the females of the high dose group showing the effect. Coupled with the fact that these animals display very large spontaneous incidences of pituitary adenomas, the DS considers these pituitary tumours are ***not related to treatment*** with acetochlor. The RAC concurs with this view.

(e) SD Rat: Femur tumours.
The original diagnosis is considered erroneous and ***not indicative of a neoplastic effect***. The RAC concurs with this view.

(f) SD Rat: Stomach tumours.
Basal cell tumours were initially detected in the stomach of one male and one female at the dose of 1750 ppm in the study by Broadmeadow, (1989). Subsequently it was recognised that the lesions in the forestomach were squamous cell carcinomas. Historical control data showed that these tumours are not a common finding (global incidence of 0% in 16 studies of 104 week conducted from 1983 to 1989 at Huntington Life Sciences). In addition, there were no pre-neoplastic lesions present (Hardisty, 2001b) which are a hallmark of chemical induction of neoplasia in the rodent forestomach. The DS concludes that these tumours were **spontaneous neoplasms unrelated to acetochlor administration**. RAC concurs with this view.

(g) CD-1 Mouse: Histiocytic sarcomas.
Histiocytic sarcoma is a spontaneous tumour in aged mice, and the incidence rapidly increases after 18 months, especially in females. The incidence varies greatly according to strain, age and sex of the mice examined. Reported incidence for mice older than 24 months is 10.4% in C57BL/6J female and 22.2% in C57BL/6J male, compared to 0.6% in BALB/c female, 0.7% in BALB/c male and 0.8% in CBA females for the same age group (Frith *et al*., 1990, 1993, 2001).

Histiocytic sarcoma is a systemic tumour that is commonly noted in the liver of males, and the liver or uterus of females. This neoplasm is not a primary tumour that arises from the uterus, it is considered one of the nonlymphoid haematopoietic sarcomas with histogenesis from a cell of the mononuclear phagocyte system or monocyte/macrophage lineage. Its aetiology is unknown.

A higher incidence of histiocytic sarcomas was reported in female CD-1 mice from the 1500 and 5000 ppm groups of the Ahmed (1983b) mouse oncogenicity study. However, there was no clear dose-response relationship. Historical control data for the testing laboratory was not available. However, data from other testing facilities indicated that the incidence of this type of tumour in female mice is quite variable, occurring in up to 18% of tested animals.

Incidence of histiocytic sarcomas in females from a 23-month mouse feeding study with acetochlor (Ahmed, 1983)

	Interim Sacrifice				**Main Study**			
Dose Level (ppm)	0	500	1500	5000	0	500	1500	5000
No. of animals	10	10	10	10	50	50	50	50
Histiocytic sarcomas	0	0	1	0	0 (0%)	3 (6%)	7** (14%)	6* (12%)

Incidence of histiocytic sarcomas in females from an 18-month mouse feeding study with acetochlor (Amyes, 1989)

	Interim Sacrifice				Main Study			
Dose Level (ppm)	0	10	100	1000	0	10	100	1000
No. of animals	10	10	10	10	50	50	50	50
Histiocytic Sarcomas	0	0	0	0	2 (4%)	1 (2%)	0 (0%)	5 (10%)

Historical control data for histiocytic sarcomas in female CD-1 mice (Table 48 in the CLH report)

Source	Approx. time period	Study duration	No. studies	Incidence (%)	
				Range	Mean
Charles River (1995)	1981-1991	18 months	12	0-10.0	2.2
		21 months	3	0-6.0	3.8
		24 months	8	0-10.0	3.8
Charles River (2005)	1987-2000	78 weeks	25	0-15.0	2.7
		91-104 weeks	29	0-18.3	6.5
Inveresk Research (1996)	1990-1993	18 months	5	0-2.0	0.8
		24 months	8	0-10.0	4.0

Historical control incidence data reported in contemporary studies for lung tumours in female CD-1 mice

Source	Approx. time period	Study duration	No of 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

* testing laboratory that performed the 18 months mice study

In the second long-term mouse study by Amyes (1989), there was a 10% incidence in the high dose female group. The DS presented the views of the PWG and the US EPA, a European expert pathology and toxicology panel and the ECBI SE. These groups concluded that the histiocytic sarcomas were either equivocal or generally unrelated to treatment, their incidences fell within historical controls or they were not indicative of any carcinogenic potential of acetochlor.

The DS makes a few noteworthy points:

- The histiocytic sarcoma is not considered to be a primary uterine sarcoma.
- There was a highly variable spontaneous incidence.
- An unusually low incidence in the Ahmed (1983b) concurrent control group (0% vs. 4% in the Amyes, 1989 study).
- No clear dose-response relationship.
- No increase in tumour multiplicity.
- No decrease in tumour latency.
- No pre-neoplastic findings.
- No significant increase in incidence ($p>0.05$) in the Amyes, 1989 study.
- No increased incidence of histiocytic sarcomas in any of the carcinogenicity studies with other closely related chloroacetanilides, such as alachlor.
- Incidence within the range of more recent historical control compiled from Charles River 2005 (1.67-18.33%).
- Incidence slightly above the more relevant historical control data from Charles River Laboratories (0 – 10%).
- Lack of evidence for direct genotoxicity of acetochlor.

The DS considers the increased incidence of histiocytic sarcomas in female mice at 1500 ppm and at 5000 ppm in the Ahmed (1983b) study to be treatment-related. Toxicity at the highest dose of 5000 ppm was excessive and tumours at this dose could be considered of no relevance to humans. The incidence of histiocytic sarcomas in the second mouse study is not considered treatment-related. This tumour type was not seen with butachlor or alachlor. Taking all these points together RAC agrees with the DS that the link with acetochlor treatment is weak. If the historical control ranges are accepted then the histiocytic sarcomas are unlikely to be treatment-related and are not of concern for human exposure to acetochlor. For example, a breakdown of the incidences from 24 × 104-week mouse studies from Charles River (2005) is shown below: top row = study number, bottom row = % incidence:

30	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**
6	8	7	2	7	18	3	6	12	4	8	8	11	7	5	0	0	6	12	17	10	7	11	8	4

Alternatively, the data does show an increase in neoplasm incidence with acetochlor treatment relative to concurrent controls in two independent studies and this is an effect that cannot be ignored. Using a weight of evidence approach, RAC considers these effects borderline **but relevant to human health**.

(h) CD-1 Mouse: Lung Tumours.

Statistically significant increases in the incidence of alveolar/bronchiolar adenomas and combined adenomas and/or carcinomas were noted in all groups of females from the Ahmed (1983b) mouse oncogenicity study conducted with acetochlor. The incidence of carcinomas was also significantly increased in females, but only at the top dose of 5000 ppm.

Incidence of lung tumours in Ahmed, (1983) 23-month mouse study

	Males				Females			
Dose Level (ppm)	**0**	**500**	**1500**	**5000**	**0**	**500**	**1500**	**5000**
# tissues examined	50	50	50	50	50	50	50	50
Adenomas (%)	7 (14)	10 (20)	11 (22)	5 (10)	1 (2)	7 (14)*	9 (18)*	7 (14)*
Carcinomas (%)	6 (12)	3 (6)	3 (6)	3 (6)	0 (0)	4 (8)	1 (2)	6 (12)**
Adenomas and/or carcinomas combined (%)	12 (24)	13 (26)	14 (28)	8 (16)	1 (2)	10 (20)**	10 (20)**	11 (22)**

Incidence of lung tumours in Amyes, (1989) 18-month mouse study

	Males				Females			
Dose Level (ppm)	**0**	**10**	**100**	**1000**	**0**	**10**	**100**	**1000**
# tissues examined	50	50	50	50	50	50	50	50
Adenomas (%)	9 (18)	5 (10)	11 (22)	16 (32)	4 (8)	4 (8)	5 (10)	9 (18)
Carcinomas (%)	3 (6)	3 (6)	3 (6)	4 (8)	1 (2)	0 (0)	2 (4)	2 (4)
Adenomas and/or carcinomas combined (%)	11 (22)	8 (16)	13 (26)	18 (36)	5 (10)	4 (8)	7 (14)	11 (22)

The incidence of spontaneous lung tumours in control CD-1 mice from 18/24-months studies which have been reported in the literature shows that lung tumours are a common spontaneous tumour in CD-1 mice with a highly variable incidence that complicates the evaluation of carcinogenicity bioassays. In addition, CD-1 mice appear to be highly susceptible to chemically induced lung tumours, a feature that may be linked to the high prevalence of the *Pas1* susceptibility allele in this strain of mouse (Manenti *et al*., 2003).

Historical control incidence data reported in contemporary studies for lung tumours in female CD-1 mice

Source	**Approx. time period**	**Study duration**	**No of 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	1990 – 1993	18 months	5	0-16.0	7.6	2-10.0	4.0	6.0-18.0	11.6

(1996)		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

* testing laboratory that performed the 18 months mice study

There was no historical control data available from the testing laboratory that performed the 23 month mouse study. According to the DS, the Fifth Report of the Cancer Assessment Review Committee (US EPA 2007b) determined that the 23-month (Study 1) mouse study showed weak evidence for increased benign lung tumours in females and that the 18-month study (Study 2) showed weak evidence for increased benign lung tumours in males. Other evaluations concluded that the results were treatment related.

The DS makes several interesting points when assessing the relevance of these lung tumours:

- Lung tumours are a common spontaneous tumour in CD-1 mice with a highly variable incidence and a high susceptibility to chemically induced lung tumourigenesis.
- Unusually low incidence in concurrent control group in females of the 23-month study, for adenoma (2% vs. 8% in 18 months mice study) and for combined incidence (2% vs. 10% in 18 months mice study).
- Incidence in females (23-month study), at all dose groups for adenomas and for carcinoma fall within (using uncensored data) the range of historical control reported for contemporary studies.
- Incidence in females (23-month study), at 1500 and 5000 ppm groups for adenoma, and for carcinoma at 5000 ppm (using censored data) fall outside the range of historical control reported for contemporary studies.
- In males of 18-month mice study, adenoma incidence at 100 and 1000 ppm and combined incidence at 1000 ppm were above the more relevant HCD from the testing laboratory Life Science Research Ltd.
- Inconsistent dose-responses between the two studies.
- Lack of pre-neoplastic lung lesions in the 23-month study (while the 18-month study showed an increase in bronchiolar hyperplasia).
- Lack of evidence of direct genotoxicity of acetochlor.
- Shorter term studies did not identify lung as a target organ in mice or any other species

The DS, in considering all of the above points, suggests that the increased incidence of lung tumours is considered treatment-related, although the association is weak. Without mechanistic studies to determine the MoA, the relevance to humans cannot be ruled out. The RAC endorses this view.

(i) CD-1 Mouse: Renal tumours.

In the 23-month mice study (Ahmed, 1983b) a higher incidence of renal tumours was observed in females at 5000 ppm (2/50, 4%). There was no evidence for an increased incidence of renal tumours in males from this study, or in either sex in the other chronic mouse study. No increased incidence was observed in rats. RAC considers these effects are not relevant to human health.

(j) CD-1 Mouse: Ovarian tumours.

The DS considers that the ovarian tumours are not related to treatment, based on the following:

(1) lack of precursor lesions in this study or the other rodent studies on acetochlor;
(2) lack of tumour multiplicity or bilateral tumours;
(3) lack of linear dose-response;
(4) low incidence; and
(5) within HCD.

(k) CD-1 Mouse: Hepatic tumours.
There was an increase of hepatic adenomas and carcinomas in males and females at the highest dose tested (5000 ppm) in the 23-month mouse study by Ahmed, (1983b). This was statistically significant for males only.

Tissue	Observation	Dose (ppm)							
		Males				Females			
		0	500	1500	5000	0	500	1500	5000
	mg/kg bw/day	0	75	227	862	0	95	280	1084
Liver[a]	*# tissues examined*	50	50	50	50	50	50	50	50
	Hepatocellular adenoma	8	7	10	19*	2	0	1	5
	Hepatocellular carcinoma	4	4	4	9	0	0	0	2
	Hepatocellular adenoma/carcinoma	12	10	14	26**	2	0	1	7

Most of the evaluation reports that commented on the hepatic tumours also considered the role of excessive toxicity to the liver and questioned the relevancy of these findings. Doses of nongenotoxic chemicals which induce cytotoxic liver necrosis have been associated with hepatocellular proliferation leading to the induction of hepatic tumours if the dosing is frequent and is maintained for long periods.

Mechanistic studies evaluating the acute effects of acetochlor on rat liver showed that hepatic toxicity was associated with depletion of hepatocellular glutathione reserves at doses at which a slight increase in UDS are observed. The data provide some evidence that the UDS is secondary to depleted glutathione, rather than a consequence of direct genotoxicity. Hepatocellular proliferation was also evaluated in mice administered acetochlor in the diet for 90 days. At higher doses, BrdU incorporation was shown to increase during this time, providing some evidence that a non-genotoxic, proliferative mechanism may be involved in the formation of these hepatocellular tumours. The liver tumours are considered by the DS not to be relevant to humans. The RAC endorses this view.

Comments received during public consultation

Extensive comments were received from industry arguing against the classification proposals. An in-depth response has been written by the DS in the RCOM document and is supported by the RAC.

Two Member States commented during the public consultation. Both supported the classification proposals for human health submitted by the DS.

Assessment and comparison with the classification criteria

Comparison of acetochlor carcinogenicity data with the corresponding classification criteria is not simple because the data are complex, some results are borderline and the criteria can be interpreted in different ways.

First and foremost there are statistically significant increases in tumour incidences for several types of tumours in two different species. This is generally taken as positive evidence of a carcinogenic effect. The primary effect is treatment-related nasal tumours in rats with weak supporting evidence from mouse carcinogenic data in the lung and histiocytic tumours in the uterus.

Category 1A

As there is no epidemiological evidence regarding the carcinogenicity of acetochlor in humans, a classification in Category 1A is not appropriate.

Category 1B versus Category 2

It is therefore necessary to decide whether to classify acetochlor in Category 1B or Category 2. Since an increase in tumour incidence has been observed in two species an argument for classification in category 1B can be made. However, on consideration of the available data, there are a number of factors that indicate that classification in Category 2 may be more appropriate:

- The available genotoxicity data on acetochlor do not support a genotoxic MoA for tumour induction.
- Extensive mechanistic data suggests that acetochlor is carcinogenic in nasal tissues by a secondary mechanism with a practical threshold.
- Treatment-related increased incidence of nasal olfactory tumours is observed in all 3 rat long-term studies, at dose levels ≥ 1000 ppm (~ 54 mg/kg/day) and in the rat 2-generation reproductive toxicity study.
- High doses were required to induce some tumours, and they were generally above the MTD in the experiments conducted. Associative regenerative hyperplasia leading to tumour development cannot be ruled out in several cases.
- Excluding the nasal polypoid adenomas, the increased incidences of other tumour types are often within the normal historical control range.
- Supporting evidence for a carcinogenic effect comes from two tumour types in the mouse but the incidences are weak and inconsistent.
- The mechanism of tumour formation has been extensively explored in the rat and there is a clear species difference with respect to metabolites (and tumour susceptibility) in the plasma of rats and mice.
- Quinone imine precursors that can potentially react with cellular macromolecules and deplete cellular glutathione can be formed in the nasal turbinates of rats, mice, and monkeys.
- Both monkey and human nasal tissues are unable to para-hydroxylate the sulfoxide metabolite of acetochlor, which is the primary precursor to quinone imine in the rat. However, a low rate of para-hydroxylation (approximately 4% of the rate in rats) was observed when monkey nasal tissue was incubated with EMA.
- Human liver microsomes are capable of the EMA pathway and both ASMSO and EMA can form quinone imines.
- The activities of the enzymes involved and the particular metabolites formed can differ between species.
- There is no data in monkeys with respect to the plasma profile of metabolites following acetochlor administration. There is data for rat and mouse.
- No data with regard to how acetochlor is metabolised in humans. The relevance to humans cannot be excluded.

In reference to rat nasal polypoid adenomas, the existence of a secondary mechanism of action (quinone imine adduct formation → cytotoxicity → regenerative hyperplasia → tumours) with the implication of a practical threshold above a certain dose level (amount of available acetochlor *sec* methyl sulphoxide) indicates that consideration of Category 2 classification is appropriate.

No Classification
The weight of evidence indicates that acetochlor has a strong potential to induce nasal tumours in rats but not in mice thus indicating significant species differences must exist. The argument for no classification may be based on several points:

- Mechanistic studies have demonstrated a non-genotoxic, species-specific MoA for these tumours.
- These tumours were predominantly benign, did not progress significantly in the second year of the studies, and were not life-threatening.
- The MoA responsible for the rat nasal tumours is not relevant to humans on the basis of large quantitative species differences in toxicokinetics and/or toxicodynamics.
- p-OH sec-methyl sulfoxide is the primary route of quinone imine formation with little contribution from p-OH EMA in rats. EMA was not identified in rat plasma.
- The fact that EMA may possibly be metabolised to trace levels of quinone imine formation in humans does not necessarily mean that it should be considered carcinogenic to humans.
- The main plasma metabolite in rats is ASMSO which is converted to the p-OH sec-methyl sulfoxide in the rat nasal turbinate epithelial tissue. Much lower levels of ASMSO are found in mice and mice are not susceptible to the formation of nasal tumours.
- An increase in nasal polypoid adenomas was observed with ASMSO in a 1-year rat feeding study.
- Arylamidase experiments measuring the conversion of the sulphide to EMA were conducted in the absence of NADPH and thus in the absence of any competing cytochrome P-450 oxidative reactions. However, even under these artificial conditions which would be expected to maximize the production of EMA, no EMA was detected in monkey nasal tissue.
- There was no evidence of quinone imine-protein adducts, nasal cell proliferation or nasal tumours in mice, even though mouse nasal and liver tissues produce much higher levels of EMA and p-OH-EMA than monkey nasal tissue.
- The p-hydroxylation rate of EMA in primate nasal tissue is substantially (~24-fold) less than in rats.
- The amount of quinone imine produced in rats is significantly higher than in mice, which do not develop nasal tumours, and at least several orders of magnitude higher than in primates and humans.
- Low levels of quinone imine produced in the mouse were not sufficient to lead to detectable accumulation in nasal tissues, formation of DABQI-protein adducts, nasal cell proliferation or nasal tumours.
- The amount of quinone imine that theoretically may be produced in humans (from either sulphoxide or EMA) would be at least several orders of magnitude lower than in mice.
- Based on the substantial quantitative species differences, it can be concluded that rat nasal tumours produced by acetochlor are not relevant to humans and should not trigger classification.

Conclusion
Based on the available evidence the DS concluded that acetochlor best fits the criteria for classification as Carc. 2; H351. The RAC endorses the opinion of the DS. These conclusions are drawn from findings of rare nasal olfactory epithelial tumours in the male and female rat (where relevance to humans cannot be ruled out) and are supported by weak evidence for benign lung

tumours in male and female mice as well as histiocytic tumours in the uterus of mice (MoA unknown).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

(1) Fertility:
The effects of acetochlor on fertility have been investigated in three multigenerational studies in rats. The most recent study by Milburn (2001) was considered to be the only one acceptable by the DS from a regulatory standards point of view. The other studies provide useful supporting data.

Reproductive toxicity studies (fertility) investigating the effects of acetochlor

Study	Comments	Reference
Rat 2-generation; strain: CD (SD) IGS BR (Sprague-Dawley)	Doses (mg/kg bw/d): 0, 20/22, 61/68, 181/207, male/female Doses (ppm): 0, 200, 600, 1750 ***Acceptable*** GLP Guidelines: US EPA 83-4 OPPTS 870.3800 ≈ OECD 416 (2001)	Milburn G., 2001; DAR B.6.6.1; CLH report section 4.11.
Rat 2-generation; strain: CD (SD) IGS BR (Sprague-Dawley)	Doses (mg/kg bw/d): 0, 1.4/1.7, 14/17, 128/175, male/female, Doses (ppm): 0, 18, 175, 1750 ***Considered Not Acceptable*** (missing data for many reproductive indices) non GLP Guidelines: US EPA 83-4 ≈ OECD 416 (1983)	Willoughby C.R., 1989; DAR B.6.6.2; CLH report section 4.11.
Rat 2-generation; strain: COBS CD	Doses (mg/kg bw/d): 0, 30/45, 89/130, 325/442, male/female, Doses (ppm): 0, 500, 1500, 5000 ***Considered Not Acceptable*** (missing data for many reproductive indices), severe body weight loss at highest dose, greatest in F1 generation (19-39%), less in the F0 generation (10 – 25%) GLP Guidelines: US EPA 83-4 ≈ OECD 416 (1983)	Schardein, J.L., 1982; DAR B.6.6.3; CLH report section 4.11.

Study 1 (Milburn, 2001):
In a 2-generation study in rats (Milburn, 2001), treatment-related decreases in the number of implants (11% for F0, 13% for F1), the number of live pups (-16% on postnatal day 1 in litters from F1 parents), and the number of live and dead pups (-10% for F0 and -16% for F1) was found at 1750 ppm. A decreasing trend in these parameters was also observed at the two lower doses for both generations; see the section "Supplemental information - In depth analyses by RAC" in Annex 1 for details.

Statistically significant changes in the weight of some reproductive organs were also seen during this study. A decrease in the absolute weight of the ovaries was observed from 200 ppm in F0 parents (-25%) and at 1750 ppm in F1 parents (-10%), along with a significant reduction in the relative ovarian weight in the F0 parents only (-12%). The decrease in the relative ovarian weight of the F1 parents is not considered to be significant because no dose response is observed. However, there were no treatment-related effects on ovarian histopathology or fertility. The effect on ovarian weight was limited to the high dose animals and occurred in the presence of systemic toxicity, such as significant decreases in body weight (-10% for F0, -14% for F1) and body weight gain (-19% for F0, -11% for F1) during the pre-mating period, and increases in liver, kidney and thyroid weights, and nasal proliferative lesions (hyperplasia and polypoid adenomas).

Left epididymis absolute weight decreased at 600 ppm in F0 parents. At 1750 ppm there was decreased relative weight of the uterus and decreased absolute weight of the uterus, epididymides, testes and seminal vesicles in F0 parents. In F1 parents an increase in the relative weight of epididymis and decrease in the absolute weight of right epididymis, testes and seminal vesicles was observed at 1750 ppm.
Study 2 (Willoughby, 1989):

In a second 2-generation study in rats (Willoughby, 1989 - considered not acceptable by the DS due to guideline deviations), significant changes in the weight of some reproductive organs were observed at 1750 ppm in F1 parents, such as increases in the relative weight of epididymides, seminal vesicles and testes. These effects, in the presence of maternal toxicity, were not observed in F0 parents.

No treatment-related decrease in absolute and relative ovarian weight and no treatment-related changes in the histopathology of the ovaries, or mating and fertility indices, were observed.

This study was originally considered deficient because it did not include the newer endpoints added by the 2001 OECD guidelines (e.g. sperm parameters and developmental milestones). The absence of those parameters has no impact on the validity of the remaining data, such as ovarian weight, fertility, number of implants, litter size, etc.

Study 3 (Schardein, 1982):
Similarly, another two-generation study in rats (Schardein, 1982 - also considered not acceptable by the DS) found a decrease in the absolute weight of testes at 5000 ppm from both the F0 and F1 generations. However, there was no decrease in the relative testicular weights, thereby questioning the significance of the absolute testicular weight findings. There was also a decrease in the absolute ovarian weight (-35%) in the F1 generation with little change in the relative weights (no data was reported for the F0 parents). There were no ovarian histopathological findings of concern. There was a significant decrease in the number of live pups at birth in the F1b litter at 5000 ppm (outside of the historical control range). Significant systemic toxicity in treated females was also observed at all dose levels in this study.

Other Studies:
No treatment-related decrease in absolute or relative ovary weight or histopathological changes in the ovaries were noted in two 13-week and one 24-month studies in the rat. Treatment-related decreases in absolute ovarian weight, but not in relative ovary weight, were observed in the two other 24-month studies in the rat but were not accompanied by histopathological changes and were thus considered to be related to decreased body weights.

The DS concludes there was no evidence from the available reproductive toxicity studies in rats to warrant support for classification due to fertility impairment. The significant decrease in ovarian weight observed in one study in the presence of significant maternal toxicity is not considered indicative of a specific reproductive effect or relevant for classification.

The DS did not consider the effects on the dog testes noted in the 1-year dog studies for fertility classification. In these studies, severe toxicity to the testes was seen at doses from 40-50 mg/kg bw/day. The effects noted in a second species are however, in RAC's view, suitable grounds to warrant consideration of reproductive (fertility) toxicity classification and further discussion may be viewed in the section "Supplemental information - In depth analyses by RAC".

(2) Development:
In a developmental toxicity study in rats (Brooker *et al.*, 1989a), an increase in the number of post-implantation losses and in the early/total resorptions were observed at 600 mg/kg bw/d though both effects were in the range of historical controls. At this same dose level there was a decrease of the foetal weight and a slight increase above the historical controls in the incidence of the skeletal anomalies and in the incidence of reduced ossification of the sacrocaudal vertebral arches. The latter also occurred at 150 mg/kg bw/d but in this case the value was within the range of historical controls. The incidence of variant sternebrae was also significantly increased at 600 mg/kg bw/d. However, the developmental effects at 600 mg/kg bw/d occurred in the presence of maternal toxicity manifested by mortality, clinical signs of toxicity and decrease of body weight, body weight gain and food consumption.

In a developmental toxicity study in rats (Rodwell, 1980), dwarfism was observed in one litter at 400 and 200 ppm but the incidence fell within the range of historical controls. No

treatment-related effects were observed in the other two developmental toxicity studies in rabbits (Adam, 1986 and Brooker *et al.*, 1989b).

Other effects indicating potential developmental toxicity were observed in offspring of a 2-generation study in rats (Milburn, 2001). Toxicity was manifested by significant decrease of live plus dead pups in F1 litters at 1750 ppm and in F2 litters from 600 ppm, and also a reduction in the number of live pups at 1750 ppm in F2 litters. A delay in the time of vaginal opening was also observed at 1750 ppm in F1 pups and a reduction in the anogenital distance was seen in males in the F2 generation. There were significant variations of the litter weight in F1 pups from 600 ppm and in F2 pups at 1750 ppm and organ weight variations, bodyweight and bodyweight gain reductions in F1 pups at 1750 ppm and in F2 pups from 600 ppm. These findings occurred in the presence of maternal toxicity from 600 ppm, manifested by bodyweight reduction, changes in the weight of some organs and the occurrence of nasal hyperplasia and polypoid adenomas.

In addition, a 2-generation toxicity study in rats (Schardein, 1982), showed a significant decrease in the number of live pups at birth in the F1b litter (outside of the historical control range). These effects were observed in the presence of maternal toxicity.

According to the DS, none of the studies showed evidence of malformations and the observed foetal findings were considered to be of a secondary, non-specific consequence of the maternal toxicity rather than a direct effect on development. Acetochlor was not considered teratogenic to rats and rabbits by the DS.

Comments received during public consultation

Two Member States commented during the first public consultation. Both supported the no classification conclusion for reproductive toxicity submitted by the DS.

Following discussions at RAC-30 (September 2014), acetochlor was subjected to a second, targeted public consultation focussing specifically on the reproductive toxicity hazard class. Three comments were received, one from Industry, one from an MSCA and one from an NGO.

The MSCA considered that the data for acetochlor do not warrant classification for fertility effects. Their main points are summarised as follows:

- Effects on fertility in the 2-generation study in rats are secondary to parental toxicity.
- Testicular effects in dogs of the high dose group are also secondary to parental toxicity, i.e. severe neurological disturbances, decreased bodyweight and food consumption, all necessitating the sacrifice of 2 out of 5 males in this group.
- No effects on testes were observed in rats and mice in repeated dose studies and there was no evidence for very large reductions in sperm count (>90%) that would be expected to have an effect on fertility.

Industry replied with a comprehensive document in response to the RAC proposal to classify acetochlor as Repr. 2. In their response they addressed the three main endpoints of concern, i.e. testicular toxicity in the dog after one year of treatment, decrease in rat ovary weight, and a decrease in the number of implantations in a rat multigeneration reproductive toxicity study. Industry also suggested that a further review be conducted of the histology slides of the testes and epididymides from the 90-day, 119-day, and two 1-year dog studies by a formal Pathology Working Group that includes several experts in the histopathology of reproductive organs. However, RAC was of the view that this was not required because it is difficult to see what further data this exercise might provide other than reaffirming the results of Creasy (2003) with respect to the testicular findings in dogs at doses that are below those responsible for extensive systemic toxicity.

The NGO submitted several general comments with specific reference to five selected publications from PubMed, namely: Rollerova *et al.* (2011), Yin *et al.* (2008), Swan *et al.* (2003), Rakitsky *et al.* (2000) and Ashby *et al.* (1997).

Assessment and comparison with the classification criteria

(1) Fertility

Consideration of Category 1:

Effects are seen in two species (rat, dog), but the data do not warrant classification for fertility in Category 1A or 1B for the following reasons:

- There is no evidence from humans.
- There is no clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects. All notable effects occurred in presence of parental/systemic toxicity manifested by bodyweight reduction, reduced body weight gain, changes in the weight of some organs and the occurrence of nasal hyperplasia and polypoid adenomas from 600 ppm.
- Significant fertility parameters, such as mating, fertility and pregnancy indices, as well as sperm parameters were not altered by the administration of acetochlor.
- There are no specific reproductive studies with acetochlor on dogs to ascertain if the effect on the testes is a primary one that has a direct effect on reproductive performance.

Consideration of Category 2:

The main effects on fertility were observed in the 2-generation study in rats (Milburn, 2001) at 1750 ppm and at 600 ppm and also in the two 1-year dog studies (Ahmed, 1981; Broadmeadow, 1989) at 40 and 50 mg/kg bw/day, respectively:

- Decrease in the number of live plus dead pups at the high dose in F2 litters and in F1 litters. At 1750 ppm, decrease in the number of implantations in F0 and F1 females and in the number of live pups on postnatal day 1 in F2 litters.
- Significant changes in the absolute weight of rat ovaries at the high dose in F0 (-25%) and F1 (-22%) parents (Milburn, 2001). A significant decrease in the relative ovarian weight is seen in F0 parents only (-12%). There is no effect on reproductive performance (mating, fertility and pregnancy indices).
- Slight testicular effects in rats alone are not convincing evidence for classification though some members of the scientific community consider absolute testicular weight is significant because the testis, like the brain, is conserved despite body weight loss (Parker, 2012).
- There are significant and severe acetochlor effects on the testes in dogs with doses of 40 – 50 mg/kg bw/d in two independent 1-year studies.
- The 119-day dog study by Ahmed (1980) indicates a dose-related decreasing (but none statistically significant) trend in testicular weight: 0, -9.4%, and -24% for the 0, 25 and 75 mg/kg bw/d doses respectively.
- The histopathology confirms that severity is marked enough to indicate functional impairment in the testes of dogs at between 40 - 50 mg/kg bw/d.
- Dose-dependent increases in bilateral ovarian atrophy in the 2-year rat study by Naylor (1986), doses were 0, 2, 12 and 60 mg/kg bw/d with incidence of 1/70, 2/70, 4/70, and 5/70.

Consideration of no classification:

The weight of evidence indicates that acetochlor has only minimal potential to induce reproductive toxicity and that such effects occur only in the presence of significant systemic toxicity:

- Slight effects on several reproductive endpoints in the rat (e.g. decrease in the number of implants and litter size) occurred only at a dose level that caused significant maternal toxicity. No such reproductive effects were noted at similar or higher dose levels in two other rat reproduction studies.
- Except for a slight decrease at the high-dose level in the Milburn (2001) reproduction study, there were no meaningful, dose-related decreases in relative ovarian weights and no treatment-related ovarian histopathological lesions in any of the rat, mouse or dog studies with acetochlor.
- There is no evidence of testicular toxicity noted in any of the studies with rats or mice, and no statistically significant changes in the 90-day or 119-day studies with dogs.
- There is clear evidence of testicular toxicity (decreased testes weights and testicular histopathology) in two 1-year dog studies. However, these effects occur at the highest dose levels (40 – 50 mg/kg bw/day) against a background of significant systemic toxicity. Indeed, in one study (Broadmeadow, 1989) there is good evidence for a decline in kidney function and possibly chronic renal failure in dogs treated with acetochlor. Chronic renal failure is associated with many effects on the body including testicular atrophy and ovarian dysfunction.
- According to the guidance on the application of the CLP criteria (CLP Guidance; section 3.7.2.2.1.1) "*Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes*". The 12-month Broadmea dow (1989) study has effects only seen at the high dose, with significant mortality (60%), significant renal toxicity and significant body weight reductions (males: -16%, females: -37%).
- Histopathological lesions of the testes were initially reported at 10 mg/kg bw/d in the Broadmeadow (1989) 1-year dog study. However, this finding was contradicted by a subsequent peer review of the original histopathology slides by Creasy (2003). These conclusions were supported by a lack of testicular lesions at 12 mg/kg bw/d in the 1-year dog study by Ahmed (1981). Therefore there is no evidence for acetochlor related testicular pathology at low doses that do not cause systemic toxicity.
- Testicular lesions in one animal at 10 mg/kg bw/d in the Broadmeadow (1989) 1-year dog study were not related to treatment with acetochlor but were instead caused by lymphocytic orchitis, an autoimmune disease sometimes seen in beagle dogs (Creasy, 2003).

(1) Conclusions on fertility

The weight of evidence suggests that at high concentrations, acetochlor may affect fertility or reproductive performance in the dog. Smaller effects are seen in the rat 2-generation studies at larger doses than used in the dog studies but it is unclear if the effects in the rat alone are sufficient for classification. The effects on the dog testes are of concern, but it needs to be considered whether the effect is a primary one, i.e. whether acetochlor has a direct toxicological effect on the testes or whether it is secondary to renal insufficiency. The dog studies indicate that this species is the most sensitive. The 1-year dog study by Broadmeadow (1989) also provides evidence for (delayed onset) chronic renal failure (high water consumption, high urinary volume with low specific gravities, increased plasma urea or BUN and creatinine, increased GGT, significant renal histopathology, severe neurological involvement suggestive of uremic toxicity) though not all of the classical effects associated with renal failure are noted (e.g. haematology disturbance, plasma phosphate, calcium and other electrolytes, no decrease in the relative kidney weight). Chronic renal failure is associated with gonadal dysfunction in humans and the same may be true for dogs. There was no investigation of chronic renal failure *per se* so even the presumption of this diagnosis is a hypothetical one based on the effects noted primarily in a single 12-month dog study with some supporting but weak evidence from the 119-day dog study by Ahmed (1980).

In summary, there is sufficient concern to consider classifying acetochlor for its effects on fertility according to CLP. The effects on dog testes at 40-50 mg/kg bw/d in the 1-year studies are severe enough to cause a large reduction in mass and a suspected functional impairment. Furthermore, the 119-day dog study by Ahmed (1980) indicates a trend for a dose-related decrease (but not statistically significant) in testicular weight. There are clear indications of chronic renal failure at the high dose in one 12-month dog study but insufficient evidence to make an association between it and the testicular effects observed. The second 12-month dog study by Ahmed (1981) is more significant because there was no indication of renal failure and no lethalities, but firm evidence for testicular changes was present. There are no mechanistic studies investigating the aetiology of the testicular effects so it is not possible to be certain if they are a consequence of a primary effect by acetochlor or secondary to renal insufficiency.

The RAC therefore concluded that acetochlor should be classified as ***Repr. 2; H361f***.

(2) Conclusions on developmental toxicity:

Adverse effects on development regarded as significant and biologically relevant were the following:

- Increased incidence, slightly above the historical controls, of reduced ossification in sacrocaudal vertebral arches and of skeletal variations, increased incidences of variant sternebras and foetal weight reduction observed at 600 mg/kg bw/d in the teratogenicity study in rat in the presence of maternal toxicity (Brooker *et al.*, 1989a).
- In a 2-generation study in rat (Milburn, 2001), decrease of live pups in F1 litters at 1750 ppm and in F2 litters from 600 ppm and a reduction in the number of live pups at 1750 ppm in F2 litters. Also observed at 1750 ppm was a delay of the vaginal opening time in F1 pups and reduction in the anogenital distance in males in F2 pups. Decrease of litter weight, bodyweight, bodyweight gain and organ weight variations were observed in pups from 600 mg/kg bw/d. All these findings were seen in the presence of maternal toxicity, manifested by bodyweight reduction, changes in the weight of some organs and the occurrence of nasal hyperplasia and polypoid adenomas from 600 ppm.
- In another two-generation toxicity study in rats (Schardein, 1982), a significant decrease in the number of live pups at birth of the F1b litter, outside of the historical control range, was observed in presence of maternal toxicity.

According to section 3.7.2.4 of CLP Regulation and the CLP Guidance, in the interpretation of the developmental outcome to decide classification for developmental effects, it is important to consider the possible influence of maternal toxicity. Adverse developmental effects after acetochlor treatment were observed at doses with marked maternal toxicity (i.e. mortality, clinical signs, bodyweight reductions, food consumption decrease, variations in organ weights, occurrence of nasal hyperplasia and nasal adenomas). The DS is of the opinion that these reproductive effects can be regarded as irrelevant for classification since they are a secondary consequence of this marked maternal toxicity.

The severity of the effects observed is not sufficient for classification of acetochlor for developmental toxicity. The criteria in section 3.7.2.4.3 of the CLP states that *"Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity".* Section 3.7.2.4.2 of the CLP Regulation states that *"Classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies"*.

All the effects occurred at doses that caused marked maternal toxicity, as explained above. In addition, reproductive effects were limited to one species (rat, not rabbit).

The DS considered that there is no evidence from the animal studies to warrant a classification for acetochlor, and therefore classification for developmental toxicity is not necessary. **RAC agreed with the DS that acetochlor does not require classification for developmental toxicity.**

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed to classify acetochlor as Aquatic Acute 1; H400, M=1000 and Aquatic Chronic 1; H410, M=100. The classification was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms. The lowest acute toxicity value was a 72-h ErC50 of 0.00052 mg/L for algae and the lowest chronic toxicity value was a 72-h NOErC of 0.00013 mg/L, also for algae.

Degradation

The water solubility of acetochlor is 282 mg/L at 20°C (pH 6.89). In the 31-day hydrolysis test (US EPA Subdivision N 161-1) no loss of acetochlor was detected which shows that the substance is hydrolytically stable within the pH range from 5 to 9. Acetochlor is photolytically stable in aqueous solutions at 25°C. Only 8% of the applied substance degraded photolytically during a period equivalent to 30 days of Florida summer sunlight (EPA Pesticide assessment guidelines 161-2, 1982). The photolytic half-life on a soil surface is equivalent to 134 days of Florida summer light (US EPA Pesticide Assessment Guideline Subdivision N 161-3. No degradates of greater than 4% of AR (applied radioactivity) were observed in either test.

In topsoil experiments carried out under aerobic conditions in the laboratory the predominant pathway of acetochlor degradation was microbially intermediated oxidative dechlorination to t-oxanilic acid (max 11-17.1% AR) subsequently forming t-sulfinylacetic acid (max 9.2-18% AR); t-sulfonic acid (max 5.9-11.8 AR) and s-sulfonic acid (max 1.5-9.8% AR). The metabolite t-norchloro acetochlor was only present at relatively low levels. Mineralisation to carbon oxide accounted for only 11-15% AR after 84 days and 0.3-3.1 AR after 90 days. The formation of residues not extracted was 15-41% AR after 84-90 days. Acetochlor showed low to moderate persistence in soil with the single first order DT50 of 3.4-29 days (DT90 11.1-96 days) after normalisation to FOCUS reference conditions. In four field dissipation studies from Europe single first order DT50 was estimated to be in the range 7-17 days (DT90 23-56 days).

The water/sediment study comprising two systems in laboratory demonstrated that acetochlor exhibited moderate persistence by dissipating in the total systems with estimated single first order DT50 of 17-22 days (DT90 56-75 days). ModelMaker compartment model used resulted in degradation DT50 of 26-55 days in the water compartment and 9.6-7.5 in the sediment compartment. The metabolites t-oxanilic acid and t-norchloro acetochlor were identified as significant degradation products representing maxima of 13.1 and 10.44% AR in water and 2.9 and 19.2% AR in sediment, respectively. Mineralisation to carbon dioxide accounted for only 1.4-2.7% of the AR after 100 days. Residues not extracted from sediment were the most significant sink for radioactivity representing 24-50% AR at study end after 100 days. Degradation of acetochlor was extensive with numerous minor products including: sulfonic acid, thioacetic acid derivative and t-sulfinylacetic acid. None of these products accounted for more than 7%.

No ready biodegradability test is presented in the CLH dossier. According to the results of the BIOWIN models included in Epiweb™ the substance can be considered as not readily biodegradable. Altogether the DS concluded that aacetochlor is persistent in the environment for classification purposes.

Bioaccumulation

The log Pow is 4.14 at 20°C (pH ~ 6.5). The only available bioconcentration study followed U.S.EPA FIFRA 165-4 guidelines. There were some deviations from the OECD 305 guideline (1996)

but these were not considered to have affected the outcome of the study. There were two test systems in the study. Test system I contained smaller fish (~1 g) for monitoring accumulation and elimination of total ^{14}C residues. In test system II the fish were larger (~ 25g) in order to extract and characterise the residues. The characterisation of tissue residues demonstrated that acetochlor was present in all tissues, ranging from 4 to 25% of total radioactivity. In addition to acetochlor, three major metabolites were present which together accounted for the majority of the extractable radioactivity. The whole fish BCF from test system I and test system II were 132 and 20, respectively. The DS concluded that although the log Pow is greater than 4, the study shows a BCF value of 20 which shows that acetochlor is non-bioaccumulative.

Aquatic Toxicity
There are altogether three acute toxicity studies for fish, one for Daphnia, seven for algae and one for *Lemna*. The lowest values are presented in Table 1.

Table 1. Lowest acute aquatic toxicity data available

Species	Test guideline	Test type and duration	Result
Oncorhynchus mykiss	OECD 203, GLP	96h, static	LC50 0.36 mg/L (mean measured 94-111% of nom.)
Daphnia magna	ASTM (1980) and US EPA (1975), in accordance with OECD 202 (1981), GLP	48h, static	EC50 8.6 mg/L (mean measured 88-93% of nom.)
Pseudokirchneriella subcapitata	**US EPA FIFRA Subdivision J Guideline 123-2, OECD 201, EEC Method C.3, GLP**	**72h, static**	**ErC50 0.00052 mg/L (mean measured 91-100% of nom.)**
Lemna gibba	US EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD 221, GLP	7d, static	ErC50 0.0074 mg/L (mean measured 86-95% of nom.)

There are two chronic toxicity studies for fish, one for Daphnia, seven for algae, one for Lemna and one for a midge *Chironomus*. The *Chironomus* test is done using OECD TG 219, namely Sediment/water chironomid toxicity test using spiked water. The 28-day development EC50 is > 10 mg/L and the respective NOEC is 1.6 mg/L. The lowest toxicity values are presented in Table 2.

Table 2. Lowest chronic aquatic toxicity data available

Species	Test guideline	Test type and duration	Result
Oncorhynchus mykiss	US EPA FIFRA Subdivision E, 72-4 guideline and ASTM E 1241-88 (1988), GLP	32d, flow-through	NOEC 0.13 mg/L (mean measured 100-110% of nom.)
Daphnia magna	US EPA 72-4 (b) guideline in agreement with OECD 202 Part II, GLP	21d, flow-through	NOEC reproduction 0.0221 mg/L (mean measured 91-105% of nom.)

Pseudokirchneriella subcapitata	**US EPA FIFRA Subdivision J Guideline 123-2, OECD 201, EEC Method C.3 guideline, GLP**	**72h static**	**NOErC 0.00013 mg/L (mean measured 91-100% of nom.)**
Lemna gibba	US EPA FIFRA Subdivision J Guidelines 122-2 and 123-2, OECD 221, GLP	7d static	NOErC <0.00085(* mg/L (mean measured 86-95% of nom.)

(* This is the value corrected in the public consultation and accepted by the DS. In the CLH Report the value is <0.085 mg/L. However, in the DAR 2005 the value is also 0.00085 mg/L.

The lowest acute toxicity value for acetochlor is a 72-hour ErC50 of 0.00052 mg/L. The lowest chronic toxicity value for acetochlor is a 72-hour NOErC of 0.00013 mg/L.

The 7-day NOEC for growth rate in the *Lemna gibba* study was <0.00085 mg/L. After 7 days of exposure inhibition at this particular concentration was 37%, 16% and 31% for frond number, growth rate, and dry weight, respectively. This concentration was the lowest concentration tested.

Table 3. Toxicity values available for degradation products

	t-oxanilic acid	t-sulfinylacetic acid	t-sulfonic acid	t-norchloro acetochlor
Fish, 96h, LC50	> 93 mg/L	> 120 mg/L	> 180 mg/L	42 mg/L
Daphnia, 48h, EC50	> 120 mg/L	> 120 mg/L	> 120 mg/L	170 mg/L
Algae, 72h, ErC50	42 mg/L	68 mg/L	17 mg/L	0.49 mg/L
Algae, 72h, NOErC	32 mg/L	56 mg/L	3.2 mg/L	0.24 mg/L

One of the metabolites of aacetochlor, namely t-norchloro acetochlor, shows high toxicity to algae.

Comments received during public consultation

Three MS agreed with the proposed classification. One MS gave corrections to the toxicity results in Table 75 concerning toxicity of acetochlor to algae and aquatic plants toxicity. The DS took note of the corrections informed. The corrected numbers are used in the Tables above.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider acetochlor as not rapidly degradable. The substance is hydrolytically stable. There is no ready biodegradability test available. In a water/sediment study the dissipation DT50 for the total system was 17-22 days. Non-extractable residues represented 24-50% AR in sediment and mineralisation was negligible. Two metabolites, t-oxanilic acid and t-norchloro acetochlor, were identified as significant degradation products. T-norchloro acetochlor is very toxic to algae in acute tests and thus classifiable for environment.

Bioaccumulation

RAC agrees that acetochlor has a low potential to bioaccumulate based on the fish BCF values of 20 (based on residues) and 132.

Aquatic toxicity

There are adequate acute and chronic toxicity data available on fish, Daphnia, algae and the aquatic plant *Lemna*. The lowest acute toxicity value was ErC50 of 0.00052 mg/L for algae and the lowest chronic toxicity NOEC value was of 0.00013 mg/L for algae. In addition, the 7-day NOEC for growth rate of <0.00085 mg/L was the lowest value for Lemna. Because this concentration was the lowest one tested and resulted in a 16% reduction in growth rate, a lower value could be possible if a more reliable test result became available. This might have an impact on the M-factor for Aquatic Chronic classification.

Conclusion on classification

RAC agrees with the DS proposal to classify acetochlor as follows according to the CLP Regulation:

Aquatic Acute 1; H400, M=1000 and

Aquatic Chronic 1; H410, M=100

The classification is based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report and responses to comments provided by the Dossier Submitter and by RAC (excl. confidential information).

Annex 3 Comments and RAC's response to comments received during the targeted public consultation on the reproductive toxicity of Acetochlor (ISO)