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Chemical Review Committee
Fourth meeting
Geneva, 10–13 March 2008
Item 5 (b) (i) of the provisional agenda*

Inclusion of chemicals in Annex III of the Rotterdam Convention: review of notifications of final regulatory action to ban or severely restricted a chemical: alachlor

Alachlor: supporting documentation provided by European Community

Note by the Secretariat

The Secretariat has the honour to provide, in the annex to the present note, the supporting documentation provided by the European Community in support of its notification of final regulatory action on alachlor.

* UNEP/FAO/RC/CRC.4/1

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Annex

- **Commission decision 2006/966/EC December 2006**
- **Review report for the active substance Alachlor (SANCO/4331/2000-Final - 10 January 2007)**
- **Opinion of scientific Panel of Plant Health, Plant Protection Products and their residues on a request from the Commission related to the evaluation of Alachlor in the context of the Council Directive 91/414/EEC – The EFSA Journal 2004, 11, 1-34**
- **Addenda End-points (January 205, page 1-34)**
- **ALACHLOR: Reasoned Statement for the Overall Conclusions – April 1999 (Monograph, Vol. 1, level 2)**

COMMISSION

COMMISSION DECISION

of 18 December 2006

concerning the non-inclusion of alachlor in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance

(notified under document number C(2006) 6567)

(Text with EEA relevance)

(2006/966/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market [1], and in particular the fourth subparagraph of Article 8(2) thereof,

Whereas:

(1) Article 8(2) of Directive 91/414/EEC provided for the Commission to carry out a programme of work for the examination of the active substances used in plant protection products which were already on the market on 25 July 1993. Detailed rules for the carrying out of this programme were established in Regulation (EEC) No 3600/92 of 11 December 1992 laying down the detailed rules for the implementation of the first stage of the program of work referred to in Article 8(2) of Council Directive 91/414/EEC concerning the placing of plant protection products on the market [2].

(2) Commission Regulation (EC) No 933/94 of 27 April 1994 laying down the active substances of plant protection products and designating the rapporteur Member States for the implementation of Commission Regulation (EEC) No 3600/92 [3], designated the active substances which should be assessed in the framework of Regulation (EEC) No 3600/92, designated a Member State to act as rapporteur in respect of the assessment of each substance and identified the producers of each active substance who submitted a notification in due time.

(3) Alachlor is one of the 89 active substances designated in Regulation (EC) No 933/94.

(4) In accordance with Article 7(1)(c) of Regulation (EEC) No 3600/92, Spain, being the designated rapporteur Member State, submitted on 20 July 1999 to the Commission the report of its assessment of the information submitted by the notifiers in accordance with Article 6(1) of that Regulation.

(5) On receipt of the report of the rapporteur Member State, the Commission undertook consultations with experts of the Member States as well as with the main notifiers as provided for in Article 7(3) of Regulation (EEC) No 3600/92. It appeared that further data were required. Commission Decision 2001/810/EC [4] laid down a deadline for data submission by the notifier, which expired 25 May 2002. The same decision set a further deadline of 31 December 2002 for specified long term studies.

(6) The Commission organised a tripartite meeting with the main data submitters and the rapporteur Member State for this active substance on 19 December 2003.

(7) The assessment report prepared by Spain has been reviewed by the Member States and the Commission within the Standing Committee on the Food Chain and Animal Health. This review was finalised on 4 April 2006 in the format of the Commission review report for alachlor.

[1] OJ L 230, 19.8.1991, p. 1, Directive as last amended by Commission Directive 2006/75/EC (OJ L 248, 12.9.2006, p. 3).

[2] OJ L 366, 15.12.1992, p. 10, Regulation as last amended by Regulation (EC) N° 2266/2000 (OJ L 259, 13.10.2000, p. 27.)

[3] OJ L 107, 28.4.1994, p. 8, Regulation as last amended by Regulation (EC) N° 2230/95 (OJ L 225, 22.9.1995, p. 1.).

[4] OJ L 305, 22.11.2001, p. 32.

(8) The review of alachlor revealed a number of open questions which were addressed by the Scientific Panel on Plant health, Plant protection products and their Residues. The Scientific Panel was asked to comment on two questions. The first question was whether the occurrence of nasal turbinate tumours observed in the rat carcinogenicity study was relevant to humans and, if so, whether a genotoxic mechanism is involved. The second question was whether the information presented for the metabolites 65, 85, 54, 25, 76 and 51, which exceed the level of 0,1 µg/l, was sufficient to demonstrate that they are not relevant. In its opinion [1] on the first question, the Scientific Panel concluded that the strength of the evidence suggests that a mode of action other than genotoxicity is involved in the occurrence of nasal turbinate tumours observed in the rat carcinogenicity studies. While the mode of action could be relevant to humans, it is extremely unlikely that concentrations of the active metabolite would be achieved to initiate the chain of events terminating in cancer. On the second question, the Scientific Panel concluded that metabolites 65, 54 and 25 have been adequately tested for toxicity, but the toxicity database is inadequate in the case of the soil metabolites 85, 76 and 51. The genotoxicity database is also inadequate for soil metabolites 85, 76 and 51. For metabolite 25 the Scientific Panel was unable to conclude that this metabolite was safe from the point of view of genotoxicity. It is concluded that while the information presented for metabolites 65 and 54 is sufficient to demonstrate that they are not relevant, a similar conclusion cannot be reached for metabolites 85, 76, 51 and 25.

(9) During the evaluation of this active substance, other areas of concern have been identified. It was found that the expected concentration in groundwater of some of the above metabolites exceed the maximum acceptable limit of 0,1 µg/l. In addition, it could not be precluded that alachlor has a carcinogenic potential. In this context, alachlor has been classified as a carcinogen of category 3 by Commission Directive 2004/73/EC [2] of 29 April 2004 adapting to technical progress for the 29th time Council Directive 67/548/EEC [3] on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. In this case, it was considered appropriate to increase the safety factors used in the setting of an acceptable operator exposure level (AOEL). The exposure resulting from the handling of the substance and its application at the rates, i.e. the intended doses per hectare, proposed by the notifier, would exceed this level and, in other words, lead to an unacceptable risk for the operators.

(10) Consequently, as these concerns remain unresolved, assessments made on the basis of the information submitted have not demonstrated that it may be expected that, under the proposed conditions of use, plant protection products containing alachlor satisfy in general the requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC

(11) Alachlor should therefore not be included in Annex I to Directive 91/414/EEC.

(12) Measures should be taken to ensure that existing authorisations for plant protection products containing alachlor are withdrawn within a prescribed period and are not renewed and that no new authorisations for such products are granted.

(13) Any period of grace for disposal, storage, placing on the market and use of existing stocks of plant protection products containing alachlor allowed by Member States, should be limited to a period no longer than 12 months to allow existing stocks to be used in no more than one further growing season.

(14) This Decision does not prejudice any action the Commission may undertake at a later stage for this active substance within the framework of Council Directive 79/117/EEC of 21 December 1978 prohibiting the placing on the market and use of plant protection products containing certain active substances [4], as last amended by Regulation (EC) No 850/2004 [5].

(15) This decision does not prejudice the submission of an application for alachlor according to the provisions of Article 6 (2) of Directive 91/414/EEC in view of a possible inclusion in its Annex I.

(16) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on the Food Chain and Animal Health,

HAS ADOPTED THIS DECISION:

Article 1

Alachlor shall not be included as active substance in Annex I to Directive 91/414/EEC.

Article 2

Member States shall ensure that:

(a) Authorisations for plant protection products containing alachlor are withdrawn by 18 June 2007;

[1] Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the evaluation of alachlor in the context of Council Directive 91/414/EEC (Question No EFSA-Q-2004-48) adopted on 28 October 2004.

[2] OJ L 152, 30.4.2004, p. 1.

[3] OJ 196, 16.8.1967, p. 1.

[4] OJ L 33, 8.2.1979, p. 36.

[5] OJ L 158, 30.4.2004, p. 7.

(b) from 19 December 2006 no authorisations for plant protection products containing alachlor are granted or renewed under the derogation provided for in Article 8(2) of Directive 91/414/EEC.

Article 3

Any period of grace granted by Member States in accordance with the provisions of Article 4(6) of Directive 91/414/EEC, shall be as short as possible and shall expire not later than 18 June 2008.

Article 4

This Decision is addressed to the Member States.

Done at Brussels, 18 December 2006.

For the Commission
Markos KYPRIANOU
Member of the Commission

EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate D - Food Safety: Production and distribution chain
Unit D.3 - Chemicals, contaminants and pesticides

Review report for the active substance **alachlor**

finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 4 April 2006
in support of a decision concerning the non-inclusion of alachlor in Annex I of Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance

1. Procedure followed for the re-evaluation process

This review report has been established as a result of the re-evaluation of alachlor, made in the context of the work programme for review of existing active substances provided for in Article 8(2) of Directive 91/414/EEC concerning the placing of plant protection products on the market, with a view to the possible inclusion of this substance in Annex I to the Directive.

Commission Regulation (EEC) No 3600/92([1]) laying down the detailed rules for the implementation of the first stage of the programme of work referred to in Article 8(2) of Council Directive 91/414/EEC, as last amended by Regulation (EC) No 2266/2000([2]), has laid down the detailed rules on the procedure according to which the re-evaluation has to be carried out. Alachlor is one of the 90 existing active substances covered by this Regulation.

In accordance with the provisions of Article 4 of Regulation (EEC) No 3600/92, Phytorus SA on 26 July 1993, Monsanto SA on 19 July 1993, I.Pi.Ci. Industria Prodotti Chimici on 30 July 1993, ACI International on 30 July 1993, Makhteshim Agran on 20 July 1993, Industrias Químicas del Vallés on 28 July 1993, Pilar Ibérica SL on 23 July 1993, Helm AG on 23 July 1993, Calliope SA on 21 July 1993, SA John & Stephen B. on 29 July 1993, Tradi-Agri SA on 29 July 1993 and B.V. Luxan on 21 July 1993 notified to the Commission of their wish to secure the inclusion of the active substance alachlor in Annex I to the Directive.

In accordance with the provisions of Article 5 of Regulation (EEC) No 3600/92, the Commission, by its Regulation (EEC) No 933/94([3]), as last amended by Regulation (EC) No 2230/95([4]), designated Spain as rapporteur Member State to carry out the assessment of alachlor on the basis of the dossiers submitted by the notifiers. In the same Regulation, the Commission specified furthermore the deadline for the notifiers with regard to the submission to the

[1] OJ No L 366, 15.12.1992, p.10.

[2] OJ No L 259, 13.10.2000, p.27.

[3] OJ No L 107, 28.04.1994, p.8.

[4] OJ No L 225, 22.09.1995, p.1.

rapporteur Member States of the dossiers required under Article 6(2) of Regulation (EEC) No 3600/92, as well as for other parties with regard to further technical and scientific information; for alachlor this deadline was 31 October 1995.

Monsanto SA, SA John & Stephen B, Sabachem International LTD, Phytorus SA and Makhteshim Agan submitted each in time a dossier to the rapporteur Member State. However, according the Spanish Regulation (Article 4 of Orden del Ministerio de la Presidencia de 28 de Marzo de 1996 – BOE 3.04.96) Phytorus SA, as notifier of the active substance Alachlor should have paid the fees for doing the assessment of its dossier, as Phytorus SA did not pay these fees, Phytorus SA must not be considered as notifier. In May 1998 Dow AgroSciences informed the Commision and Member States that Dow AgroSciences would deal in future with all matters concerning the reviews of the dossiers submitted on behalf of Sanachem. In November 1999 Dow AgroSciences informed the Commission, The Regulatory Authorities of the Member States and the Joint Research centre of the European Chemicals Bureau that Dow agroSciences does not wish to proceed any further with its support of the review of Alachlor.

Monsanto SA submitted in time a dossier to the rapporteur Member State which did not contain substantial data gaps, taking into account the supported uses. Therefore Monsanto SA was considered to be the main data submitter.

In accordance with the provisions of Article 7(1) of Regulation (EEC) No 3600/92, Spain submitted on 20 July 1999 to the Commission the report of its examination, hereafter referred to as the draft assessment report, including, as required, a recommendation concerning the possible inclusion of alachlor in Annex I to the Directive. Moreover, in accordance with the same provisions, the Commission and the Member States received also the summary dossier on alachlor from Monsanto SA, on 27 June 2000.

In accordance with the provisions of Article 7(3) of Regulation (EEC) No 3600/92, the Commission forwarded for consultation the draft assessment report to all the Member States on 28 January 2000 as well as to Monsanto SA being the main data submitter, on 08 February 2000.

The Commission organised an intensive consultation of technical experts from a certain number of Member States, to review the draft assessment report and the comments received thereon (peer review), in particular on each of the following disciplines:

- identity and physical /chemical properties ;
- fate and behaviour in the environment ;
- ecotoxicology ;
- mammalian toxicology ;
- residues and analytical methods ;
- regulatory questions.

The meetings for this consultation were organised on behalf of the Commission by the Pesticide Safety Directorate (PSD) in York, United Kingdom, from January to July 2001.

The report of the peer review (i.e. full report) was circulated, for further consultation, to Member States on 27 June 2001 and the main data submitter on 25 August 2001 for comments and further clarification.

In accordance with the provisions of Article 6(4) of Directive 91/414/EEC concerning consultation in the light of a possible unfavourable decision for the active substance the

Commission organised a tripartite meeting with the main data submitter and the rapporteur Member State for this active substance on 19 December 2003.

In accordance with the provisions of Article 7(3) of Regulation (EEC) No 3600/92, the dossier, the draft assessment report, the peer review report (i.e. full report) and the comments and clarifications on the remaining issues, received after the peer review were referred to the **Standing Committee on the Food Chain and Animal Health**, and specialised working groups of this Committee, for final examination, with participation of experts from all Member States. This final examination took place from December 2004 to April 2005, and was finalised in the meeting of the **Standing Committee** on 4 April 2006.

These documents were also submitted to the Scientific Committee for Plants for separate consultation. The report of this Committee was formally adopted on 28 October 2004 (Question N° EFSA-Q-2004-48[5]). The review of alachlor revealed a number of open questions which were addressed by the Scientific Panel on Plant health, Plant protection products and their Residues (PPR). The Scientific Panel was asked to comment on two questions: Is the occurrence of nasal turbinate tumours observed in the rat carcinogenicity study relevant to humans and, if so, is a genotoxic mechanism involved? The second question was whether the information presented for the metabolites 65, 85, 54, 25, 76 and 51, which exceed the level of 0,1 µg/l, sufficient to demonstrate that they are not relevant? In its Opinion[6] to the first question, the Scientific Panel concludes that the strength of the evidence suggests that a mode of action other than genotoxicity is involved in the occurrence of nasal turbinate tumours observed in the rat carcinogenicity studies. While the mode of action could be relevant to humans, it is extremely unlikely that concentrations of the active metabolite would be achieved to initiate the chain of events terminating in cancer. On the second question, the Scientific Panel concluded that metabolites 65, 54 and 25 have been adequately tested for toxicity, but the toxicity database is inadequate in the case of the soil metabolites 85, 76 and 51. The genotoxicity database is also inadequate for soil metabolites 85, 76 and 51. For metabolite 25 the Scientific Panel was unable to conclude that genotoxicity testing was adequate. It is concluded that whether the information presented for metabolites 65 and 54 is sufficient to demonstrate that they are not relevant, a similar conclusion cannot be reached for metabolites 85, 76, 51 and 25.

The present review report contains the conclusions of the final examination; given the importance of the draft assessment report, the peer review report (i.e. full report) and the comments and clarifications submitted after the peer review as basic information for the final examination process, these documents are considered respectively as background documents A, B and C to this review report and are part of it.

2. Purposes of this review report

This review report including the background documents has been developed and finalised in support of Commission Decision 2006/966/EC concerning the non-inclusion of alachlor in Annex I to Directive 91/414/EEC.

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[5] Opinion of the Scientific Committee on Plants regarding the inclusion of alachlor in Annex I to Council Directive 91/414/EEC concerning the placing of plant protection products on the market.

[6] Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the evaluation of alachlor in the context of Council Directive 91/414/EEC (Question N° EFSA-Q-2004-48)adopted on 28 October 2004

In accordance with the provisions of Article 7(6) of Regulation (EEC) No 3600/92, Member States will keep available or make available this review report for consultation by any interested parties or will make it available to them on their specific request. Moreover the Commission will send a copy of this review report (not including the background documents) to all operators having notified for this active substance under Article 4(1) of this Regulation.

3. Overall conclusion in the context of Directive 91/414/EEC

The overall conclusion of this evaluation, based on the information available and the proposed conditions of use, is that:

- **the information available is insufficient** to satisfy the requirements set out in Annex II and Annex III Directive 91/414/EEC in particular with regard to
    - the environmental fate and toxicology/ecotoxicology of the substance and its metabolites
    - the exposure of operators, workers and bystanders.
- **concerns were identified with regard to**
    - the fate and behaviour of the substance in the environment, in particular the formation of a large variety of degradation products, some of which are of toxicological and/or ecotoxicological concern;
    - its possible impact on operators, workers and bystanders.

In conclusion from the assessments made on the basis of the submitted information, no plant protection products containing the active substance concerned is expected to satisfy in general the requirements laid down in Article 5 (1) (a) and (b) of Council Directive 91/414/EEC.

Alachlor should therefore not be included in Annex I to Directive 91/414/EEC.

The EFSA Journal (2004), 111, 1-34.

Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from the Commission related to the evaluation of alachlor in the context of Council Directive 91/414/EEC[1].

(Question N° EFSA-Q-2004-48)

adopted on 28 October 2004

SUMMARY OF OPINION

The Scientific Panel on Plant health, Plant protection products and their Residues (PPR) concludes that the strength of the evidence suggests that a mode of action other than genotoxicity is involved in the occurrence of nasal turbinate tumours observed in the rat carcinogenicity studies. While the mode of action could be relevant to humans, it is extremely unlikely that concentrations of the active metabolite would be achieved to initiate the chain of events terminating in cancer.

The PPR Panel also concludes that metabolites 65, 54 and 25 have been adequately tested for toxicity, but the toxicity database is inadequate in the case of the soil metabolites 85, 76 and 51. The genotoxicity database is also inadequate for soil metabolites 85, 76 and 51. For metabolite 25 the PPR Panel was unable to conclude that genotoxicity testing was adequate. It is concluded that the information presented for metabolites 65 and 54 is sufficient to demonstrate that they are not relevant; a similar conclusion cannot be reached for metabolites 85, 76, 51 and 25.

Key words : alachlor, acetochlor, chloroacetanilide, 2,6-diethyl aniline, quinoneimine, herbicide, rat, nasal turbinate mucosal tumour, carcinogenicity, genotoxicity, toxicity, metabolism, relevant metabolite, mode of action.

[1] For citation purposes: Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of alachlor in the context of Council Directive 91/414/EEC, *The EFSA Journal* (2004) 111, 1-34.

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BACKGROUND[2]

Alachlor is used as an herbicide and is included in the first list of active substances referred to in Article 8(2) of Directive 91/414/EEC[3] concerning the placing of plant protection products on the market. On the basis of the evaluation report prepared by Spain as Rapporteur Member State (RMS), the substance has been peer reviewed with Member State experts and consequently discussed in the working group "Plant Protection Products–Evaluation" on 17/18.9.2003 and in the working group "Plant Protection Products – Legislation" of the Standing Committee on the Food Chain and Animal Health on 2 and 3 October 2003.
A tripartite meeting with the RMS and the main data supplier was organised on 19 December 2003.
The peer review identified several data gaps that were addressed by the notifier. All information submitted has been evaluated and discussed with Member States in the Working Groups "Evaluation".
Some outstanding issues were identified and may trigger a broader discussion.
The notifier provided mechanistic studies, which would confirm that the observed nasal tumours are specific to the rat (2-years rat study) and have no relevant effects to humans.

[2] Background delivered by the European Commission.
[3] OJ No L 230, 19.08.1991, p.1.

However, several Member States consider alachlor and/or some of its metabolites to be genotoxic carcinogens, for which no threshold value can be set. They observed that it cannot be excluded that these observed tumours in rats may be relevant to humans.
Furthermore, studies show that alachlor degrades rapidly in aerobic soil to a large number of metabolites.
Following the applied scenarios for groundwater, alachlor does not leach to shallow groundwater at levels above 0.1 µg/l. However some of the metabolites may exceed significantly this level (metabolites 65, 85, 54, 25, 76, 51).
The Commission Guidance document (SANCO/221/2000 rev 10 final 25 February 2003) on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC provides that in such cases sufficient information has to be made available to demonstrate that the concerned metabolites leaching above 0.1 µg/l are not relevant.

TERMS OF REFERENCE

Question 1: Is the occurrence of nasal turbinate tumours observed in the rat carcinogenicity study relevant to humans? If so, is a genotoxic mechanism involved?

Question 2: Is the information presented for the metabolites listed above sufficient to demonstrate that they are not relevant ?

ASSESSMENT QUESTION 1

Question 1: Is the occurrence of nasal turbinate tumours observed in the rat carcinogenicity study relevant to humans? If so, is a genotoxic mechanism involved?

1.1. Introduction

Alachlor is one of several chloracetanilide herbicides that have been associated with increased tumour incidences in a number of organs in rodents submitted to long-term, dietary exposure experiments. Epigenetic modes of action have been suggested for this activity, but there has been concern in some Member States that genotoxic mechanisms have not been adequately considered as the more likely mode of action in the development of tumours of the nasal turbinates. This concern is captured in the two, clearly related questions put to the PPR Panel. The opinion of the PPR Panel is based on the experimental data and arguments described under the following headings:
- Kinetic and metabolic studies on alachlor;
- Carcinogenicity studies of alachlor in rats and mice;
- DNA interaction and other genotoxicity studies *In vitro* and *In vivo* with alachlor and its major mammalian metabolites;
- Studies of epigenetic modes of action in the nasal turbinates.

1.2. Kinetic and metabolic studies on alachlor

The absorption, distribution and excretion of alachlor have been studied in rat, mouse, Syrian hamster and rhesus or squirrel monkeys. Metabolism *In vivo* and *In vitro* has been studied in rat, mouse, squirrel monkey and man. Alachlor is well absorbed from the gastro-intestinal tract (GIT) in all species tested. Tissue distribution and whole body autoradiography studies show that alachlor metabolites accumulate in the nasal turbinates of rats (particularly in Long-Evans as opposed to either Sprague-Dawley or F344 strains), but not of CD-1 mice, Syrian hamsters and squirrel monkeys (Ribelin & Wilson, 1985; Hall & Wilson, 1992). Accumulation of radioactivity was found in blood and in the GIT of all species investigated. Haemoglobin binding is stronger in

rats than in other species. Accumulation of radioactivity in the GIT was most pronounced in the rat, as a result of extensive enterohepatic circulation. Excretion in urine and faeces respectively, as a percentage of the dose, was about 45 % and 42 % in rats, about 20 % and 60 % in mice and about 78 % and 17 % in rhesus monkeys. The high faecal elimination in mice is a result of enterohepatic circulation and binding of metabolites to intestinal material, rather than poor absorption. Urinary elimination kinetics in rats are biphasic, the half-life times being about 7 h for the α-phase and about 100 h for the β-phase. The urinary elimination half-life time in rhesus monkeys is about 5 h.

Alachlor is extensively metabolised in rats and mice through a complex network of pathways, whereas metabolism in monkeys appears to be simpler (see Appendix Figures 1, 2 and 3) Studies with liver and kidney homogenates have led to the identification of two major metabolic pathways in all of these species. One of these is oxidative dealkylation (loss of the methoxymethyl group) by cytochrome P450 enzymes to form the secondary chloramide (metabolite 13) (Feng & Patanella, 1988) that is then hydrolysed by microsomal arylamidases to 2,6-diethylaniline (Feng *et al.*, 1990). These reactions can be catalysed by microsomal preparations from rat and mouse liver and nasal fractions and the oxidation products can be conjugated with glucuronic acid or glutathione. The other metabolic pathway requires conjugation with glutathione, with loss of chlorine, a reaction that is mediated by cytosolic glutathione *S*-transferases (Feng & Patanella, 1988). The secondary chloramide product of oxidative dealkylation (metabolite 13) is also a substrate for this conjugation, again with loss of chlorine.

The conjugates are excreted in bile into the GIT, where the corresponding thiol metabolites are formed by cleavage of the C-S bond and then reabsorbed. These thiols are *S*-methylated in the liver to form tertiary and secondary methyl sulphides that undergo further metabolism by *S*-oxidation and side-chain hydroxylation. They are largely eliminated in urine. The influence of sex, dose and route of administration is relatively small.

The specificity of rat liver microsomal arylamidases for several of these potential substrates (6 methylthio compounds, including sulphides, sulphoxides and sulphones, and 2 mercapturates) was examined (Feng *et al.*, 1990). The formation of 2,6-diethylaniline was observed only from the methyl sulphide secondary amide metabolite. Thus, two secondary amides, one formed from each of the metabolic pathways described are substrates for arylamidase. These are the *N*-dealkylated methylsulphide and the *N*-dealkylated chloroacetanilide, which form a common product, 2,6-diethylaniline (Kimmel *et al.*, 1986; Feng *et al.*, 1990). This appears to be a key metabolite in the toxicology of alachlor. Oral dosing of rats and mice with the homologous 2,6-dimethyl-[^{14}C-phenyl]-aniline (a carcinogen of nasal tissue in male and female CD rats) led to a very intense localisation of radioactivity in the nasal mucosa of male and female rats at 24 h. Mice showed only slight localisation in nasal mucosa, but appeared to have a higher level of radioactivity in liver (Hall, 1991). In addition, the administration of radiolabelled metabolites, alachlor-methyl sulphide and 2,6-diethylaniline, also resulted in marked radioactivity in the nasal tissue (Wilson & Hall, 1988), whereas radioactivity did not accumulate in nasal tissue of mice following administration of 2,6-diethylaniline (Hall & Wilson, 1993). A proposed unstable metabolic derivative of 2,6-diethylaniline in rats is 2,6-diethylnitrosobenzene (Kimmel *et al.*, 1986; Wratten *et al.*, 1987), but its significance is unclear. Much more firmly established is the oxidation of 2,6-diethylaniline by hepatic and nasal microsomal (aniline) hydroxylase to 4-amino-3,5-diethylphenol (metabolite 86). The *in vivo* relevance of this pathway was confirmed by the presence of the sulphate conjugate of 4-amino-3,5-diethylphenol in the urine of rats orally administered the methyl sulphide secondary amide metabolite. The initial rate of this hydroxylation reaction in rat preparations is greater when catalysed by the nasal enzyme than when the hepatic enzyme is used, by about seven-fold in one study (Feng *et al.*, 1990) and two-fold in another (Li *et al.*, 1992).

Comparisons of nasal tissue metabolism of alachlor or some of its metabolites to 4-amino-3,5-diethylphenol have been made using tissue from rat, mouse, squirrel monkey and man (Asbury *et al.*, 1994). The activity of rat arylamidase, which metabolises secondary amide metabolites of alachlor to 2,6-diethylaniline has been found to be 20-fold and four-fold higher than that of the mouse or squirrel monkey, respectively while the aryl hydroxylase activity of rat nasal tissue is two-fold and more than seven-fold higher than in nasal tissue from the other two species, respectively. In contrast, species differences in liver enzyme reaction rates with these substrates are small (Feng *et al.*, 1990).

The relative rates of reaction for four important steps in alachlor metabolism in hepatic and nasal tissue of different species can be summarised, as in Table 1.

Table 1: Ratios of initial reaction velocities for hepatic and nasal enzyme preparations from various species *(Asbury et al., 1994; Feng & Patanella, 1988; Li et al., 1992).*

	Rat/Human	Rat/Squirrel monkey	Rat/Mouse
Alachlor GSH* conjugation			
Hepatic	4.0	3.9	0.5
Nasal	32.5	114.3	0.8
Secondary sulphide hydrolysis			
Hepatic	N/A	0.9	2.2
Nasal	5.8	4.0	20.0
Secondary amide hydrolysis			
Hepatic	N/A		
Nasal	3.7		
DEA** hydroxylation			
Hepatic	7.5	3.0	0.3
Nasal	129.8	7.6	1.9

*GSH : reduced glutathione, **DEA : diethylaniline

These ratios clearly demonstrate that at least for nasal tissue, the rat enzymes have a greater activity than human, squirrel monkey and (except for alachlor conjugation with glutathione) mouse enzymes. This is supportive of a species distinction, with rat nasal tissues forming 4-amino-3,5-diethylphenol more rapidly than the other nasal tissues preparations. It can also be demonstrated that the important 2,6-diethylaniline hydroxylation reaction has higher activity in rat nasal tissue than in rat liver (Table 2).

Table 2 : Ratios of initial reaction velocities for hepatic and nasal enzyme preparations in rats

	Rat hepatic/Rat nasal
Alachlor GSH conjugation	0.45
Secondary sulphide hydrolysis	4.77
Secondary amide hydrolysis	8.0
DEA hydroxylation	0.12

4-Amino-3,5-diethylphenol (metabolite 86) either conjugates with sulphate (metabolite 20) or, upon further oxidation, rearranges to 3,5-diethylbenzoquinone 4-imine (DEIQ), which is suspected of being the metabolite that causes damage in the nasal mucosa. Quinone-imines are electrophilic, can deplete cellular antioxidants (Tee *et al.*, 1987) and covalently bind to reduced glutathione and protein sulphydryl groups (Feng & Wratten, 1987; Feng *et al.*, 1990).

Species differences in the ability to form DEIQ correlate with the ability to form protein adducts in nasal tissue. Phenyl-[^{14}C]-labelled alachlor administered in the diet to female Long-Evans rats (126 mg/kg bw/day) for up to 13 days was covalently bound to proteins in nasal tissue. Hydrolysis and subsequent hplc analysis showed that most of the radioactivity co-eluted with the acid-hydrolysed acetylated cysteine-DEIQ standard. A minor radioactive fraction co-eluted with 2,6-diethylaniline standard. Its source must have been different from cysteine-DEIQ, which was stable under the conditions of acid hydrolysis (Lau *et al.*, 1995). Similar studies with male rhesus monkeys (126 mg/kg bw/day for 14 days) and female CD-1 mice (50 mg/kg bw/day for 14 days) failed to demonstrate any cysteine-DEIQ adduct in proteins from nasal tissue (Mehrsheikh & Lau, 2001 a, b).

1.3. Carcinogenicity studies of alachlor in rats and mice

Alachlor has been studied in six experiments that could give information on its carcinogenic potential: four with rats (Daly *et al.*, 1981a; Stout *et al.*, 1983; Stout *et al.*, 1984; Genter *et al.*, 2000, Genter *et al.*, 2002) and two with mice (Daly *et al.*, 1981b; Rouloff & Thake, 1984).

1.3.1 Rats

It is noted that, although the Long-Evans strain has been used in all of the rat studies, it should not be assumed that this strain is particularly sensitive to the development of the types of lesions described: Sprague-Dawley rats used in studies with the closely related chloroacetanilide, acetochlor, developed the same nasal lesions (Ashby *et al.*, 1996).

1.3.1.1 Study 1:

Long-Evans rats (50/sex/group) were fed alachlor diets delivering doses of 0, 14, 42 and 126 mg/kg bw/day for two years. Of the neoplastic lesions described, there were treatment related increases in adenomas of the nasal turbinate mucosa in males, thyroid follicular cell adenomas in males and adenomas and carcinomas combined in females and various tumours of the glandular stomach in males and females (Table 3). The malignant mixed gastric tumours were unusual and pluripotent. In addition, hepatocellular adenomas were more common, but not significantly elevated, in treated rats (Daly *et al.*, 1981a).

Table 3: Tumour incidences in Long-Evans rats fed alachlor continuously for 2 years (Study 1)

	Males				Females			
Dose (mg/kg bw/day)	0	14	42	126	0	14	42	126
Stomach								
Any type of tumour	0/50	0/50	0/50	17/50	0/50	0/50	1/50	23/50
Malignant mixed gastric tumour	0/49	0/50	0/50	11/50	0/50	0/50	1/50	17/49
Leiomyosarcoma	0/24	0/33	0/32	1/31	0/17	0/23	0/18	1/29
Osteosarcoma	0/24	0/33	0/32	3/31	0/17	0/23	0/18	4/29
Gastric adenocarcinoma	0/49	0/50	0/50	2/50	0/50	0/50	0/50	1/49
Thyroid follicular epithelium								
Adenoma	1/48	0/50	1/49	11/50	0/49	0/44	2/46	2/49
Carcinoma	0/48	0/48	0/48	0/48	0/48	0/48	0/48	2/49
Nasal turbinate mucosa								
Adenoma	0/46	0/46	10/41	23/42	0/49	0/47	4/45	10/48
Adenocarcinoma	0/46	0/46	1/41	0/46	0/49	0/47	1/45	0/48

Nasal turbinate adenomas showed a dose dependant increase in males and females at doses of 42 and 126 mg/kg bw/day, which was statistically significant in all of these groups except in females at 42 mg/kg bw/day.

1.3.1.2 Study 2:
Long-Evans rats (100/sex/group) were fed alachlor diets delivering 126 mg/kg bw/day. After 5–6 months, 49 females and 19 males were switched to control diet and maintained for 19 additional months (group III). After 7 months, other groups of 10 males and 20 females were killed (group II). The remaining 70 males and 31 females were maintained on the alachlor diet for 2 years (group I). A small control group (6/sex) was also included, but this size is inadequate for any statistical comparisons of tumour incidence. The data from Groups I and III are presented in Table 4, (Stout *et al.*, 1984).

Table 4: Tumour incidences in Long-Evans rats fed alachlor, either continuously for 2 years or for 5 – 6 months followed by 19 months on control diet (Study 2).

	Group I (2 years)		Group III (5-6 months)	
Dose: 126 mg/kg bw/day	Males	Females	Males	Females
Stomach				
Malignant tumours	3/68	19/31	0/20	1/49
Thyroid follicular epithelium				
Adenoma	8/69	4/31	1/20	2/49
Carcinoma	10/69	0/31	1/20	2/49
Nasal turbinate mucosa				
Adenoma	42/61	11/25	10/17	19/46
Adenocarcinoma	7/61	2/25	0/17	1/46

Adenomas of the nasal turbinates were increased in male and female rats after 5-6 months exposure to 126 mg/kg bw/day followed by control diet for 19 months, to incidences similar to those observed after two years of continuous exposure. This unusual response was not observed for tumours of the stomach and thyroid. Adenocarcinomas of the nasal turbinates also were increased in male rats after 2 years exposure.

1.3.1.3 Study 3:
Long-Evans rats were fed alachlor diets delivering 0, 0.5, 2.5 or 15 mg/kg bw/day for 25 months. Tumours of the nasal turbinates were not increased at 2.5 mg/kg bw/day and below. Adenomas of this organ were increased in both males (15/45) and females (14/48) in the 15 mg/kg bw/day dose group. There were no carcinomas and there were no increases in tumour incidence in stomach or thyroid (Stout *et al.*, 1983).

1.3.1.4 Study 4:
Male Long-Evans rats (70/group) were fed alachlor diets delivering 0 or 126 mg/kg bw/day for up to 24 months, with sub-groups of about 10-15 killed at 1, 6, 12 and 18 months. In addition, a group of Long-Evans rats was administered the same dose of alachlor for just one month and then maintained on the control diet for five months. In the first study, at 6 months, 50% of the rats treated with alachlor developed ≥ 1 tumours of the ethmoid turbinates, demonstrating that it was not necessary to hold the rats on control diets for several months in order to allow tumours to develop (as might be assumed from the data of Stout *et al.*, 1984, above). The increase in tumour burden was dramatic between 6 and 12 months of alachlor exposure, and by ≥ 12 months of alachlor exposure, rats typically had 5–20 tumours, with a significant portion of the nasal passages occupied by tumours, which ranged from small polyps to vast glandular, often haemorrhagic tumours at any of the ≥ 6 month time points examined. In the second

study, rats treated with alachlor for just one month and then maintained on the control diet for 5 more months had no detectable olfactory mucosal lesions, (Genter *et al.*, 2000; Genter *et al.*, 2002).

In the main study, in addition to these observations, at one month it was found that there was neither any histological abnormality nor evidence of enhanced cell proliferation (assessed by BrdU[4] incorporation) in any region of the nasal cavity; but after 6 months exposure there was proliferation of basal and non-basal cells in the olfactory mucosa. The masses that were recorded (above) ranged from dysplastic plaques to polyploid adenomas that originated in the olfactory regions. Both plaques and neoplasms were associated with regions of respiratory metaplasia and were often covered with a low columnar-to-pseudostratified, poorly ciliated epithelium. The tumour cells no longer expressed characteristics of the olfactory mucosa, including olfactory marker protein (OMP, for mature sensory neurons) and Nma (antibody recognising CYP2A3, an orthologue of human CYP2A6, which is found in the subepithelial Bowman's glands of rats). The sites of plaque and tumour development coincided with regions of Nma immunoreactivity, i.e., lateral and ventral quadrants of the nasal cavity, but not the mucosa lining the dorsal medial meatus and the dorsal septum. These data suggest that local metabolism is important in alachlor-induced tumours and support the concept that regions of altered epithelial differentiation give rise to small raised plaques, which progress to elevated neoplastic polyps and finally to well-differentiated adenomas.

1.3.2 Mice.

1.3.2.1 Study 1:

CD-1 mice (50/sex/group) were fed alachlor diets delivering doses of 0, 26, 78 and 260 mg/kg bw/day for 18 months. Of the neoplastic lesions described, there were insignificant excesses of liver tumours and an excess of lung tumours in high dose females, the latter being within the historical control range, (Daly *et al.*, 1981b).

1.3.2.2 Study 2:

CD-1 mice (60/sex/group) were fed diets containing alachlor at concentrations of 0, 100, 400 or 1600 ppm (M: 0, 16.6, 65.4, 262 mg/kg bw/d, F: 0, 23.7, 90.3, 399 mg/kg bw/d) for up to approximately 18 months. Ten mice per sex/group were killed and examined after 12 months. A number of histological changes were more frequent in the treated groups than in the controls. These included chronic nephritis, centrilobular hypertrophy and eosinophilic foci in the liver and accumulation of eosinophilic globules in the olfactory epithelium of male mice and fibrous osteodystrophy of the sternum in females. Increased tumour incidences were observed in the lungs of mice, which were significantly elevated in males of the 400 ppm group (Table 5), (Roloff & Thake, 1984).

Table 5: Incidences of selected lesions in CD-1 mice exposed to alachlor (expressed as %)

Dose (ppm)	Male Mice				Female Mice			
	0	100	400	1600	0	100	400	1600
Nasal turbinates Eosinophilic globules in olfactory epithelium	0	0	0	11	2	2	0	9
Lung Bronchoalveolar								
Hyperplasia	2	3	2	5	2	2	0	0
Adenoma	7	18	27	22	5	14	10	17
Carcinoma	2	0	5	0	2	2	2	5
All tumours	7	18	32	22	7	15	12	20

[4] BrdU:Bromodeoxyuridine

Thus, in the studies in mice, no tumours of the nasal turbinates were reported.

1.4. Occupational Epidemiology

In the most recent up-date study of an industrial cohort with occupational and environmental exposure to alachlor (Leet *et al.*, 1996), there was no evidence for nasal cancers (or thyroid or gastric cancers), but there was an elevated risk of colorectal cancer. The study conducted in Iowa (USA), followed 943 workers with at least one year of cumulative employment from start up of the alachlor manufacturing process in March 1968 through December 1990. Approximately 96% of all workers were successfully traced to determine their last known residence and cancer status. Eighteen workers were diagnosed with cancer during the follow-up period, based on pathology information from the state-wide cancer registry maintained by the State Health Registry of Iowa. The standardised incidence ratio (SIR) for all cancers was 1.5 (95% CI[5] 0.9-2.4) for all workers exposed to alachlor, which was due primarily to elevated rates for colorectal cancer and chronic myeloid leukemia. Workers with 5 or more years in estimated high alachlor exposure jobs had elevated rates of colorectal cancer (3 cases, SIR = 5.2, 95% CI 1.1-15.1). Interpretation of the study results was limited by the small size of the study population and minimal length of follow-up. Nonetheless, the findings suggest the need for continued evaluation of this and other alachlor-exposed cohorts.

1.5. DNA interaction and other genotoxicity studies *in vitro* and *in vivo* with alachlor and its major mammalian metabolites

In evaluating the mutagenicity data, two general factors have been considered: the complexity of alachlor metabolism in rats in particular, with the likely involvement of metabolites generated in liver that have subsequently undergone entero-hepatic circulation; and the postulated tissue specificity (nasal tissue) for the ultimate metabolite-tissue interactions. These factors detract from the ability of *in vitro* supplemented activation systems to generate the ultimate reactive metabolite(s) and of *in vivo* systems in which organs other than the nasal turbinates are the target to detect any activity. The former problem might be surmountable if intermediate metabolites are studied, rather than alachlor itself.

A selected compilation of the genotoxicity data available on alachlor is summarised in the Appendix in Table 1. Studies not listed included those that are not acceptable for clear, technical reasons. There may, however, be others that should not be included (e.g., commercial preparations).

Most studies (6/10) of alachlor for mutagenic activity in bacteria showed no activity. Two studies, however, reported activity in frameshift-sensitive strains of *S. typhimurium* in the absence of any additional metabolic activation system (Mirkova & Zaikov, 1986; Njagi & Gopalan, 1980). The latter of these was with a commercial preparation. In addition, significant responses were obtained in two other studies with *S. typhimurium* TA100 using additional activation systems, one from plants (Plewa *et al.*, 1984) and others from rat liver and rat olfactory mucosa (Wetmore *et al.*, 1999). This last result contradicts earlier ones in which the activation system was based on nasal turbinate tissue from rats, mice and monkeys (Kier & Stegeman, 1990). In the study by Wetmore *et al.* (1999), the rat liver preparation was effective only at exceedingly high doses of alachlor (8430 and 15000 μg/plate), whereas a significant increase was observed with olfactory epithelium S9 at an alachlor dose of 1250 μg/plate, which was close to a toxic dose. There was no effect of respiratory epithelium S9 at any alachlor dose level in the same study. Since there were no differences in either the strains or dose ranges

[5] CI: Confidence Interval

used between those studies from which significant results were reported and those that did not, the mutagenicity of alachlor in bacteria remains unclear.

Genotoxic activity was observed in one study of alachlor with mouse lymphoma cells and using rat olfactory mucosal S9 (Wetmore *et al.*, 1999). The significant response occurred at a single, toxic dose (5.6 μg/ml, however, cloning efficiency was not unduly affected) and was mainly attributable to an increase in small colonies (indicating damage greater than that due to a few base changes). No effect was seen in another study with the same test system, but using rat liver S9 (Enninga *et al.*, 1987). In contrast to these uncertain results, several studies of chromosomal aberration in cultured mammalian cells have yielded significant responses to alachlor exposure. It is likely that this activity is dependent upon the chloroacetamide function. Studies with another chloroacetamide, acetochlor, have shown that it is clastogenic too, whereas des-chloro-acetochlor, which is, other than the lack of the chlorine atom, the identical molecule, has no clastogenic activity (Ashby *et al.*, 1996). The same group survives in *N*-dealkylated chloracetanilide, which could therefore be reactive in nasal tissue before it is metabolised to 4-hydroxy-3,5-diethylaniline by arylamidase. However, alachlor clastogenicity has not been reliably reproduced *in vivo*, (only one study reporting a positive response, while five others did not) and since the metabolites retaining chlorine are generated in liver, any of them (in addition to unmetabolised alachlor) that are potentially clastogenic in the nose should also be available for similar activity in the bone marrow, the usual target for such assays. The absence of demonstrable clastogenic activity *in vivo* suggests either a lack of sensitivity of the assays (because cellular dose levels easily reached *in vitro* cannot be reached *in vivo*) or the function of protective mechanisms that are not normally available in the *in vitro* assays. Alkaline elution assays conducted *in vivo*, including a single-cell alkaline elution assay on rat nasal epithelium have not demonstrated significant responses, but significant responses were obtained in two of three assays for unscheduled DNA synthesis (UDS) in rat liver following oral dosing by gavage that probably depleted glutathione reserves (by analogy with acetochlor, Ashby *et al.*, 1996). A potentially important result is the finding of binding to DNA in the nasal turbinates, but not in the liver of rats dosed orally with alachlor (Asbury & Wilson, 1994); however, the mean radioactivity for DNA from the hepatic and nasal tissues were reported to be only 92.83 ± 8.63 and 205 ± 58.90 fmol alachlor equivalent/mg DNA, respectively.
The covalent binding indices (CBI)[5] after correction for protein contamination was −0.13 ± 0.89 for liver DNA and 1.66 ± 1.24 for nasal DNA. This is a very low CBI and is unlikely to account for the neoplasic response observed. Of much greater toxicological significance is the protein binding in nasal tissue. In contrast to this low level (if real) of DNA adducts, feeding of female Long-Evans rats with diets delivering 126 mg/kg bw/day for up to 13 days resulted in average levels of DEIQ-cysteine adducts in nasal tissue of 70, 88 and 218 pmoles/mg protein after 3, 7 and 13 days, respectively (Lau *et al.*, 1995). On the other hand, similar studies with male rhesus monkeys (126 mg/kg bw/day for 14 days) and female CD-1 mice (50 mg/kg bw/day for 14 days) failed to demonstrate any cysteine-DEIQ adduct in proteins from nasal tissue (Mehrsheikh & Lau, 2001 a, b).

Because of the complexity of alachlor metabolism, of special interest are genotoxicity studies on mammalian metabolites. Bacterial tests have generally been restricted to the use of *S. typhimurium* TA100 and TA 98 in the presence and absence of S9 preparations from rat liver, although other strains were also used in some cases. Weak or very weak positive results have been obtained in TA100 with CP101384 (35), CP97230 and CP101394 (27); in TA100 and TA1535 with 2,6-diethylaniline and in TA1535 with 2,6-diethyl-2-methylthioacetanilide. Apart from CP97230, the positive responses occurred in both the presence and absence of S9. Other tests conducted with these metabolites were: CP101394 (27), bone marrow micronucleus test

[5] CBI = μmole chemical bound per mole DNA phosphate/mmole chemical administered per kg bw. CBI values in rat liver for strong hepatocarcinogens are > 1000, e.g., dimethylnitrosamine, aflatoxin B1; moderate hepatocarcinogens CBI of 150 – 600, e.g., 2-acetylaminofluorene, *N*-nitrosopyrrolidine; weak hepatocarcinogens CBI of 10 –240, e.g., urethane, 4-dimethylaminoazobenzene, vinyl chloride; non-hepatocarcinogens CBI 1 – 20, benzene, benzo(a)pyrene; doubtful or non-carcinogens CBI of < 0.05 – 1.5, e.g., saccharin, toluene, ethinyloestradiol, oestrone (Lutz & Schlatter, 1979).

in mice, negative (Flowers, 1990); 2,6-diethylaniline, gene mutation assay in Chinese hamster ovary (CHO) cells (*hprt* locus), inconsistent results in four experiments (Flowers, 1987); 2,6-diethylaniline, *in vivo* alkaline elution assay in rat liver, negative (Taningher *et al.*, 1993).

1.6. Studies of epigenetic modes of action in the nasal turbinates

The *in vitro* cytotoxicity effects of alachlor, DEA, sec-amide methyl sulphide, and sec-amide chloride were assessed by evaluating the leakage of acid phosphatase from olfactory and respiratory explant cells. Results showed increased acid phosphatase leakage from olfactory but not respiratory cells following exposure to alachlor; and from both olfactory and respiratory cells following exposure to 2,6-diethylaniline, but not from either olfactory or respiratory cells following exposure to the sec-amide methyl sulphide or sec-amide chloride (Asbury *et al.*, 1995).

Cell proliferation assays were performed in the respiratory and olfactory epithelium of rats and mice exposed to alachlor. Results showed a dose-related increase in cell proliferation in olfactory but not respiratory epithelium in rats administered 42 or 126 mg/kg bw/day. This cell proliferation was reversible after 60-day recovery period. On the other hand, there was no cell proliferation in mice.

The effects of alachlor upon cellular stress response genes in rat nasal turbinate tissue were also evaluated. A significantly increased expression of NMO[6] and HSP70[7] was observed in rat nasal epithelium after 60 days exposure at 126 mg/kg/day. This response was not observed at 30 days (Curtiss *et al.*, 1995)

Alachlor doses of about 1000 mg/kg bw, which are close to those causing lethality, are required to deplete reduced glutathione (GSH) in rat liver (Heydens *et al.*, 1999). In contrast, Burman *et al.* (2003) found that both reduced glutathione and ascorbic acid concentrations in olfactory mucosa from male Long-Evans rats rapidly decreased following alachlor exposure for up to 10 days (10 – 126 mg/kg bw/day), with a subsequent increase in both antioxidants to ~ 160% of control levels in the highest dose group and recovery to control levels in all groups by 10 days. These changes in GSH concentrations are associated with up-regulation in olfactory mucosa of glutamine-cysteine ligase. This is the rate-limiting enzyme in GSH biosynthesis and it remained elevated throughout the 10-day dosing period. While GSH was not depleted at all doses, ascorbate concentrations were, and they did not return to normal levels. Ascorbate is important in the maintenance of extracellular matrix proteins, including collagen IV (Chernousov *et al.*, 1998; Hospelhorn *et al.*, 1992; Kalcheim *et al.*, 1985; Kim & Peterkofsky, 1997). Alachlor disrupts basal cell orientation in the olfactory mucosa (Genter *et al.*, 2000), possibly due to the partial loss of collagen IV following ascorbate depletion, but also possibly due to up-regulated matrix metalloproteinases (Genter *et al.*, 2002) resulting in a more general and sustained degradation of the extracellular matrix.

1.7. Discussion (based on the IPCS Mode of Action Framework)

Alachlor reproducibly induces tumours of the olfactory mucosa in rats, but not in mice. The incidence is higher in males than in females. Adenocarcinomas were induced in one of the three experiments, otherwise, progression did not continue beyond the generation of adenomas.

The proposed mode of action for production of nasal tumours in rats is the local generation of cytotoxic metabolite(s) that can interact with cellular macromolecules and induce a sustained

[6] NMO : NAD(P)H Menadione Oxidoreductase 1
[7] HSP70 : Heat Shock Protein 70

cell proliferation, neoplasia arising out of this proliferating cell population. Mutagenesis induced by the cytotoxic metabolite(s) may or may not be part of this process.

The following important steps are involved in the process.
The generation of 2,6-diethylaniline *via* two metabolic pathways, one involving conjugation with glutathione, with the subsequent degradation of the conjugate to a methyl sulphide secondary amide, while the other involves oxidative dealkylation by cytochrome P450 enzymes to a secondary chloramide (metabolite 13). Both of these products are substrates for microsomal arylamidases (the methyl sulphide being the only reactive substrate among several methylthio-metabolites tested) resulting in the formation of their common metabolite, 2,6-diethylaniline.

Both the methyl sulphide metabolite and 2,6-diethylaniline become strongly localised in nasal tissue.

2,6-Diethylaniline can be further oxidised by microsomal aniline hydroxylase to 4-amino-3,5-diethylphenol (metabolite 86). It is proposed that this is a local, nasal reaction. This phenol can rearrange to the sulphydryl-reacting, cytotoxic DEIQ.

At this point, in the sequence of events, there is a reduction in nasal ascorbate concentrations, changes in glutathione concentrations and cytotoxicity followed by cell proliferation, which is presumably a reparative/regenerative response. It has also been demonstrated in one study that mutagenesis can occur, at least *in vitro*, with alachlor as the substrate in the presence of olfactory tissue preparations. This observation seems to undermine the significance of all the earlier, extra-nasal steps in metabolism, since the mutagenic metabolite has not been identified and while it could be DEIQ, it may equally be another metabolite or even reactive oxygen species, the concentrations of which could increase subsequent to perturbations in antioxidant status: quinoneimines can deplete cellular antioxidants. However, while mutagenesis may occur, it does not necessarily mean that this is involved in the neoplastic mechanism.
From within this proliferating cell population, neoplastic transformation occurs, giving rise to adenomas and, in some experiments, carcinomas in the olfactory tissue. Whether this neoplastic transformation is a genotoxic or an epigenetic event is not known. One possibility is that the accelerated cell cycle time reduces the opportunity for repair of genetic damage, which may be either "spontaneous," induced by some alachlor metabolite or as a result of reactive oxygen species. However, it is clear that the dominant reaction with macromolecules in nasal tissue is not with DNA (there is a lack of significant covalent binding with DNA) but with sulphur in proteins or glutathione. The latter would be a detoxifying reaction, but arylation of proteins could lead to a number of biologically relevant responses. Among these, adduction to proteins in chromatin could result in altered gene expression and differentiation control. It is known that alachlor interferes with the maintenance of extracellular matrix proteins and disrupts basal cell orientation in the olfactory mucosa. These possible effects are consistent with the transdifferentiation that is observed in olfactory epithelium (to respiratory epithelium). Other, but unstudied changes that could occur would be a loss of genetic stability or ability to repair the spontaneous genetic damage that occurs daily in all cells.

Adenomas are reproducibly induced by exposure to diets delivering 126 mg/kg bw/day. The incidence is lower when exposure is reduced to 42 mg/kg bw/day. Studies that have demonstrated cell proliferation in the rat nasal turbinates have only used exposures of 126 mg/kg bw/day. Lower doses were not tested and so there has been no demonstration that accelerated cell proliferation always occurs at doses that induce tumours.

No evidence of cell proliferation or histological change was found after exposure for 1 month. The earliest tumours of the nasal turbinates of rats were found after 6 months exposure, with rapid expansion in incidence at later times. Also at 6 months, transdifferentiated tissue and dysplasia were evident in the olfactory region, as was cell proliferation.

The appearance of preneoplastic lesions, as well as neoplasia, are consistent findings in rats, and they have not been observed in mice. The putative proximate metabolite, 2,6-diethylaniline, is strongly localised in rat nasal tissue, but not in mouse nasal tissue, and is therefore consistent with the species difference in neoplastic response. In addition, the metabolic steps leading to the proximate metabolite can occur *in vitro* with hepatic as well as nasal tissue from rats, but not from mice, and is therefore consistent with the species specificity of the carcinogenic response. While this observation does not support an absolute tissue specificity, the enzymatic activity that produces the penultimate reactive metabolite (4-amino-3,5-diethylphenol) from 2,6-diethylaniline is much greater in rat nasal tissue than in rat hepatic tissue. DEIQ, the putative ultimate metabolite, reacts strongly with proteins in nasal tissue, the dominant adduct being DEIQ-cysteine.

The proposed chain of events – metabolism to a proximate metabolite that is strongly localised in nasal turbinates of the susceptible species, but not of non-susceptible species, rearrangement to a strongly electrophilic substance that can then react with both glutathione and macromolecules in the target tissue, thereby causing impairment of antioxidant status and cytoxicity and other tissue damage that can lead to neoplasia – is a plausible mechanism and the available data do not conflict with it. Precisely how the damage leads to neoplasia is not indicated by the data, although plausible hypotheses are available and have been described above.

Other modes of action should be considered, one of which is genotoxicity of alachlor or its metabolites generated either in the liver or locally, in the olfactory nasal mucosa. One study has reported mutagenic responses *in vitro* in bacteria and, at a single concentration, in a mammalian cell line when the incubations were with alachlor and a metabolic activation system based on rat olfactory epithelial tissue, but either not at all or only at extremely high dose levels when rat liver was used. The result with rat nasal tissue contradicts another study that found no mutagenic effect in bacteria when the activation system was based on nasal turbinate tissue from rats, mice and monkeys. It is possible, however, that in the negative study, the active olfactory epithelium may have been diluted by respiratory epithelium (presumed to be inactive). The evidence for a mutagenic effect on the target tissue is, therefore, unclear. Other evidence for or against a mutagenic effect *in vivo* comes from alkaline elution and UDS assays in rats. Alkaline elution assays, including a single-cell (comet) assay with rat nasal epithelium have not provided any evidence for genotoxicity, but while two of three UDS assays in rat liver have given significant results, the doses applied were predicted also to cause significant glutathione depletion. There is a lack of significant covalent binding with DNA. Hence a direct genotoxic mode of action is not sustained by the available evidence.

It is concluded, therefore, that the data are consistent with the proposed mechanism of action, this being as follows. The occurrence of a concentration of a metabolite of alachlor in the olfactory mucosa and its further metabolism to a sulphydryl-reacting, cytoytoxic product that induced cell proliferation and changes in gene expression. Neoplasia arises from this metaplastic or transdifferentiated tissue. The evidence in favour of a genotoxic mode of action is weak.
While the mode of action could be relevant to humans, it is extremely unlikely (based on considerations discussed in section 1.2) that concentrations of the active metabolite would be achieved to initiate the chain of events terminating in cancer.

Conclusions and Recommendations

The Scientific Panel on Plant health, Plant protection products and their Residues (PPR) concludes that the strength of the evidence suggests that a mode of action other than genotoxicity is involved in the occurrence of nasal turbinate tumours observed in the rat carcinogenicity studies. While the mode of action could be relevant to humans, it is extremely

unlikely that concentrations of the active metabolite would be achieved to initiate the chain of events terminating in cancer.

ASSESSMENT QUESTION 2

Question 2: Is the information presented for the metabolites listed above sufficient to demonstrate that they are not relevant ?

2.1. Introduction

In responding to this question, the PPR Panel took notice of "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC (Sanco/221/2000-rev.10-final, 25 February 2003)". The opinion of the PPR Panel is based on the available experimental data obtained with the significant, aerobic soil metabolites of alachlor that address the headings listed in this document:

- Screening for biological activity
- Screening for genotoxicity
- Screening for toxicity

It has been brought to the attention of the PPR Panel that two additional alachlor metabolites, notably alachlor *t*-oxanilic acid (metabolite 70) and metabolite 39, that were not mentioned in the background delivered by the Commission, may exceed significantly 0.1 µg/l level in groundwater.

2.2. Screening for biological activity

Data on the biological activity of metabolites 65, 85, 54, 25, 76 and, 51 of alachlor are available from two field studies, which permit this issue to be addressed. In these studies, eight warm season plant species and eight cool season plant species were tested (i.e., conditions related to the growing of maize and soybean on one hand and to the growing of wheat and oilseed rape on the other). The dose rate tested was 3.36 kg/ha, which is in excess of the dose of metabolite that might be anticipated from the metabolism of alachlor in field conditions. None of the tested metabolites had any biologically relevant effect on terrestrial plant species in either study (Prosch, 2001; Moran, 2002).

2.3. Screening for genotoxicity

Relevant metabolites are to be screened for their genotoxicity by at least the following *in vitro* tests: Ames' test, gene mutation test with mammalian cells and a chromosomal aberration test. Should any of these give equivocal results, then they should be tested by *in vivo* experiments. Where data on DNA interactions and other genotoxicity studies exist for the six listed soil metabolites, they are negative (Table 5). Only for the *t*-sulphonic acid metabolite, (65), is there a study *in vivo*: a mouse bone marrow micronucleus induction test. In addition, this metabolite (65) and the *t*-sulphinylacetic acid metabolite (54) have been tested for gene mutation induction in bacteria and mammalian cells *in vitro* and for clastogenicity in mammalian cells *in vitro*. It is considered that the level of testing exercised is adequate with regard to metabolites (65) and (54).

Of the remaining four soil metabolites of concern, the *t*-methylsulphoxide metabolite (25) has only been tested in *S. typhimurium* TA100 and TA98, although with negative results. Direct testing is therefore inadequate. Although metabolite 25 is a product of alachlor in rats in the absence of information on the extent of formation of this metabolite in rats, the PPR Panel was unable to conclude that genotoxicity testing of metabolite 25 was adequate.

While the *s*-sulphonic acid metabolite (85) has not been tested at all, it was argued in the dossier that it is structurally sufficientlysimilar to the *t*-sulphonic acid metabolite (65) - which has been well tested and has shown no mutagenic activity - as to provide sufficient confidence that (85) also is likely to be non-mutagenic. While this may appear to be a reasonable position,

as noted in the next section there may be toxicological differences between these metabolites, metabolite 85 having an acute LD_{50} value about one-quarter that of metabolite 65.

Similarly, the *s*-hydroxyalachlor metabolite (76) has not been tested at all, but it has been argued that it is structurally similar to the *t*-hydroxyalachlor metabolite (39). This metabolite (39) is included in the Table 6 to provide information, but is not a metabolite of concern and gave negative results in three assays *in vitro*. While it might be predicted that metabolite (76) would also give negative results in these assays, it has a possibly reactive hydrogen atom bonded to the nitrogen. Metabolite 76 is also similar to metabolite 51.

In the case of *s*-norchloroalachlor metabolite (51), recourse has been made to *t*-norchloracetochlor for comparison (Ashby *et al.*, 1996) This compound is referred to as the des-chloro-analogue of acetochlor that, like (51), lacks the chlorine atom of the parent compound, but unlike metabolite 51 does not possess a possibly reactive hydrogen bonded to the nitrogen atom; however, there is no information available regarding the activity of metabolite 51 in tests with mammalian cells for the induction of either gene mutations or chromosomal aberrations. Testing is therefore inadequate with this metabolite.

Table 6: Genotoxicity data on aerobic soil metabolites of alachlor predicted to occur at toxicologically significant concentrations

Assay			Result	Reference
S-acid class				
t-sulphonic acid (65): major in soil, groundwater PEC > 0.1 µg/L				
In vitro	Gene mutation	Ames test in *S. typhimurium* strains	Negative	Kier, 1984 (IIA, 5.8.1/11)
	Gene mutation	Mouse lymphoma/TK test	Negative	Cifone, 2000 (IIA, 5.8.1/22)
	Chromosome aberrations	Cytogenetic test in human lymphocytes	Negative	Murli, 2000 (IIA, 5.8.1/26)
In vivo	Chromosome aberrations	Micronucleus test in mouse bone marrow	Negative	Stegeman *et al.*, 1995 (IIA, 5.8.1/32)
s-sulphonic acid (85): minor in soil, groundwater PEC > 0.1 µg/L				
Data might be extrapolated from its N-alkylated structural analogue, alachlor t-sulphonic acid (65).				
t-sulphinylacetic acid (54): minor in soil, groundwater PEC > 0.1µg/L				
In vitro	Gene mutation	Ames test in *S. typhimurium* and *E. coli* strains.	Negative	Stankowski, 2001 (IIA, 5.8.1/33)
	Gene mutation	Mouse lymphoma/TK test	Negative	Cifone, 2000 (IIA, 5.8.1/21)
	Chromosome aberrations	Cytogenetic test in human lymphocytes	Negative	Murli, 2000 (IIA, 5.8.1/28)
S-methyl class				
t-methylsulphoxide (25): very minor in soil, groundwater PEC > 0.1 µg/L				

Assay			Result	Reference
In vitro	Gene mutation	Ames test in *S. typhimurium* TA98 and TA100 strains	Negative	Kier, 1985 (IIA, 5.8.1/12)
Alachlor class				
t-hydroxyalachlor (39): very minor in soil (Here for comparison with 76), groundwater PEC > 0.1 µg/L				
	Gene mutation	Ames test in *S. typhimurium* strains	Negative	Kier, 1984 (IIA, 5.8.1/10)
In vitro	Gene mutation	CHO/HPRT test	Negative	Stankowski, 2001 (IIA, 5.8.1/38)
	Chromosome aberrations	Cytogenetic test in human lymphocytes	Negative	Murli, 2001 (IIA, 5.8.1/37)
s-hydroxyalachlor (76): very minor in soil, groundwater PEC> 0.1 µg/L				
Data might be extrapolated from its N-alkylated structural analogue, t-hydroxyalachlor (39).				
s-norchloroalachlor (51): very minor in soil, groundwater PEC > 0.1 µg/L				
In vitro	*Chromosome aberrations*	*Cytogenetics test in human lymphocytes*	*Negative*	*Bridged from acetochlor t-NCA study by Ashby et al., 1996, (Monsanto IIA, 5.4/34; Human & Exp. Toxicol. 15: 702-735 - Table 7)*

2.4. Screening for toxicity

Metabolites are considered "relevant" if their toxicological properties lead to a classification as toxic or very toxic (T or T+) according to Directive 67/548/EEC. The available toxicity data are summarised in Table 7.

Table 7: Toxicity data on aerobic soil metabolites of alachlor predicted to occur at toxicologically significant concentrations

Assay		Result (mg/kg bw or mg/kg bw/day)	Reference
S-acid class			
t-sulphonic acid (65): major in soil, groundwater PEC > 0.1 µg/L			
Oral LD_{50}	Rat	> 6000.	Bonette, 1993 (Monsanto report report SB-92-131)
90-day feeding	Rat	NOAEL 157-207	Siglin, 1993 (Monsanto report SB-92-383)
Developmental toxicity	Rat	Parental NOAEL 1000 Developmental NOAEL 1000	Holson, 1995 (Monsanto report WI-95-068)

Assay		Result (mg/kg bw or mg/kg bw/day)	Reference
PCNA staining (nasal turbinates)	Rat	Negative at 2000	
s-sulphonic acid (85): minor in soil, groundwater PEC > 0.1 μg/L			
Oral LD_{50}	Rat	1548	Blaszcak, 1993 (Monsanto report PL-94-191)
90-day feeding	Rat	No data	
Developmental toxicity	Rat	No data	
t-sulphinylacetic acid (54): minor in soil, groundwater PEC > 0.1μg/L			
Oral LD_{50}	Rat	> 5000	Blaszcak, 1993 (Monsanto report PL-94-192)
28-day feeding	Rat	738-776	Stout & Thake, 2000 (Monsanto report MSL-16608)
90-day feeding	Rat	240-296	Bechtel *et al.*, 2001 (Monsanto report MSL-17122)
Developmental toxicity	Rat	No data	
S-methyl class			
t-methylsulphoxide (25): very minor in soil, groundwater PEC > 0.1 μg/L			
		No data	
Alachlor class			
s-hydroxyalachlor (76): very minor in soil, groundwater PEC > 0.1 μg/L			
		No data	
s-norchloroalachlor (51): very minor in soil, groundwater PEC > 0.1 μg/L)			
		No data	

None of the tested metabolites was classified as toxic or very toxic.

Studies that have been conducted with metabolite 65, the alachlor *t*-sulphonic acid, show that it is very poorly absorbed and excreted much more quickly than alachlor, with only minimal metabolism. In contrast to alachlor, it does not accumulate in nasal tissues. When administered to rats in a 91-day study (Siglin, 1993), *t*-sulphonic acid (65) produced adverse effects only at the highest tested dose (10000 ppm, equivalent to 896 and 1108 mg/kg bw/day in males and females, respectively). No alachlor-related changes in thyroid weights or pathology were noted. In the absence of toxicity in these tissues, a neoplastic response secondary to target organ toxicity seems unlikely.

In a study by Hotz (1995) alachlor *t*-sulphonic acid (65) did not show any increase in PCNA[8] staining of the olfactory septum or turbinates of male rats administered 2000 ppm alachlor *t*-sulphonic acid (65) in the above-mentioned 91-day study (Siglin, 1993), as compared with those of rats from the control group, suggesting that alachlor *t*-sulphonic acid (65) did not induce an

[8] PCNA : Proliferating Cell Nuclear Antigen

increase in cell proliferation. Given that, in the case of alachlor, the central process in the formation of nasal tumours is local regenerative cell proliferation after the death of cells whose structure and function is disturbed by protein binding of 3,5-diethylbenzoquinone 4-imine, the absence of proliferation in nasal tissues of alachlor *t*-sulphonic acid (65)-treated rats suggests that no oncogenic potential exists for this metabolite in nasal tissues.
Study of developmental toxicity resulted in NOAEL values for both parental toxicity and developmental toxicity of 1000 mg/kg bw. All of these data indicate that metabolite 65 has been tested adequately.

Of the remaining metabolites, *t*-sulphinylacetic acid (54) also shows no evidence of significant toxicity in acute and repeated dose studies of up to 90 days duration. This also has been adequately tested, although not so thoroughly as metabolite 65.

The *s*-sulphonic acid metabolite (85) has only been tested in an acute oral toxicity assay. Arguments were presented in the dossier for toxicity in other assays similar to metabolite 65. However, it is noted that the LD_{50} value was 1548 mg/kg bw for 85, whereas it was > 6000 mg/kg bw for metabolite 65. Although the acute toxicity of 85 remains low, it is clearly greater than for 65, suggesting that close comparisons should not be drawn.

There are insufficient toxicological data for metabolites 76 and 51. In the case of metabolite 25, it has most probably been adequately tested because it is a metabolite of alachlor found in the urine of rats.

CONCLUSIONS AND RECOMMENDATIONS

The Scientific Panel on Plant health, Plant protection products and their Residues (PPR) concludes on the basis of the reasons stated above that metabolites 65, 54 and 25 have been adequately tested for toxicity, but the toxicity database is inadequate in the case of the soil metabolites 85, 76 and 51. The genotoxicity database is also inadequate for soil metabolites 85, 76 and 51. For metabolite 25 the PPR Panel was unable to conclude that genotoxicity testing was adequate. It is concluded that the information presented for metabolites 65 and 54 is sufficient to demonstrate that they are not relevant; a similar conclusion cannot be reached for metabolites 85, 76, 51 and 25.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from Mr A. Checchi-Lang from the Health & Consumer Protection Directorate-General requesting a consultation EFSA on alachlor, with ref. E1/DVB D/510337(04), 23 March 2004.
2. Guidance document (SANCO/221/2000 rev 10 final 25 February 2003 on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC. pp 1-14.
3. Scientific Committee on Plants (SCP) opinion on the use of alachlor as an herbicide, adopted on 25 October 1988, pp 53-77.
4. Draft Assessment Report on alachlor, annex B, Volume III, Chapter 5: Toxicology and metabolism, April 1999, pp 134-413.
5. Addendum to the Draft Assessment Report on alachlor, annex B, Volume III Chapter 6, Toxicology and metabolism. May 2001, pp 1-266.

6. Addendum to the Draft Assessment Report on alachlor, annex B, Volume III Chapter 1, Identity. May 2001, pp 1-266.
7. Addendum to the Draft Assessment Report on alachlor, Volume I, Relevant metabolites, July 2003, pp 1-22.
8. Addendum to the Draft Assessment Report on alachlor, annex B, Volume III, Chapter 5: Toxicology and metabolism, July 2003, pp 1-75.
9. Addendum to the Draft Assessment Report on alachlor, annex B, Volume III Chapter 6, Toxicology and metabolism. November 2001, pp 4-27.
10. Report summary of the Ministerio de Agricultura, Pesca y Alimentacion on the available informations on metabolites of alachlor, October 2003, pp 1-17.
11. Belgian comments on the Addendum to annex B 6, 30 October 2001, pp 1-2.
12. German comments on the Addendum of November 2001, section toxicology, 30 October 2001, pp 1-2.
13. Danish comments on the draft report of April 1999, impact on human health and animal health, 08 January 2001, pp 1-2.
14. Danish comments on the toxicology of alachlor for evaluation meeting, 22 August 2003, p 1.
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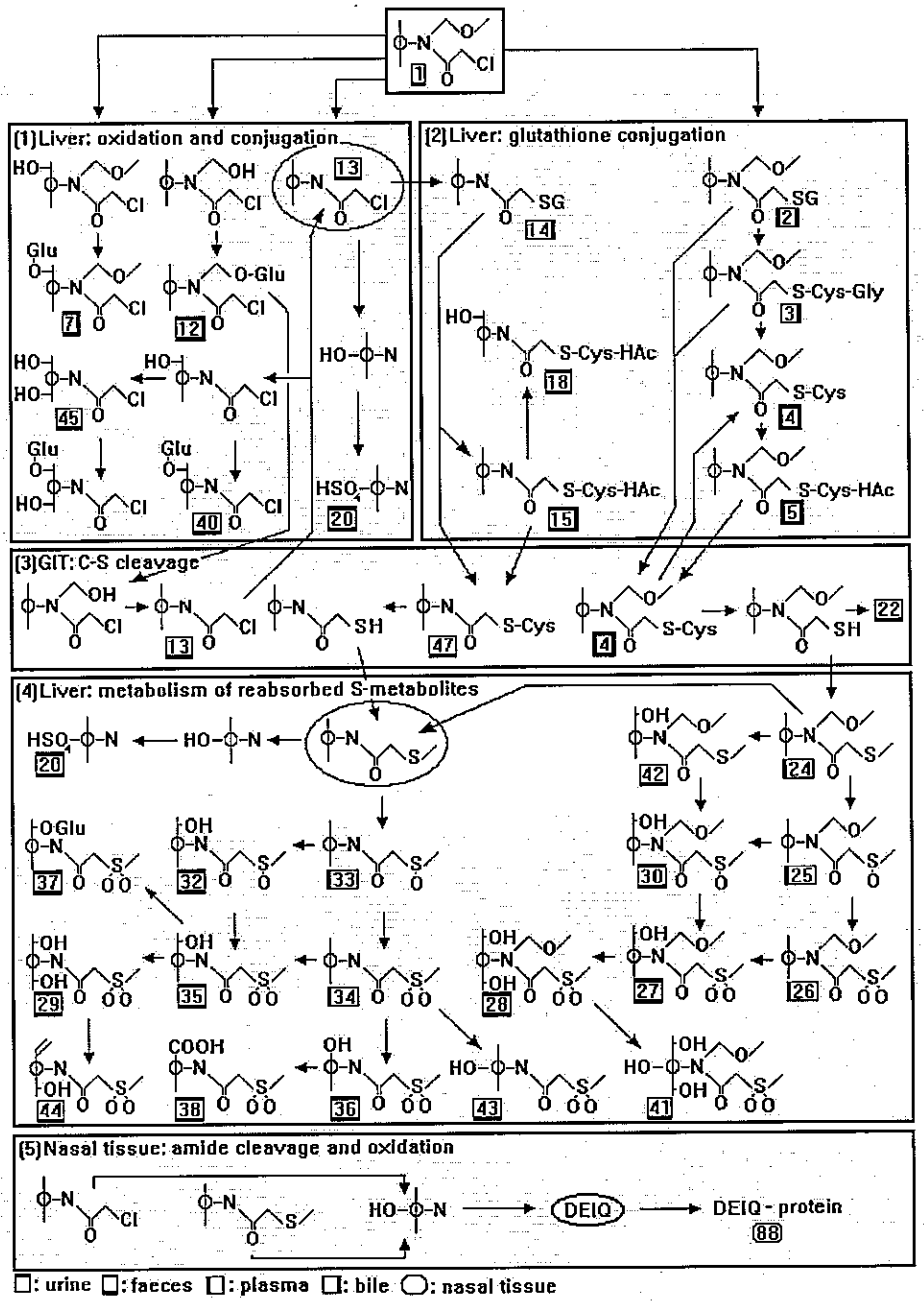
APPENDIX : FIGURES AND TABLES

Figure 1: The metabolic pathway of alachlor in rat



N.B.: The metabolite circled in section 4 is methyl sulphide secondary amide.

Figure 2: The metabolic pathway of alachlor in mouse

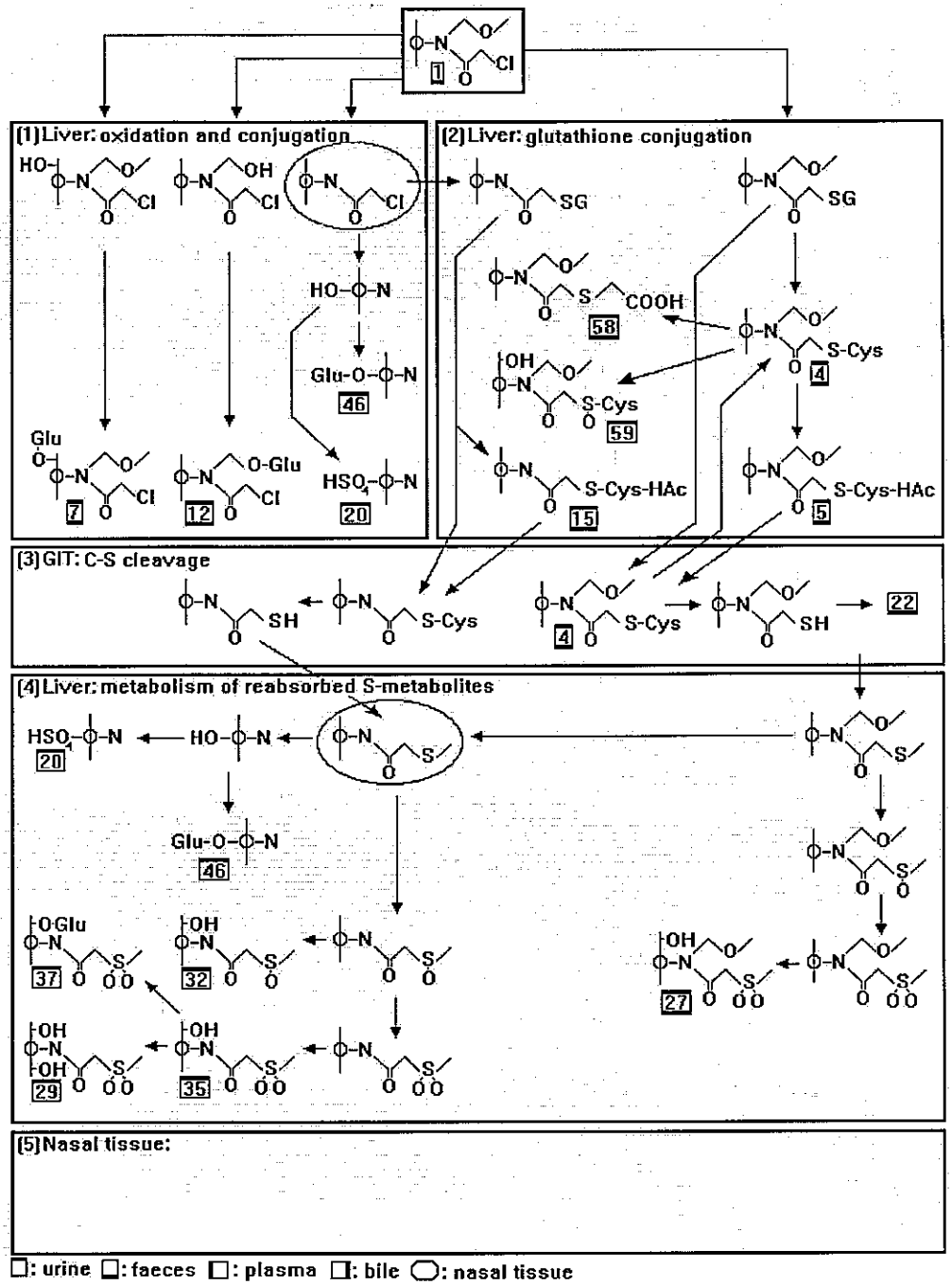
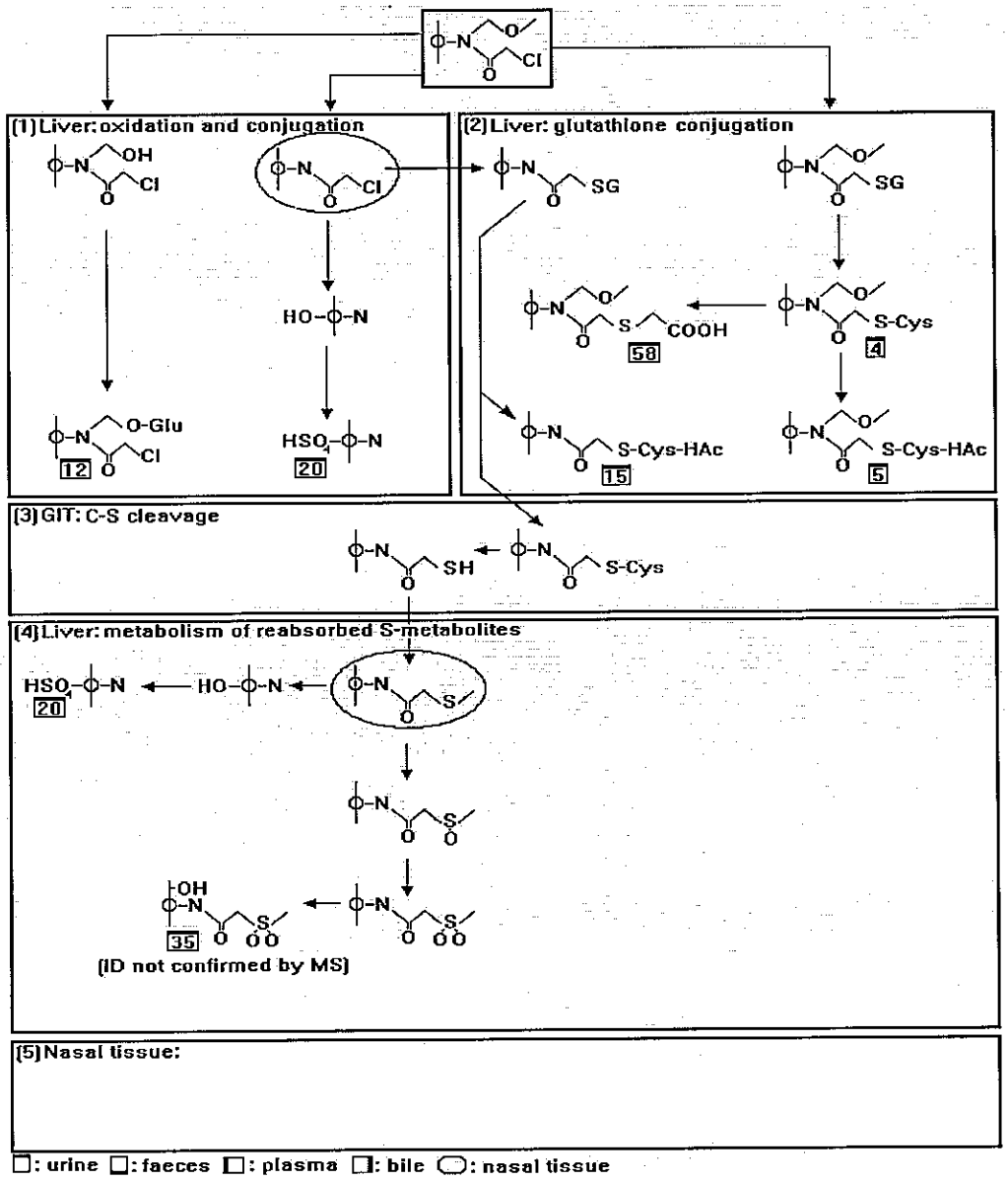


Figure 3: The metabolic pathway of alachlor in monkey



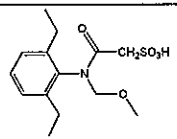
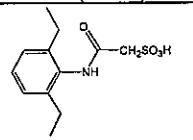
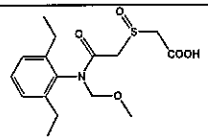
Appendix table 1: Genetic effects of alachlor

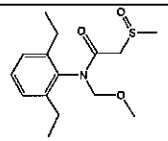
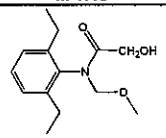
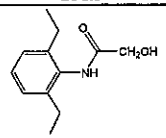
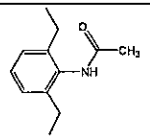
Test system	Result[a]		Test Material and Dose Range	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1535, TA1537, TA1538, TA98 & TA100 ; *Escherichia coli* WP2uvrA, reverse mutation	–	–	10 - 5000 µg/plate	Shirasu *et al.*, 1980
Salmonella typhimurium G46, C3076, D3052, TA1535, TA1537, TA1538, TA98 & TA100 ; *Escherichia coli* WP2 & WP2uvrA, reverse mutation	–	-	NG	Probst *et al.*, 1981
Salmonella typhimurium TA98 & TA100, reverse mutation	–	–	0.2 - 500 µg/plate	Wildeman & Nazar, 1982
Salmonella typhimurium TA1535, TA1537, TA1538, TA98 & TA100 ; *Escherichia coli* WP2uvrA, reverse mutation	–	–	NG	Moriya *et al.*, 1983
Salmonella typhimurium TA1538, reverse mutation	+	–	8 – 100 µg/plate	Mirkova & Zaikov, 1986
Salmonella typhimurium TA1535, TA1537, TA98 & TA100, reverse mutation	–	–	8 – 100 µg/plate	Mirkova & Zaikov, 1986
Salmonella typhimurium TA1537, TA98 & TA1978, reverse mutation	+ ?	+ ?	Commercial preparation 0.01 – 1.0%	Njagi & Gopalan, 1980
Salmonella typhimurium TA1535, TA1538, & TA100, reverse mutation	-	-	Commercial preparation 0.01 1.0%	Njagi & Gopalan, 1980
Salmonella typhimurium TA100, reverse mutation	-	+ (plant activation)	Commercial preparation, NG?	Plewa *et al.*, 1984
Salmonella typhimurium TA1535, TA1537, TA1538 & TA98, reverse mutation	-	–	Commercial preparation, NG	Plewa *et al.*, 1984
Salmonella typhimurium TA1535, TA1537, TA1538, TA98 & TA100 ; *Escherichia coli* WP2uvrA, reverse mutation	-	-	10 – 15000 µg/plate	Chesters *et al.*, 1989
Salmonella typhimurium TA1535, TA1537, TA98 & TA100, reverse mutation	–	- (S9 from nasal turbinates of rat, mouse,	5 – 5000 µg/plate	Kier & Stegeman, 1990

		monkey)		
Salmonella typhimurium TA100, reverse mutation	–	+ (S9 rat olfactory mucosa)	500 – 1500 µg/plate	Wetmore *et al.*, 1999
Drosophila melanogaster, somatic mutation (*white/white+)*	+		1 – 6 mM feed	Aguirrezabalaga *et al.*, 1994
Drosophila melanogaster, somatic mutation (*mwh+/+flr*^{3})	+		1 – 10 mM feed	Torres *et al.*, 1992
DNA strand breaks, rat hepatocytes *in vitro*	+?	NT	10 – 400 mM	Bonfanti *et al.*, 1992
Unscheduled DNA synthesis, male F344 rat primary hepatocytes *in vitro*	–	NT	0.5 – 10 nM ??	Probst *et al.*, 1981
Single-cell alkaline elution, human lymphocytes *in vitro*	+??	+??	5 – 20 µg/ml	Ribas *et al.*, 1995
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	-	-	1 – 100 µg/ml	Enninga *et al.* 1987 (not published)
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	-	+ (S9 rat olfactory mucosa)	1 – 5.6 µg/ml	Wetmore *et al.*, 1999
Gene mutation, CHO-K1-BH4 cells, hprt locus, in vitro	-	-	1-330 µg/ml	Godek et al., 1984
Chromosomal aberrations, human lymphocytes *in vitro*	+	??	1 – 40 µg/ml	Georgian *et al.*, 1983
Chromosomal aberrations, human lymphocytes *in vitro*	+	??	10 – 1000 µg/ml	Meisner *et al.*, 1992
Chromosomal aberrations, human lymphocytes *in vitro*	-	NT	2 – 40 mg/ml (or µg/ml??)	Erexon *et al.*, 1993
Chromosomal aberrations, human lymphocytes *in vitro*	+	??	1 – 20 µg/ml	Ribas *et al.*, 1996
Micronucleus induction, human lymphocytes *in vitro*	-	NT	2 – 40 mg/ml (or µg/ml??)	Erexon *et al.*, 1993
Micronucleus induction, human lymphocytes *in vitro*	+	??	1 – 20 µg/ml	Ribas *et al.*, 1996
Micronucleus induction, human lymphocytes *in vitro*	+	±	1 – 320 µg/ml	Surralés *et al.*, 1995
Covalent bidnig to DNA, nasal turbinates of male Fischer 344 rats *in vivo*	+		125 mg/kg bw x 1 (po)	Asbury & Wilson, 1994
Covalent bidnig to DNA, liver of male Fischer 344 rats *in vivo*	-		125 mg/kg bw x 1 (po)	Asbury & Wilson, 1994
Alkaline elution, liver cell nuclei from BALB/c mice *in vivo*	-		1 mmol/kg bw x 1 (ip); 0.5 mmol/kg bw x 5 (ip)	Taningher *et al.*, 1993
Alkaline elution, liver cell nuclei from Sprague-Dawley rats *in vivo*	-		2 mmol/kg bw x 1 (ip); 1 mmol/kg bw x 5 (ip); 1.5 mmol/kg bw x 1 (po)	Taningher *et al.*, 1993
Single-cell alkaline elution, nasal epithelium, male Alpk:ApfSD rats *in vivo*	-		1070 ppm, 7 days diet	Ashby *et al.*, 1997 (not published)

Unscheduled DNA synthesis, Fischer 344 rat liver cells *in vivo*	+	50 – 1000 mg/kg bw x 1 (po)	Mirasalis & Tyson, 1984
Unscheduled DNA synthesis, rat liver cells *in vivo*	w+	1000 mg/kg bw x 1 (po)	Chesters *et al.*, 19894
Unscheduled DNA synthesis, Fischer 344 rat liver cells *in vivo*	-	50 – 1000 mg/kg bw (po)	Hamilton, 1992
Chromosomal aberrations, bone marrow cells, Wistar rats	+	1250 – 5000 mg/kg bw x 1 (ip)	Georgian *et al.*, 1983
Chromosomal aberrations, bone marrow cells, Wistar rats	-	200 ppm, 280 days, diet	Georgian *et al.*, 1983
Chromosomal aberrations, bone marrow cells, Sprague-Dawley rats	-	100 – 1000 mg/kg bw x 1 (po)	Farrow & Cortina 1984
Chromosomal aberrations, bone marrow cells, male & female B6C3F1 mice	-	20 ppm, 30 or 90 days, drinking water	Meisner *et al.*, 1992
Chromosomal aberrations, bone marrow cells, SD rats	-	312.5, 625 & 1250 mg/kg bw x 1 (po)	Erexson, 2001
Micronucleus test, bone marrow cells, male & female Long-Evans rats *in vivo*	-	150 – 600 mg/kg bw × 1 (ip)	Kier, 1992 (not published)
Micronucleus test, bone marrow cells, male CD-1 mice *in vivo*	-	250 – 1000 mg/kg bw × 1 (po)	Stegeman *et al.*, 1995 (not published)
Dominant lethal effects, male albino mice	-	15 & 30 mg/kg bw x 1 (ip)	Arnold, 1972 (not published)

Appendix table 2: Structures of the six soil metabolites of alachlor predicted to be toxicologically important in ground water

Name (EU n°.)	Structure, Formula, MW	Chemical name	Synonyms
t-sulfonic acid (65)	 $C_{14}H_{21}NO_5S$ **315.42** **337.4 (Na salt)**	2-[(2,6-diethylphenyl) (methoxymethyl) amino]-2-oxo-ethanesulfonic acid (Sodium salt)	*t*-ESA *tert*-ESA *tert*-amide sulfonic acid CP 108065 (Na salt) MON 5775
s-sulfonic acid (85)	 $C_{12}H_{17}NO_4S$ **271.37**	2-[(2,6-diethylphenyl) amino]-2-oxo-ethanesulfonic acid	*s*-ESA *sec*-ESA *sec*-amide sulfonic acid CP 76082 (acid) MON 5767
t-sulfinylacetic acid (54)	 $C_{16}H_{23}NO_5S$ **341.43** **363.4 (Na salt, anh.)**	[[[2-[(2,6-diethylphenyl) (methoxymethyl)]amino-2-oxo]ethyl]sulfinyl] acetic acid (Sodium salt)	*t*-SAA *tert*-SAA *tert*-amide sulfinylacetic acid CP 147920 (Na salt) MON 5768 (Na salt)

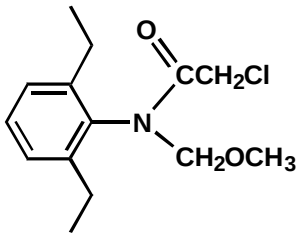
Name (EU n°.)	Structure, Formula, MW	Chemical name	Synonyms
t-methylsulfoxide (25)	 $C_{15}H_{23}NO_3S$ 296.41	N-(2,6-diethylphenyl)-N-(methoxymethyl)-2-(methylsulfinyl) acetamide	CP 76097
t-hydroxyalachlor (39) [included for comparison with (76)]	 $C_{14}H_{21}NO_3$ 251.3	N-(2,6-diethylphenyl)-2-hydroxy-N-(methoxymethyl) acetamide	*t*-OH *tert*-OH *tert*-alachlor alcohol CP 51214 MON 52707
s-hydroxyalachlor (76)	 $C_{12}H_{17}NO_2$ 207.59	N-(2,6-diethylphenyl)-2-hydroxy-acetamide	*s*-OH *sec*-OH CP 51215
s-norchloroalachlor (51)	 $C_{12}H_{17}NO$ 191.3	N-(2,6-diethylphenyl)-acetamide	*s*-NCA *sec*-NCA *sec*-acetamide CP 58997

Chapter 1: **Identity, Physical and Chemical Properties, Details of Uses, Further Information and Proposed Classification and Labelling**

Active substance (ISO Common Name)	Alachlor
Function (*e.g.* fungicide)	Herbicide

Rapporteur Member State	Spain

Identity (Annex IIA, point 1)

Chemical name (IUPAC)	2-chloro-2',6'-diethyl-N-methoxymethylacetanilide
Chemical name (CA)	2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)acetamide
CIPAC No	204
CAS No	15972-60-8
EEC No (EINECSor ELINCS)	240-110-8
FAO Specification (including year of publication)	Not less than 900 g / kg (FAO 1991)
Minimum purity of the active substance as manufactured (g/kg)	900 g / kg
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	FAO: 2-chloro-2',6'-diethylacetanilide(2-chloro-*N*-(2,6-diethylphenyl)acetamide); maximum: 30 g/kg. 2-chloro-N-(2-ethyl-6-(1-methylpropyl)phenyl)-N-(methoxymethyl)acetamide, maximum 19 g / Kg
Molecular formula	$C_{14}H_{20}NO_2Cl$
Molecular mass	269.77
Structural formula	

Physical-chemical properties (Annex IIA, point 2)

Melting point (state purity if not purified)	41.5 °C (Sinon)
Boiling point (state purity if not purified)	-
Temperature of decomposition	-
Appearance (state purity if not purified)	White crystalline solid. Munsell N9.5/90%R. (Sinon)
Relative density (state purity if not purified)	1.2254 (20 °C) (Sinon)

Surface tension	9.129×10^{-7} (Sinon)
Vapour pressure (in Pa. State temperature)	p (20 ºC) = 2.7×10^{-5} hPa p (25 ºC) = 5.5×10^{-5} hPa
Henry's law constant (Pa m^3 mol^{-1})	9.129×10^{-7} (Sinon)
Solubility in water (g/l or mg/l state temperature)	pH 5: 0.188 g / l (20 °C) (Monsanto)
	pH 7: 0.170 g / l (20 °C) (Monsanto)
	pH 9: 0.179 g / l (20 °C) (Monsanto)
Solubility in organic solvents (in g/l or mg/l state temperature)	Methanol > 803 g / l (20 °C) (Monsanto)
	Acetone > 827 g / l (20 °C) (Monsanto)
	Ethyl acetate > 761 g / l (20 °C) (Monsanto)
	1, 2 Dichloroethane > 749 g / l (20 °C) (Monsanto)
	Xylene > 723 g / l (20 °C) (Monsanto)
	n-Heptane = 130 g / l (20 °C) (Monsanto)
Partition co-efficient (log P_{ow}) (state pH and temperature)	2.97 (20 °C) (Sinon)
Hydrolityc stability (DT_{50}) (state pH and temperature)	pH 5- pH 9 : half life > 1 year (Monsanto)
Dissociation constant	Not measurable constant between pH 2.6 to pH 12.2 (Monsanto)
UV/VIS absortion (max.) (if absortion > 290 nm state ε at wavelength)	λ = 264 nm; ε = 493.45
Photostability (DT_{50}) (aqueous, sunlight, state pH)	**Not required**
Quantum yield of direct phototranformation in water at λ > 290 nm	**Not required**
Flammability	Flash point required for Monsanto, Sinon and Phytorus. Flash point Sanachem 51 °C. (Flammable)
Explosive properties	Not explosive

List of supported uses: Only use alachlor products in one out of two years

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks :
					Type	Conc.of as, g/l	method kind	growth stage	number	kg as/hl	water l/ha	kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	min-max.	min-max.	min-max.	min-max.	(k)	(l)
Soybean	France	Lasso EC	F	Weeds	EC	480	OMS	PE	1	0.4-0.8	300-600	2.40	n.a.	Monsanto
	France	Lasso MT	F	Weeds	CS	480	OMS	PE	1	0.4-0.8	300-600	2.40	n.a.	Monsanto
	Portugal	Lasso EC	F	Weeds	EC	480	HVS	PE	1	0.2-0.7	350-1000	1.92-2.40	n.a.	Monsanto
	Spain	Lasso MT	F	Weeds	CS	480	HVS	PE-	1	0.3-2.4	100-600	1.92-2.40	n.a.	Monsanto
	France	Reneur	F	Weeds	EC	480	Spray	PE	1	0.4-1.6	150-600	2.4	n.a.	Phytorus
Sunflower	Spain	Lasso MT	F	Weeds	CS	480	HVS	PE-	1	0.3-2.4	100-600	1.92-2.40	n.a.	Monsanto
Cotton	Greece	Lasso EC	F	Weeds	EC	480	Broad.	PE-	1	0.42-0.60	400	1.68-2.40	n.a.	Monsanto
	Greece	Lasso MT	F	Weeds	CS	480	Broad.	PE-	1	0.42-0.60	400	1.68-2.40	n.a.	Monsanto
	Greece	Alanex	F	Weeds	EC	480	Broad	PE-	1			1.70-2.40	n.a.	M-agan
Maize (grain & sillage)	France	Lasso MT	F	Weeds	CS	480	OMS	PE	1	0.4-0.8	300-600	2.40	n.a.	Monsanto
	France	Lasso EC	F	Weeds	EC	480	OMS	PE	1	0.4-0.8	300-600	2.40	n.a.	Monsanto
	France	Alanex	F	Weeds	EC	480	Spray	PE	1		400			M-Agan
Maize	Greece	Lasso EC	F	Weeds	EC	480	Broad.	PD	1	0.6	400	2.40	n.a.	Monsanto
	Greece	Lasso MT	F	Weeds	CS	480	Broad.	PD	1	0.6	400	2.40	n.a.	Monsanto
	Greece	Alanex	F	Weeds	EC	480	Broad	PE	1			2.40	60	M-Agan
	Italy	Lasso MT	F	Weeds	CS	480	Broad	PD PE	1	0.6	200-600	2.4	n.a.	Monsanto
	Italy	Alanex Micro	F	Weeds	ME	480	HVS	PE	1		400-600	1.68	n.a.	M-Agan
	Portugal	Lasso EC	F	Weeds	EC	480	HVS	PE	1	0.192-	350-1000	1.92-2.40	n.a.	Monsanto
	Spain	Lasso MT	F	Weeds	CS	480	HVS	PE	1	0.3-2.4	100-600	1.92-2.40	n.a.	Monsanto
	France	Reneur	F	Weeds	EC	480	Spray	PE	1	0.4-1.6	150-600	2.4	n.a.	Phytorus
	Greece	ALanex	F	Weeds	EC	480	Spray	Pre – Post sowing	1			2.4-	60	M-Agan
Sweet corn	France	Lasso EC	F	Weeds	EC	480	OMS	PE	1	0.04-0.8	300-600	2.40	n.a.	Monsanto

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application			Application rate per treatment			PHI (days)	Remarks:
					Type	Conc.of as, g/l	method kind	growth stage	number	kg as/hl	water l/ha	kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	min-max.	min-max.	min-max.	min-max.	(k)	(l)
	France	Lasso MT	F	Weeds	CS	480	OMS	PE	1	0.4-0.8	300-600	2.40	n.a.	Monsanto

Remarks:
(a) The EU and Codex classifications (both) should be used
(b) Outdoor or field use, glasshouse application (G) or indoor application (I)
(c) e.g., biting and suckling insects, soil-borne insects, foliar fungi, weeds
(d) e.g., wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GIFAP Codes - GIFAP technical Monograph No. 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g., high-volume spraying, low-volume spraying, spreading, dusting, drench
(h) Kind, e.g., overall, broadcast, aerial spraying, row, individual plant, between the plants
(i) g/kg or g/l
(j) Growth stage at last treatment
(k) PHI - Pre-harvest Interval
(l) Remarks may include: Extent of use/economic importance/restrictions (e.g., feeding/grazing/minimal intervals between applications)
(m) BBCH scale is used for growth stage identification

PE: Preemergence; POST: Postemergence; PD: Pre drilling; Post D: Post drilling; OMS: Overall medium spray; Broad: Broadcasting; HVS: High volume spray.; n.a.: not applicable

Classification and proposed labelling (Annex IIA, point 10)

With regard to physical/chemical data	None
With regard to toxicological data	Xn; Harmful Carcinogenic Cat 3 R22; Harmful if swallowed. R43; May cause sensitisation by skin contac R40, Limited evidence of a carcinogenic effect
With regard to fate and behaviour data	N - Dangerous for the environment
With regard to ecotoxicological data	R 50/53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment

Chapter 2: Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (principle of method)	Dissolved in toluene and analysed by capillary GC (DB-5, 30 m x 0.25 mm i.d.). FID.
Impurities in technical as (principle of method)	Dissolved in toluene and analysed by capillary GC (DB-5, 30 m x 0.25 mm i.d.). FID.
Plant protection product (principle of method)	Dissolved or extracted in acetone (depending formulation) and analysed by GC-FID. CIPAC 1988.

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	FDA / USDA multiresidue method (validated for pinto beans). LOQ = 0.1 ppm. Parent compound measured. (Monsanto). Confirmatory method available by GC-MS. Residues are hydrolysed and the resulting anilines analysed by HPLC-OCED (validated for maize, sunflower, soybean, cotton). LOQ = 0.01 ppm. (Monsanto). Confirmatory method available: derivatization with heptafluorobutyric anhydride and quantitated with GC-MS.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	**Parent + aniline metabolites** Milk, liver, kidney, cattle and chicken: [redacted] **Parent alone** Kidney, liver, muscle, egg, fat: [redacted]
Soil (principle of method and LOQ)	**Alachlor parent** Residue is extracted with methanol : water (9:1), filtered trough C18 solid phase where alachlor is retained . Eluted with ethyl acetate : iso-octane (2 : 8) and quntified with GC-ECD. LOQ = 0.005 ppm. (Monsanto) **Metabolites** DM-oxanilic acid, oxanilic acid, sulfinylacetic acid and sulfonic acid. Residues extracted from soil (acetronitrile : water), cleaned-up, dissolved in a pH 6 buffer and quantified with HPLC-UV. New, validated methods of analysis by LC/MS-MS in soil were developed for soil metabolites t-sulfonic acid (**65**), t-sulfinylacetic acid (**54**), t-oxanilic acid (**70**), s-oxanilic acid (**78**), t-hydroxyalachlor (**39**) and t-norchloroalachlor (**52**)
Water (principle of method and LOQ)	**Parent** Method of analysis by GC/MS (Monsanto IIA, 4.2.3/08) was developed for alachlor (**1**) in groundwater, surface water and drinking water. The LOQ was 0.05 µg/L. A confirmatory method of analysis is not required as this is a method based on mass spectrometry. An independent lab validation (ILV) was included. **Parent and metabolites.** Method of analysis by LC/MS-MS (Monsanto IIA,

	4.2.3/09) was developed for alachlor (**1**) and its metabolites **52**, **54**, **65**, **70**, **78** and **39** in groundwater, surface water and drinking water. The LOQ was 0.05 µg/L. A confirmatory method of analysis is not required as this is a method based on mass spectrometry. An independent lab validation (ILV) was included.
Air (principle of method and LOQ)	Absorption on TENAX tubes, extraction with acetonitrile and analysis with HPLC-UV. LOQ = 3 x 10^{-3}
Body fluids and tissues (principle of method and LOQ)	Not required. **Not Toxic or Very Toxic.**

Chapter 3: Impact on human and animal Health

Absorption, distribution, excretion and metabolism in mammals (Annex IIA, point 5.1)

Rate and extent of absorption:	Rat: fast and extensive oral absorption (in the range of 79 to 96%) within 96 h, based on estimation of bioavailability at 96 h, using excretion data following oral and i.v. administration. Monkey: 90%.
Distribution:	Rat: Widely distributed, in RBC (covalent binding to haemoglobin)
Potential for accumulation:	Rats: accumulation in turbinate nasal/RBC. Not in mice or monkey.
Rate and extent of excretion:	Rat: Mainly urine (42.7-47.5%), faeces (41-42.6%), and bile (17.6%), within 120 h Monkeys: 78% (within 168 h)
Metabolism in animals	Extensively metabolised by N-dealkylation, O-dealkylation, side chain hydroxylation, conjugation and S-oxidation
Toxicologically significant compounds (animals, plants and environment)	Parent compound and a number of metabolites including DEIQ and its precursors (rat).

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	1350 mg/kg bw (Xn- R22)
Rat LD_{50} dermal	4982 mg/kg bw
Rat LC_{50} inhalation	> 4.67 mg/l air/4h (nose-only)
Skin irritation	Non-irritant
Eye irritation	Non-irritant
Skin sensitisation (test method used and result)	Sensitising (M&K); R43

Short term toxicity (Annex IIA, point 5.3)

Target / critical effect	Hematotoxicty (RBC)
Lowest relevant oral NOAEL / NOEL	1 mg/kg bw/day (1-year dog)
Lowest relevant dermal NOAEL / NOEL	200 mg/kg bw/day (21 days, rabbit)
Lowest relevant inhalation NOAEL / NOEL	0.06 mg/l/day (28 days, rat)

Genotoxicity (Annex IIA, point 5.4)

	Positive *in vitro*, negative *in vivo*. No genotoxic potential.

Long term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target / critical effect	Rats: nasal epithelium, liver, eye, stomach and thyroids. Mice: Liver, bone, kidney, and nasal olfactory mucosa.
Lowest relevant NOAEL / NOEL	14 mg/kg bw/day (116-weeks, rat)
Carcinogenicity	Rats: nasal turbinate (it can not be discarded that the tumours are relevant to humans), stomach and thyroid tumours, not relevant to human (species-specific effect, not observed in mice, human or monkey). Carcinogenic cat.3, R40

Reproductive toxicity (Annex IIA, point 5.6

Reproduction target / critical effect	No effects on reproduction parameters. Body and organ weight changes in F0, F2 and F3b generations at maternal toxic doses in rat.
Lowest relevant reproductive NOAEL / NOEL	Reproduction NOAEL=30 mg/kgbw/day Parental NOAEL=10 mg/kgbw/day Developmental NOAEL=10 mg/kgbw/day (3-generation, rats)
Developmental target / critical effect	Rats: increased resorptions and decreases in the mean foetal body weight. Rabbits: no effects.
Lowest relevant developmental NOAEL/NOEL	150 mg/kgbw/day (teratology, rat)

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

No evidence

Other toxicological studies (Annex IIA, point 5.8)

Mechanistic studies in rats	Nasal tumours: This mechanism is based on the production of iminoquinone molecular species, which bind to tissue proteins causing disturbances in cell function and structure and ultimately leading to cell death and regenerative cell proliferation. Iminoquinone protein adducts have not been observed in mice and monkeys. Besides, human nasal tissue was not capable to form the iminoquinone precursor (the p-hydroxy derivative). The mechanism of action could be relevant to humans, although it is unlikely that concentrations of the active metabolite would be achieved to initiate the chain o events termination in cancer. Gastric tumours are generated at very high dose levels through a gastrin-mediated mechanism that does not appear to be operative in primates at similar doses. Thyroid tumours: TSH produces thyroid tumours following chronic stimulation at very high dose levels of the thyroid as a consequence of increased thyroid hormone excretion. This mechanism is not considered relevant to humans.
Toxicity of plant metabolites	Non-toxicologically relevant metabolites
***t*-sulfinyllactic acid** [55]: 9.3% in corn leaves	LD_{50} =>6000; NOAEL=157-207 mg/kgbw/day (90 day rats
***t*-hydroxyoxanilic acid** [67]: 10% in soybean leaves and seeds	LD_{50}= >5000; NOAEL=835-1008 mg/kgbw/day(90 day rats
***t*-hydroxysulphone sugar conjugate** [66]: 13% in soybean leaves	Similar to alachlor
Toxicity of soil metabolites	Non-toxicologically relevant metabolites.
Major metabolites: ***t*-oxanilic acid** ([70] MON 5760, CP10864)	LD_{50}= >5000; NOAEL=835-1008 mg/kgbw/day(90 day rats) No genotoxic potential
***t*-sulfonic acid** ([65] MON 5775, CP108065)	LD_{50} =>6000; NOAEL=157-207 mg/kgbw/day (90 day rats) No genotoxic potential

Transient metabolites: ***t*-hydroxyalachlor** ([39], CP51214)	$LD_{50=}$ >500 to<2000; No genotoxic potential
Minor metabolites: ***s*-sulfonic acid [85]**(MON 5767) **CP108267** ***t*-sulfinylacetic acid** ([54] MON 5768) ***s*-oxanilic acid** ([78] MON 5769)	$LD_{50=}$1548 mg/kg bw/day ("harmfull if swallowed") Non-mutagenic in bacteria. Further genotoxicity data has been received out of date. LD_{50} =>5000; NOAEL=240-296 mg/kgbw/day (90 day rats) No genotoxic potential LD_{50} =>3333; NOEL=500 mg/kgbw/day (teratology, rats) No genotoxic potential
Genotoxicity of mammalian metabolites	No genotoxic potential.
Endocrine disruption potential	Data indicating potential endocrine disrupting activities are not conclussive, and their relevance for risk assessment is controversial. Point open until a developed risk assessment strategy for evaluation of endocrine disrupting chemicals, and/or 2) formal OECD Guidelines for specific tests for endocrine disrupters, become available

Medical data (Annex IIA, point 5.9)

No evidence of toxicological concern from medical surveillance of manufacturing plant personnel.

Summary (Annex IIA, point 5.10)

	Value	**Study**	**Safety factor**
ADI:	0.0025 mg/kg bw/day	2-year, rat	200
AOEL	0.0025 mg/kg bw/day	2-year, rat	200
Drinking water limit	Not allocated		
ArfD (acute referenece dose)	Not allocated		

Dermal absorption (Annex IIIA, point 7.3)

9% (based on Rhesus monkey study *in vivo*)

Acceptable exposure scenarios (including method of calculation)

Lasso EC Operator	Not accepted for proposed uses with UK POEM and German model
Workers	Accepted for proposed uses
Bystanders	Accepted for proposed uses

Estimation of exposure based on bio-monitoring data	Not accepted for proposed uses.

Chapter 4: Residues

Metabolism in plants (Annex IIA, point 6.1 and 6.7; Annex IIIA, point 8.1 and 8.6)

Plants group covered	Cereals (C), Pulses and oilseeds (P/O)
Rotation crops	Radish, wheat, lettuce
Plant residue definition for monitoring	Alachlor and derived metabolites containing the intact aniline moiety or the 1-ethyl hidroxilated aniline moiety, determined as the sum of chromophores DEA (2, 6-diethylaniline and EA (2-Ethylaniline) when acid hydrolisis is employed or DEA and 1'-HEEA (2-(1'-hydroxyethyl)-6-ethylaniline upon basic hydrolisis, expressed as parent alachlor.
Plant residue definition for risk assessment	Idem
Conversion factor (monitoring to risk assessment)	-

Metabolism in livestock (Annex IIA, point 6.2 and 6.7; Annex IIIA, point 8.1 and 8.6)

Animals covered	Animal intake very low (not required)
Animal residue definition for monitoring	
Animal residue definition for risk assessment	
Conversion factor (monitoring to risk assessment)	-
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Yes

Residues in succeeding crops (Annex IIA, point 6.6; Annex IIIA, point 8.5)

Only cereal as rotational crop

Stability of residues (Annex IIA, point 6 introduction; Annex IIIA, point 8 introduction)

DEA and HEEA alachlor metabolites stable in field corn forage; field corn grain; milo forage; milo fodder, milo grain; and soyabean under –18ºC during 689-1394 days.

Residues from livestock feeding studies (Annex IIA, point 6.4; Annex IIIA, point 8.3)

Intakes by livestock < 0.1 mg/kg diet/day	Ruminant: yes/no	Poultry: Yes/no	Pig: Yes/no
Muscle			
Liver			
Kidney			
Fat			
Milk			
Eggs			

Summary of critical residues data (Annex IIA, point 6.3; Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region	Trials results revelant to the critical GAP [(a)]	Recommendation/comments	MRL	STMR [(b)]
Cotton	S (W)	11 trials (all of them < 0.02*)		0.02*	<0.02
Maize/corn	N	21 trials (all of them < 0.02*)	PHI = 90 days	0.02*	<0.02
Maize/corn	S	9 trials (all of them < 0.02*)	PHI = 90 days	0.02*	<0.02
Maize/corn	W	36 trials (all of them < 0.02*)	PHI = 90 days	0.02*	<0.02
Soyabean	S (W)	22 trials (3x0.02, 10x0.04, 2x0.05, 0.06, 0.08, 0.09, 3x0.11, 0.12)		0.2	0.06
Sunflower	S (W)	7 trials (0.06, 0.11, 2x0.12, 0.22, 0.45, 0.67)		1.0	0.25
Sweet corn	S	12 x < 0.05*		0.05*	<0.05
Peanut			Residue trials required to M-Agan and Sinon		
Cabbage			Residue trials required to M-Agan and Sinon		
Cauliflower			Residue trials required to M-Agan and Sinon		
Peas			Residue trials required to M-Agan		

Consumer risk assessment (Annex IIA, point 6.9; Annex IIIA, point 8.8)

ADI	0.0025 mg/kg bw/day
TMDI (WHO)(% ADI)	2.64 %
TMDI (Chlidren) (%ADI)	5.68%
IEDI (European Diet) (% ADI)	
Factors included in IEDI	
ARfD	Not allocated
Acute exposure (% ArfD)	

Processing factors (Annex IIA, point 6.5; Annex IIIA, point 8.4)

Crop/proccessed crop	Number of studies	Transfer factor	% Transference
Maize deodorised oil	2		Not transference
Maize starch	6		Not transference
Soybean protein concentrate	15		Not transference
Soyabean protein isolate	15		Not transference

[(a)] Numbers of trial in which particular residue levels were reported *e.g.* 3 x <0.01, 1 x 0.01, 6 x 0.02, 1 x 0.04, 1 x 0.08, 2 x 0.1, 2 x 0.15, 1 x 0.17

[(b)] Supervised Trials Median Residue *i.e.* the median residue level estimated on the basis of supervised trials relating to the critical GAP

Soyabean refined oil	19		20%
Soyabean deodorised oil	5		Not transference
Sunflower deodorised oil	3		Not transference

Proposed MRLs (Annex IIA, point 6.7; Annex IIIA, point 8.6)

Cotton (seed)	0.02*
Maize (grain)	0.02*
Sweet corn (grain)	0.05*
Soyabean (grain)	0.2
Sunflower (seed)	1.0

Chapter 5: Fate and behaviour in the environment

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)
Data from reviews has not been included.

Mineralization after (120) days	1 study with 4 soils 6.9%, 28,6%, 30.9%, 22.0% (120 days)
Non-extractable residues after 120 days	1 study with 4 soils 42.2%, 37.9%, 33.0%, 49.9% (120 days)
Major metabolites – name and/or code, % of applied (range and maximum)	1 study with 4 soils t-sulfonic acid (Met **65**) 18.0-12.2% (29-58 DAT) t-oxanilic acid (Met **70**) 14.3-10.6% (30-23 DAT) s-sulfonic acid (Met **85**) 13.2-12.0% (86-30 DAT) t-sulfinylacetic acid (Met **54**) 9.4% (58 DAT)

Route of degradation in soil – Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation	No studies on the anaerobic degradation of alachlor in soils has been presented. No required
Soil photolysis (30 d)	1.1 % volatiles; 3.9 % unextracted; 90% a.s.; 3.7 % metabolite ketoalachlor (max), 9 unknowns, sum of all < 10%.

Rate of degradation in soil (Annex IIA, point 7.1.1.2; Annex IIIA, point 9.1.1)

Method of calculation	Gustafson-Holden
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab}(20ºC aerobic): 6.1-15.8 d; n = 2. DT_{50lab}(18-30ºC aerobic): 8-43d; n = 7. DT_{50lab} (20°C aerobic): 9-24 d, n = 3 transformed to 20º C DT_{50lab} (20°C aerobic): 7.8-15.3 d, n = 3 n=3 r^2=0.98-0.99
	DT_{90lab} (20°C aerobic): 30-80 d DT_{90lab} (20°C aerobic): 25.8-56.9d n=3 r^2=0.98-0.99
	DT_{50lab} (10°C aerobic): 46.8d n=1 r^2=0.99 DT_{90lab} (20ºC aerobic): 129d n=1 r^2=0.99
	DT_{50lab} (20°C anaerobic): Not required
	Degradation in the saturated zone: no data
Metabolites	t-sulfonic acid (**65**): DT_{50}=55.4-76.8; n =3; r^2=0.97-0.85 s-sulfonic acid (**85**): DT_{50}=62.9d; n=1; r^2=0.82 t-Oxanilic acid (**70**): DT_{50}=2.9-9.4; n=3; r^2=0.99-0.92 s-Oxanilic acid (**78**): DT_{50}=3-17; n=3; r^2=0.98-0.99 t-Sulfonil Acetic Acid (**54**): DT_{50}=21-7-51.2d; n=2; r^2=0.97-0.99 t-Hydroxy Alchlor (**39**): DT_{50}=18.1-34.1d; n=3; r^2=0.94-0.84 s-Hydroxy Alachlor (**76**): DT_{50}=38.5-75.0d; n=3; r^2=0.82-0.74 t-Norchloro Alachlor (**52**): DT_{50}=61.3-64.7d; n=2; r^2=0.73-0.83 s-Norchloro Alachlor (**51**): DT_{50}=16.8-76.4d; n=3; r^2=0.60-0.71 t-Methylsulfone (**26**): DT_{50}=25.3d; n=1; r^2=0.98 t-Methylsulfoxide (**25**): DT_{50}=26.4-126.4; n=4; r^2=0.99-0.81
Field studies (state location, range or median with n value)	DT_{50f}: California 5.7 d, n = 1 DT_{50f}: 11-24 d, one soil, three different application dates. DT_{50f}: 4.0-13 d, n = 5
t-sulfonic acid (Met 65)	DT_{50f}: California 27.3 d, n = 1 (First order since maximum)

t-oxanilic acid (Met 70)	DT_{50f}: California 42.6 d n = 1 (First order since maximum)
t-sulfinylacetic acid (Met 54)	DT_{50f}: California 38.5 d n = 1 (First order since maximum)
Alachlor	DT_{90f} California 42 d DT_{90f}: 37-80 d, one soil, three different application dates
Soil accumulation and plateau concentration	No accumulation is expected

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f / K_{oc}

Alachlor (only desorption)

Soil type	K_f	K_{oc}	1/n
silt loam	1.85	103.9	0.85
silt loam	0.97	131.1	0.92
sandy loam	0.87	150.0	0.94
loamy sand	1.17	101.7	0.83
Loamy sand	3.32	152	0.96
Loam	1.91	157	0.99
Clay loam	3.90	131	0.96
Silt loam	3.78	192	0.96

K_d

Alachlor (only desorption)

Soil type	Kd	Koc
silt loam	1.39	78.1
silt loam	0.84	113.5
sandy loam	0.78	134
loamy sand	0.85	73.9
Loamy sand		213
Loam		198
Clay loam		196
Silt loam		313

Metabolites

t-sulfonic acid (**65**): 7.8 L/kg
t-oxanilic acid (**70**) : 2 L/kg
t-sulfinylacetic acid (**54**): 10.8 L/kg
s-oxanilic acid (**78**): 24.3 L/kg
s-sulfonic acid (**85**): 0 L/kg (estimated by HPLC)
t-methylsulfoxide (**25**): 39 L/kg (estimated by HPLC)
t-methylsulfone (**26**): 98 L/kg (estimated by HPLC)
t-hydroxyalachlor (**39**): 78 L/kg
s-hydroxyalachlor (**76**): 22 L/kg (estimated by HPLC)
t-norchloroalachlor (**52**): 75 L/kg
s-norchloroalachlor (**51**): 26 L/kg (estimated by HPLC)

pH dependence (yes / no) (if yes type of dependence)

Yes, however desorption has been studied in soils with pH =6.8 to 8.0. K_{oc} increased with pH.

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching

Soil	% OM	K	% Leached beyond 30 cm soil
Drummer	3.4	3.7	0.5
Spinks	2.4	1.3	42.5
Ray	1.2	0.9	82.0
Lintonia	0.7	0.3	92.0

Spinks, Ray and Lintonia a 99 % of leached residue was extractable in CH_2Cl_2. This extract is mainly alachlor.
Drummer: of the eluted radioactivity a 20 % was soluble in water, 23% was 2'6'-diethyl-N-methoxymethyl-acetanilide (52), 19% was 2-chloro-2'6'-diethylacetanilide and 5% was 2'6'-diethyl-N-methoxymethyl-2-methyl-thio-acetanilide (24).

Aged residues leaching

%OM 2.4 (Recommended 0.25-0.75)

	Organic matter has a very high effect on the mobility of alachlor. Thus the mobility observed in this study should be significantly lower than that expected for soils with organic matter content requested by the Annex II. At the end of the study (75 DAT) a 30% of TAR in leachate while 61% of TAR in soil column. t-sulfonic acid and t-oxanilic acid in leachate (both 15.3% of TAR)
Lysimeter/field leaching studies	Location: English Midlands Applied dose 1.92 kg/ha Field leaching study (Autumn Oilseed Rape) Clayey soil Averaged yearly concentration in soil water (μg/L)

Depth	1990	1991	1992
1 m	<0.05		

Sandy soil Averaged yearly concentration in soil water (μg/L)			
Depth	1990	1991	1992
1.0 m	<0.05		
1.5 m		0.62- 34.3*	0.06- 1.35

* Faulty installation suspected for increased leaching below topsoil

	Location: English Midlands Applied dose 1.92 kg/ha Field leaching study (Spring Fodder Maize) Clayey soil Averaged yearly concentration in soil water (μg/L)

Depth	1991	1992	1993
1.0 m		0.08- 0.16	

Sandy soil Averaged yearly concentration in soil water (μg/L)			
Depth	1991	1992	1993
1.0 m	<0.05		
1.5 m		0.09- 0.73	<0.05 -0.37

Alachlor PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	First order kinetics
Application rate	3.36 Kg a.s./ha, (Restricted to OM higuer than 4%) DT50=30 days. 0% interception, 5 cm soil density 1.5 g/mL

$PEC_{(s)}$ mg/Kg		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial		4.480	-		
Short term	24h	4.378	4.429		
	2d	4.278	4.378		
	4d	4.085	4.279		
Long term	7d	3.811	4.136		
	28d	2.346	3.299		
	50d	1.411	2.656		
	100d	0.444	1.747		

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. 5 cm

$PEC_{(s)}$	Single	Single	Multiple	Multiple

mg/Kg		application Actual	application Time weighted average	application Actual	application Time weighted average
Initial		3.2	-	-	
Short term	24h	3.127	3.163		
	2d	3..055	3.127		
	4d	2.917	3.056		
Long term	7d	2.722	2.954		
	28d	1.675	2.356		
	50d	1.007	1.897		
	100d	0.317	1.247		

Alachlor Metabolites PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception.

Initial PEC soil:

Metabolites	Maximum concentration (% of applied radioactivity)	Correction factor for Molecular weight	PECs initial (0-20cm) (mg/kg)
t-Sulfonic acid(**65**)	18.0	1.1692	0.1684
s-Sulfonic acid (**85**)	13.2	1.006	0.1062
t-Sulfinylacetic acid (**54**)	9.4	1.2656	0.0952
t-Methylsulfoxide (**25**)	3.4	1.0988	0.0299
t-Methylsulfone (**26**)	3.1	1.1618	0.0288
t-Oxanilic acid (**70**)	14.3	0.9835	0.1125
s-Oxanilic acid (**78**)	4.6	0.8202	0.0302
t-Hydroxyalachlor (**39**)	1.7	0.9315	0.0127
s-Hydroxyalachlor (**76**)	3.6	0.7695	0.0222
t-Norchloroalachlor (**52**)	1.6	0.8723	0.0112
s-Norchloroalachlor (**51**)	3.5	0.7091	0.0199

t-Sulfonic acid (65)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=73.7d

PECs		Single application Actual (mg/kg)	Single application Time Weighted Average (mg/kg)
Initial	**0-20 cm**	0.1684	-
Short term	**24h**	0.1669	0.1676
	2d	0.1654	0.1669
	4d	0.1624	0.1654
Long term	**7d**	0.1581	0.1632
	28d	0.1308	0.1488
	50d	0.1072	0.1355
	100d	0.0683	0.1109

s-Sulfonic acid (85)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=62.9d

PECs	Single application Actual (mg/kg)	Single application Time Weighted Average

			(mg/kg)
Initial	**0-20 cm**	0.1062	-
Short term	**24h**	0.1051	0.1056
	2d	0.1039	0.1051
	4d	0.1016	0.1039
Long term	**7d**	0.0983	0.1022
	28d	0.0780	0.0914
	50d	0.0612	0.0817
	100d	0.0353	0.0644

t-Sulfinylacetic acid (54)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=51.2d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	**0-20 cm**	0.0952	-
Short term	**24h**	0.0939	0.0945
	2d	0.0926	0.0939
	4d	0.0902	0.0926
Long term	7d	0.0866	0.0908
	28d	0.0651	0.0792
	50d	0.0484	0.0692
	100d	0.0246	0.0521

t-Methylsulfoxide (25)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=126.4d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0299	-
Short term	24h	0.0297	0.0298
	2d	0.0296	0.0297
	4d	0.0292	0.0296
Long term	7d	0.0288	0.0293
	28d	0.0256	0.0277
	50d	0.0227	0.0261
	100d	0.0173	0.0230

t-Methylsulfone (26)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=25.3d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0288	0.0288
Short term	24h	0.0280	0.0284
	2d	0.0273	0.0280
	4d	0.0258	0.0273
Long term	7d	0.0238	0.0262
	28d	0.0134	0.0201
	50d	0.0073	0.0157
	100d	0.0019	0.0098

t-Oxanilic acid (70)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=11.8d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.1125	0.1125
Short term	24h	0.1061	0.1093
	2d	0.1000	0.1062
	4d	0.0889	0.1003
Long term	7d	0.0746	0.0922
	28d	0.0217	0.0552
	50d	0.0060	0.0363
	100d	0.0003	0.0191

s-Oxanilic acid (78)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=16.3d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0302	-
Short term	24h	0.0290	0.0296
	2d	0.0278	0.0290
	4d	0.0257	0.0279
Long term	7d	0.0227	0.0263
	28d	0.0097	0.0180
	50d	0.0040	0.0129
	100d	0.0005	0.0073

t-Hydroxyalachlor (39)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=34.1d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0127	-
Short term	24h	0.0124	0.0125
	2d	0.0122	0.0124
	4d	0.0117	0.0122
Long term	7d	0.0110	0.0118
	28d	0.0072	0.0097
	50d	0.0046	0.0080
	100d	0.0017	0.0054

s-Hydroxyalachlor (76)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=75d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0222	-
Short term	24h	0.0220	0.0221

	2d	0.0218	0.0220
	4d	0.0214	0.0218
Long term	7d	0.0208	0.0215
	28d	0.0171	0.0195
	50d	0.0140	0.0177
	100d	0.0088	0.0145

t-Norchloroalachlor (52)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=64.7d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0112	-
Short term	24h	0.0110	0.0111
	2d	0.0109	0.0110
	4d	0.0107	0.0109
Long term	7d	0.0104	0.0108
	28d	0.0083	0.0096
	50d	0.0065	0.0086
	100d	0.0038	0.0069

s-Norchloroalachlor (51)

Method of calculation	First order kinetics
Application rate	2.4 Kg a.s./ha 0% interception. DT50=76.4d

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	0-20 cm	0.0199	0.0199
Short term	24h	0.0195	0.0198
	2d	0.0191	0.0197
	4d	0.0181	0.0195
Long term	7d	0.0186	0.0192
	28d	0.0154	0.0175
	50d	0.0126	0.0160
	100d	0.0080	0.0131

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	pH = 5 DT50 > 1 y, 25 ºC
	pH = 7 DT50 > 1 y, 25 ºC
	pH = 9 DT50 > 1 y, 25 ºC
	No data available for metabolites
Photolytic degradation of active substance and relevant metabolites	$\varepsilon < 10$ at $\lambda = 290$ nm.
Readily biodegradable (yes/no)	Non-ready biodegradable
Water-Sediment Degradation in -DT_{50} water	23.7-22.24 d, n=2, r^2=0.93-0.91(First order kinetics)
Water/sediment -DT_{90} water	78.7-73.9 d, n=2, r^2=0.93-0.91(First order kinetics)
- DT_{50} whole system	21.1-41.7 d, n=2, r^2= 0.98-0.98 (First order kinetics)
- DT_{90} whole system	70.22-138.7 d, n=2, r^2=0.98-0.98 (First order kinetics)

Distribution in water / sediment systems (active substance)	1.08% – 3.75% (100 days)
Distribution in water / sediment systems (metabolites)	**(52) *t*-acetamide**: 8.23% (water) (14 days) 15.15%-19.68% (sediment) (100 days) No degradation was observed in the system

PEC (surface water) (Annex IIIA, point 9.2.3)

Method of calculation	a s, 1.Order, DT_{50} 42 d, depth of water 0.3 m (drift values Ganzelmeier et al.2000)
Application rate	3360 g/ha. Restricted to soils with OM > 4%.
Main routes of entry	Spray drift

$PEC_{(sw)}$ µg/l		Single application Actual 1 m distance	Single application Time weighted average 1 m distance
Initial		31.02400	-
Short term	24 h	30.51620	30.76940
	2 d	30.01671	30.51758
	4 d	29.52539	30.26851
Long term	7 d	29.04212	30.02216
	14 d	27.63924	29.29904
	21 d	26.30413	28.59918
	28 d	24.62377	27.70076
	42 d	21.93728	26.21873

Method of calculation	a s, 1.Order, DT_{50} 42 d, depth of water 0.3 m (drift values Ganzelmeier et al.2000)
Application rate	2400 g/ha
Main routes of entry	Spray drift

$PEC_{(sw)}$ µg/l	Single application Actual 1 m distance	Single application Time weighted average 1 m distance	Single application Actual 5 m distance	Single application Time weighted average 5 m distance
Initial	22.16	-	4.56	-
Short term				
24 h	21.80	21.98	4.49	4.52
2 d	21.44	21.80	4.41	4.49
4 d	20.74	21.44	4.27	4.41
Long term				
7 d	19.74	20.93	4.06	4.31
14 d	15.67	18.73	3.22	3.85
21 d	13.96	17.75	2.87	3.65
28 d	11.08	15.99	2.28	3.29
42 d	22.16	22.16	4.56	4.56

Method of calculation	a s, 1.Order, DT_{50} 42 d
Application rate	2400 g a.s./ha
Main routes of entry	run-off; 0.1 dilution factor

$PEC_{(sw)}$		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial		2.65000	-		
Short term	24h	2.60662	2.62825		
	2d	2.56396	2.60674		
	4d	2.52199	2.58547		
Long term	7h	2.48071	2.56442		
	21d	2.36088	2.50266		
	28d	2.24684	2.44288		
	42d	2.10331	2.36614		

PEC (surface water) Metabolites

Method of calculation	depth of water 0.3 m (drift values Ganzelmeier et al.2000)Maximum % of each metabolite from water/sediment study
Application rate	2400 g/ha
Main routes of entry	Spray drift

Metabolites	**$PEC_{sw\ initial}$ drift (µg/l)**
t-Sulfonic acid (65)	1.3
t-Oxanilic acid (70)	1.72
s-Oxanilic acid (78)	1.44
t-Norchloroalachlor (52)	1.58
s-Norchloroalachlor (51)	0.3

PEC (sediment) Alachlor

Method of calculation	33.1% Alachlor in sedimentSediment depth 5 cm 0.5 g/cm^3 DT50=20.9d
Application rate	2400 g/ha
Main routes of entry	Spray drift

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	**0-5 cm**	0.0880	-
Short term	**24h**	0.0851	0.0866
	2d	0.0824	0.0851
	4d	0.0771	0.0824
Long term	**7d**	0.0698	0.0785
	28d	0.0348	0.0573
	50d	0.0168	0.0430
	100d	0.0032	0.0256

PEC (sediment) Metabolite 52

Method of calculation	19.7% Met 52 in sediment. Sediment depth 5 cm 0.5 g/cm^3 DT50=87.7d
Application rate	2400 g/ha
Main routes of entry	Spray drift

PECs		**Single application Actual (mg/kg)**	**Single application Time Weighted Average (mg/kg)**
Initial	**5 cm**	0.0460	-

Short term	**24h**	0.0456	0.0458
	2d	0.0453	0.0456
	4d	0.0446	0.0453
Long term	**7d**	0.0435	0.0448
	28d	0.0369	0.0413
	50d	0.0310	0.0380
	100d	0.0209	0.0318

PEC (ground water) (Annex IIIA. point 9.2.1)

Method of calculation and type of study (*e.g.* modelling. monitoring. Lysimeter)	Modelling
Application rate	2.9 Kg/ha

$PEC_{(gw)}$

26 consecutive yearly application.
Maize FOCUS scenarios

Compounds	**80th percentile of mean annual concentrations in groundwater**							
	PELMO							
	Chateau-dun	Hamburg	Krems-munster	Okehamp-ton	Piacenza	Porto	Sevilla	Thiva
Alachlor	**0.000**	**0.000**	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.000**
t-Sulfonic acid (**65**)	10.611	25.910	20.207	20.965	11.835	6.268	0.213	1.094
s-Sulfonic acid (**85**)	7.321	15.846	14.88	12.819	6.799	7.473	0.307	0.703
t-Sulfinylacetic acid (**54**)	3.362	9.167	6.568	7.669	4.365	1.344	0.021	0.211
t-Methylsulfoxide (**25**)	0.679	1.705	1.147	1.345	1.929	0.032	0.000	0.056
t-Methylsulfone (**26**)	0.000	0.000	0.000	0.000	0.005	0.000	0.000	0.000
t-Oxanilic acid (**70**)	0.005	0.111	0.034	0.069	0.040	0.003	0.000	0.000
s-Oxanilic acid (**78**)	0.000	0.003	0.000	0.001	0.003	0.000	0.000	0.000
t-Hydroxyalachlor (**39**)	0.000	0.007	0.001	0.002	0.035	0.000	0.000	0.000
s-Hydroxyalachlor (**76**)	0.780	1.900	1.277	1.427	1.350	0.109	0.000	0.053
t-Norchloroalachlor (**52**)	0.009	0.053	0.016	0.029	0.179	0.000	0.000	0.000
s-Norchloroalachlor (**51**)	0.589	1.278	0.943	1.038	1.029	0.073	0.000	0.066
Compounds	**PEARL**							
	Chateau-dun	Hamburg	Krems-munster	Okehamp-ton	Piacenza	Porto	Sevilla	Thiva
Alachlor	**0.000**	**0.001**	**0.000**	**0.002**	**0.014**	**0.000**	**0.000**	**0.000**
t-Sulfonic acid (**65**)	21.738	35.380	23.076	21.191	*	7.422	5.431	18.334
s-Sulfonic acid (**85**)	14.775	23.539	17.957	13.149	9.518	6.265	4.380	10.888
t-Sulfinylacetic acid (**54**)	7.832	12.978	8.562	7.954	*	1.903	1.555	5.169
t-Methylsulfoxide (**25**)	2.104	2.696	1.943	2.327	2.449	0.228	0.459	1.784
t-Methylsulfone (**26**)	0.001	0.003	0.002	0.003	0.030	0.000	0.000	0.002
t-Oxanilic acid (**70**)	0.070	0.577	0.234	0.240	0.136	0.005	0.004	0.083
s-Oxanilic acid (**78**)	0.003	0.010	0.006	0.012	0.011	0.000	0.000	0.003
t-Hydroxyalachlor (**39**)	0.015	0.022	0.016	0.030	0.066	0.000	0.001	0.017
s-Hydroxyalachlor (**76**)	1.750	2.392	1.814	1.973	1.820	0.271	0.375	1.427
t-Norchloroalachlor (**52**)	0.101	0.136	0.115	0.143	0.276	0.001	0.013	0.109
s-Norchloroalachlor (**51**)	1.270	1.602	1.255	1.379	1.337	0.215	0.321	1.063

$PEC_{(gw)}$

Alachlor applied one out of two years (46 years simulation)
Maize FOCUS scenarios

Compounds	80th percentile of Groundwater concentrations							
	PELMO							
	Chateau-dun	Hamburg	Krems-munster	Okehamp-ton	Piacenza	Porto	Sevilla	Thiva
Alachlor	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**
t-Sulfonic acid (**65**)	7.217	17.885	13.709	15.240	7.858	3.838	0.04	0.527
s-Sulfonic acid (**85**)	5.118	10.102	9.477	7.810	4.667	3.974	0.065	0.276
t-Sulfinylacetic acid (**54**)	1.994	5.931	3.938	5.365	3.125	0.524	0.003	0.090
t-Methylsulfoxide (**25**)	0.331	0.885	0.446	0.752	1.257	0.012	0.000	0.021
t-Methylsulfone (**26**)	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000
t-Oxanilic acid (**70**)	0.004	0.063	0.020	0.047	0.027	0.001	0.000	0.000
s-Oxanilic acid (**78**)	0.000	0.001	0.000	0.000	0.002	0.000	0.000	0.000
t-Hydroxyalachlor (**39**)	0.000	0.003	0.000	0.001	0.020	0.000	0.000	0.000
s-Hydroxyalachlor (**76**)	0.391	0.797	0.565	0.890	0.840	0.040	0.000	0.018
t-Norchloroalachlor (**52**)	0.004	0.022	0.006	0.011	0.093	0.000	0.000	0.000
s-Norchloroalachlor (**51**)	0.308	0.611	0.430	0.575	0.587	0.030	0.000	0.024
Compounds	**PEARL**							
	Chateau-dun	Hamburg	Krems-munster	Okehamp-ton	Piacenza	Porto	Sevilla	Thiva
Alachlor	**0.000**	**0.000**	**0.000**	**0.001**	**0.007**	**0.000**	**0.000**	**0.000**
t-Sulfonic acid (**65**)	10.498	16.648	12.836	10.865	*	4.100	*	9.057
s-Sulfonic acid (**85**)	6.775	11.133	9.191	6.536	4.871	3.128	2.269	6.403
t-Sulfinylacetic acid (**54**)	3.928	6.135	4.637	4.242	*	0.937	*	3.225
t-Methylsulfoxide (**25**)	1.077	1.272	0.935	1.236	1.261	0.093	*	0.826
t-Methylsulfone (**26**)	0.000	0.001	0.001	0.001	0.015	0.000	0.000	0.001
t-Oxanilic acid (**70**)	0.038	0.285	*	0.134	*	0.003	*	0.025
s-Oxanilic acid (**78**)	0.002	0.005	0.004	0.006	0.006	0.00	0.000	0.001
t-Hydroxyalachlor (**39**)	0.007	0.010	0.007	0.014	0.038	0.000	0.001	0.006
s-Hydroxyalachlor (**76**)	0.953	1.325	0.926	0.979	*	0.147	0.199	0.693
t-Norchloroalachlor (**52**)	0.045	0.066	0.048	0.065	0.147	0.000	0.005	0.050
s-Norchloroalachlor (**51**)	0.686	0.858	0.621	0.690	0.631	0.104	*	0.534

Fate and behaviour in air (Annex IIA. point 7.2.2; Annex IIIA. point 9.3)

Direct photolysis in air	
Photochemical oxidative degradation in air (DT_{50})	2.6 h Atkinson model
Volatilization	From plant surfaces: No data available From soil: No data available

PEC (air)

Method of calculation	

Definition of the Residue (Annex IIA. point 7.3)

Relevant to the environmental	Soil: Alachlor, *t*-sulfonic acid (65), *t*-oxanilic acid (70), sulfinylacetic acid (54), *s*-oxanilic acid (78), *s*-sulfonic acid (85) Water/sediment: Alachlor, *t*-norchloroalachlor (52) Groundwater: Alachlor, *t*-sulfonic acid (65), *t*-oxanilic acid (70), sulfinylacetic acid (54) and *s*-oxanilic acid (78), *s*-sulfonic acid (85)

Monitoring data. <u>if available</u> (Annex IIA. point 7.4)

Soil (<u>indicate location and type of study</u>)	No data available

Surface water (indicate location and type of study)

Monitoring.

Location	No. of detections above 0.1 µg/L	Range (µg/L)
River Po Italy	0 of 12	
River Po	2 of 11	0.14-0.21
River Po	12 of 85	0.1-0.48
River Po	0 of 24	-
River Arno (5 sites) Italy	6 of 25	0.13-0.27
Aqua Bonifica Italy		<0.5
River Po	2 of 10	max 2.78
River Po (91-95). 4 sites	4 of 438	<1
Alessandria Province 94-95 Italy	0 of 415	
Treviso (94-95) Italy	0 of 181	
Trentino (1995) Italy	0 of 76	
Loire-Bretagne (21 sites) France	0 of 88	
Loire-Bretagne (16 sites) France	0 of 112	
Bretagne (5 sites)	3 of 55	0.2-0.23
Bretagne (7 sites)	2 of 26	max 0.2
Bretagne (1 site)	0 of 11	
Bassin Garonne France	1 of 31	0.2
Bassin Adour France	4 of 19	-
Rhin-Meuse	0 of 151	
Bassin Charante France	1 of 12	0.11
Center France	0 of 31	
France (1990-1999)	9 of 3128	
Greece (1990-1996)	23 of 388	
Italy (1992-	3 of 176	

1995)		
Spain (1991-1998)	3 of 48	

Ground water (indicate location and type of study)

Monitoring

Location	**No. of detections above 0.1 µg/L**	**Range (µg/L)**
River Po Italy 37 sites. 8 sampling times	0 of 37	-
Veneto Italy	1 of 98	-
Lombardia Italy	2 of 224	-
Marche Italy	0 of 134	
Torino Italy	0 of 90	
Vicenza (22 wells) Italy	3 of 213	0.11-0.54
N. Italy (55 sites)	4 of 220	0.11 – 0.16
Sondino Italy	0 of 4	
Trebia-Nure (28 wells) Italy	0 of 28	
Vercelli/Lomellina (Italy)	0 of 424	
Milano Italy	0 of 1753	
Arezzo Italy	0 of 113	
Alessandria Province (94-95) Italy	2 of 508	-
Treviso Italy (94-95)	0 of 1287	
Alto Adige (1995)	0 of 31	
Udine Italy Irrigation Wells	17 of 200 12 of 300	0.2-0.6 -
River Po Italy Irrigation Wells	2 of 2	0.14-1.15
France, Greece, Italy, Spain	9 of >3717	Max 0.8

Ground water Monitoring for metabolites

Analyte	**USA; 1995-2001; 188 groundwater samples**			
	Results (µg/L)			
	min	**Max**	**Average**	**95th Percentile**
t-sulfonic acid (**65**)	<0.05	14.7	0.629	3.07
t-oxanilic acid (**70**)	<0.05	3.12	<0.05	<0.05

t-sulfinylacetic acid (**54**)	<0.05	<0.05	<0.05	<0.05
s-sulfonic acid (**85**)	<0.05	0.946	<0.05	0.227
s-oxanilic acid (**78**)	<0.05	<0.05	<0.05	<0.05
t-methylsulfone (**26**)	<0.05	0.235	<0.05	<0.05

The most frequently detected compound is t-sulfonic acid (**65**), followed by the s-sulfonic acid (**85**) and the t-oxanilic acid (**70**). Traces of t-methylsulfone (**26**) and s-oxanilic acid (**78**) could be found in some samples, but t-sulinylacetic acid (**54**) was never observed. Non-polar degradates (**39, 76, 52, 51**)were not detected in groundwater samples.

The risk assessment of the relevance of metabolites should focus on metabolites **65, 85 and 70**

Air (indicate location and type of study)	No data available

Chapter 6: Effects on non-target species

Effects on terrestrial vertebrates (Annex IIA, point 8.1; Annex IIIA, points 10.1 and 10.3)

Acute toxicity to mammals	Rat LD50= 1350 mg/kg bw
Long term toxicity to mammals	Developmental NOAEL= 150 mg/kg bw/day (rat) Reproductive NOAEL= 10 mg/kg bw/day (3-generation rat study)
Acute toxicity to birds	Chicken LD50= 916 mg/kg
Dietary toxicity to birds	Bobwhite quail and mallard duck >5620 ppm (active ingredient and formulation)
Reproductive toxicity to birds	Mallard duck NOEC =50 ppm ai (4.97 mg/kg bw/day)

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Application rate (kg as/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	TER	Annex VI Trigger
2.4	All crops	Large birds eating grass	Acute	13.7	10
2.4	All crops	Large birds eating grass	Dietary	20.9	10
2.4	All crops	Large birds eating grass	Long-term	0.19	5
2.4	All crops	Earthworm-eating Birds	Long-term	19.88	5
2.4	All crops	Mammals	Acute	14.3	10
2.4	All crops	Mammals	Long-term	2.23	5
2.4	All crops	Mammals	Long-term	1.86	5
2.4	All crops	Earthworm-eating mammals	Long-term	469	5
The application of alachlor in post-emergence is not supported by the available data. Only Pre-emergence of crops and weeds application is supported.					

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
Laboratory tests				
Fish	Alachlor technical	Acute	96 h LC50	1.8
Fish	Alachlor formulation (Lasso M)	Acute	96 h LC50	1.5 (ai)
Fish	Metabolite 65	Acute	96 h LC50	>104
Fish	Metabolite 70	Acute	96 h LC50	>100
Fish	Metabolite 54	Acute	96 h LC50	>127
Fish	Metabolite 78	Acute	96 h LC50	>121
Fish	Metabolite 39	Acute	96 h LC50	55 (38-65)
Fish	Alachlor technical	Chronic	96 d NOEC	0.19
Fish	Alachlor formulation (Sanachlor 480 EC)	Chronic	14 days NOEC	0.25
Daphnia magna	Alachlor technical	Acute	48 h LC50	10
Daphnia magna	Alachlor formulation	Acute	48 h LC50	7.2 (ai)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
	(Alachlor 480 g/l EC)			
Daphnia magna	Metabolite 65	Acute	48 h LC50	>105
Daphnia magna	Metabolite 70	Acute	48 h LC50	>95
Daphnia magna	Metabolite 54	Acute	48 h LC50	> 126
Daphnia magna	Metabolite 52	Chronic	21 d NOEC	7.4
Daphnia magna	Alachlor technical	Chronic	21 d NOEC	0.23
Daphnia magna	Alachlor formulation (Salachlor 480 EC)	Chronic	21 d NOEC	0.23
Chironomus riparius	Alachlor technical	Chronic	28d NOEC	0.75
Algae	Alachlor technical	Chronic	120 h NOEC	0.00035
Algae *(Selenastrum capricornutum)*	Alachlor technical	Acute	96 h EC50	0.0029
Algae	Alachlor formulation (alachlor 480 g/l)	Chronic	72 h NOEC	0.0022 (0.001 ai)
Algae	Alachlor technical	Acute	EC50 72 h	0.0019
Algae	Alachlor formulation (alachlor 480 g/l)	Acute	EC50 72 h	0.0063 (0.003024 ai)
Algae	Alachlor formulation (alachlor 42.55%)	Acute	EC50 72 h	0.057
Algae *(Selenastrum capricornutum)*	Alachlor formulation (Lasso EC)	Acute	EC50 72 h	0.0026 (ai)
Algae (*Skeletonema costatum)*	Alachlor formulation (Lasso EC)	Acute	EC50 72 h	0.167 (ai)
Algae (*Selenastrum capricornutum)*	Alachlor formulation (Lasso MT)	Acute	EC50 72h	0.0196(ai)
Algae (*Skeletonema costatum)*	Alachlor formulation (Lasso MT)	Acute	EC50 72h	>0.226 (ai)
Aquatic plants (*Lemna gibba)*	Alachlor formulation (Lasso EC)	Acute	EC 50 7 d	0.0068 (ai)
Aquatic plants (*Lemna gibba)*	Alachlor formulation (Lasso MT)	Acute	EC 50 7 d	0.119 (ai)
Aquatic plants (*Gliceria maxima)*	Alachlor formulation (Lasso MT	Acute	EC 50 21 d NOEC 21d	>0.220 (ai) 0.220 (ai)
Aquatic plants (*Lagarosiphon major)*	Alachlor formulation (Lasso MT	Acute	EC 50 14 d NOEC 14 d	0.251 (ai) 0.0647 (ai)
Algae	Metabolite 65	Acute	72 h EC50	3.5
Algae (*Navicula pelliculosa)*	Metabolite 70	Acute	96 h EC50	>132
Algae *(Selenastrum*	Metabolite 70	Acute	72 h EC50	>123

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
capricornutum)				
Algae (*Navicula pelliculosa)*	Metabolite 54	Acute	72 h EC50	46
Algae *(Selenastrum capricornutum)*	Metabolite 54	Acute	72 and 96h EC50	>127
Algae (*Navicula pelliculosa)*	Metabolite 78	Acute	96 h EC50	>116
Algae *(Selenastrum capricornutum)*	Metabolite 78	Acute	96 h EC50	>116
Algae *(Selenastrum capricornutum)*	Metabolite 39	Acute	96 h EC50	55
Aquatic plant	Alachlor technical	Acute	14 d IC50	0.0023
Aquatic plant	Metabolite 65	Acute	14 d IC50	> 120
Aquatic plant (Lemna gibba)	Metabolite 70	Acute	7 d IC50	> 203
Aquatic plant (Lemna gibba)	Metabolite 54	Acute	7 d IC50	> 209
Aquatic plant (Lemna gibba)	Metabolite 78	Acute	7 d IC50	>204
Aquatic plant (Lemna gibba)	Metabolite 39	Acute	7 d IC50	68

Microcosm or mesocosm tests

The microcosm study with algal community showed a NOEC of 1 µg/l
An algal community assay was made with exposing two concentrations of alachlor (5 and 90 µg/l). High levels of alachlor significantly reduced community biovolume at 4 weeks. Navicula spp. and G.eximium were affected (although in the first one the effect did not remain over time).

April 2003.

Newmann et al., 2002
An outdoor microcosm test was performed to investigate the effect of a concentration series of MON 29882 (Lot A0M0808204, 43.2% w/w alachlor) on an aquatic algal community. Concentrations up to 64.8 µg a.i./L had only transient effects on unicellular algal community development and are not anticipated to limit increase of filamentous algal biomass in natural ecosystems. Although at 64.8 µg a.i./L, filamentous alga percent surface cover may be transiently decreased, no effects on floating surface cover are expected at or below 29.4 µg a.i./L. In conclusion, the NOAEC for unicellular algae (all parameters assessed) and for percent surface cover expressed as change in percent cover relative to the respective Day –1 value and fresh and dry weights of filamentous algae is 64.8 µg a.i./L. Based on mean measured concentrations, the NOEC value is 60.9 µg alachlor/L (141.0 µg MON 29882/L).
The NOAEC for percent cover of filamentous algae is 29.4 µg a.i./L. Based on mean measured concentrations, this NOEC value is 35.08 µg alachlor/L.

Kaur et al., 2002

Assessment of the toxicity of the alachlor formulation MON 29882 to aquatic macrophytes. Concentrations up to 313.1 µg a.i./L had only transient effects on the aquatic macrophytes G. maxima, L. major and M. spicatum compared to the controls. However, all these effects were not statistically different when compared to the controls. Effects observed during the exposure phase were no longer observed by the end of the exposure phase itself (Day 70). Also effects observed during the three recovery phases were no longer evident by the end of each recovery phase. In conclusion, the lowest NOAEC for the three macrophyte species is based on the results of the most sensitive species for this study (M. spicatum); so the NOAEC is 45.8 µg/l (measured concentrations).

Mesocosms
Foekema et al, 2002
Determination of the biological effect and fate of MON 39801 (43% w/w Alachlor) in outdoor ponds according to HARAP (1999) and CLASSIC (2000) guidance documents. The notifier proposes a system

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
NOEC for aquatic ecosystems exposed to MON 39801 of 7.4 µg a.i./L. (i.e. 5.65 µg a.i./L based on the mean measured initial concentrations). The rapporteur deems that the NOEC of the mesocosms of 7.4 µg a.i./L is not considered valid due to some effects were observed for this concentration and these effects continuing even in post treatment period (i.e decrease of biomass of Elodea Canadensis that was recovered from 56 to 73 days post treatment). The rapporteur believes that a valid NOEC of the system could be 0.3 µg a.i./L; in this concentrations, no significant effects were observed for any taxonomic group, and although there were some effects for the concentration of 0.1 µg a.i./L, these effects were recovered at the end of the test period. The rapporteur also considers an Environmental Acceptable Concentration from this study of 1.3 µg/l due to the low ecological relevance of the effects observed at effects for this concentration which were recovered at the end of the study. This value will be used in the aquatic environmental risk assessment for alachlor.				

Toxicity/exposure ratios for the most sensitive aquatic organism (Annex IIIA, points 10.2)

Application rate (kg as/ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI Trigger
2.4	All crops	Fish	Acute	1	56.25	100
2.4	All crops	Fish	Acute	2	140.6	100
2.4	All crops	Fish	Acute	Run-off	692.3	100
2.4	All crops	Daphnia	Acute	1	225	100
2.4	All crops	Daphnia	Acute	Run-off	2716	100
2.4	All crops	Algae	Acute	1	0.059	10
2.4	All crops	Algae	Acute	30	2.37	10
2.4	All crops	Algae	Acute	Run-off	0.71	10
2.4	All crops	Algae	Microcosm	1	0.03	1
2.4	All crops	Algae	Microcosm	30	1.25	1
2.4	All crops	Algae	Microcosm	Run-off	0.37	1
2.4	All crops	Aquatic plants	Acute	1	0.07	10
2.4	All crops	Aquatic plants	Acute	30	2.875	10
2.4	All crops	Aquatic plants	Acute	Run-off	0.86	10
2.4	All crops	Algae and aquatic plants	Mesocosms	1	0.05	1
2.4	All crops	Algae and aquatic plants	Mesocosms	20	1.08	1

Bioconcentration

Bioconcentration factor (BCF)	50 based on ^{14}C. Higher BCF were observed for formulation products.
Annex VI Trigger for de Bioconcentration factor	
Clearance time (CT_{50})	Approximately 98% was eliminated in 14 days.

(CT_{90})	

Effects on honeybees (Annex IIA, point 8.3.1; Annex IIIA, point 10.4)

Acute oral toxicity	LD50 oral formulation > 100 µg /bee
Acute contact toxicity	LD50 contact formulation >100 µg /bee
Acute oral toxicity	LD50 oral (ai) > 94 µg ai/bee
Acute contact toxicity	LD50 contact (ai) >100 µg ai/bee
Acute oral toxicity	LD50 oral formulation MT > 90 µg ai/bee
Acute contact toxicity	LD50 contact formulation MT >100 µg ai/bee
Acute oral toxicity	LD50 oral formulation EC > 90 µg ai/bee
Acute contact toxicity	LD50 contact formulation EC >100 µg ai/bee

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Application rate (kg as/ha)	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests				
2.4	All crops	Oral formulation MT	<26.6	50
2.4	All crops	contact formulation MT	<24	50
2.4	All crops	Oral formulation EC	<26.6	50
2.4	All crops	contact formulation EC	<24	50
Field or semi-field tests				

Effects on other arthropod species (Annex IIA, points 8.3.2; Annex IIIA, point 10.5)

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory Tests						
Bembidion tetracolum		Microtech alachlor formulation	7 l/ha (3.36 kg/ha)	Mortality	0 %	30%
Chrysoperla carnea		Microtech alachlor formulation	7 l/ha (3.36 kg/ha)	Mortality	0%	30%
Poecilus cupreus		Sanachlor 480 EC	2.9	Mortality	3.3 %	30%
T. pyri		Microtech alachlor formulation	2.8	Beneficial capacity	24 %	30%
A. rhopalosiphi		Microtech alachlor formulation	2.8	Mortality	100 %	30%

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect	Annex VI Trigger
A. rhopalosiphi	adults	Microtech alachlor formulation	2.8	Extended laboratory study Mortality Reproduction	0% No affected	30%
Field or semi-field tests						

Effects on earthworms (Annex IIA, point 8.4; Annex IIIA, point 10.6)

Acute toxicity	LC50 technical = 267 mg/kg (applying factor of 2 = 133.5) LC50 formulation =483 mg /kg (232 mg ai/kg) Metabolites: LC50 (metabolite 70) > 1000 ppm LC50 (metabolite 54) > 1000 ppm LC50 (metabolite 69) > 1000 ppm LC50 (metabolite 39) > 1000 ppm LC50 (metabolite 65) > 857 ppm
Reproductive toxicity	April 2003. NOEC Metabolite 70 = 1.81 mg/kg dry soil NOEC Metabolite 78 = 1.40 mg/kg dry soil NOEC Metabolite 65 = 1.86 mg/kg dry soil NOEC Metabolite 54 = 1.29 mg/kg dry soil

Toxicity/exposure ratios for earthworms (Annex IIIA, point 10.6**)**

Application rate (kg as/ha)	Crop	Time-scale	TER	Annex VI Trigger
3.36	All crops	Acute	29.7	10

Effects on soil micro-organism (Annex IIA, point 8.5; Annex IIIA, point 10.7)

Nitrogen mineralization	No relevant effects at approximately 2X proposed application rate (2.4 kg/ha)
Carbon mineralization	No relevant effects at approximately 2X proposed application rate (2.4 kg/ha)

LEVEL 2

ALACHLOR

Reasoned Statement for the Overall Conclusions

2. Reasoned statement of the overall conclusions drawn by the Rapporteur Member State

2.1 Identity

Alachlor is a herbicide, that is absorbed from the soil primarily by the shoot of emerging seedling. Following absorption, it is translocated (apoplastic) throughout the plant. The mode of action of alachlor appears to be inhibition of protein synthesis in susceptible plants. Alachlor produces a selective weed control in maize, sweet corn, soybean sunflower and cotton, controlling annual grasses and small weed broadleaf species, killing off susceptible weed species and growth suppression on some tolerant ones. One application to soil pre-emergence or early post emergence (2-3 leaf stage)is enough to achieve an effective weed control for 60-80 days after application.

A number of formulated products containing alachlor, either alone or mixed with other herbicides so as to widen the spectrum activity, are marketed throughout the Member States of the European Union (EU). The majority of the formulation types are emulsion concentrate (EC) and capsulate suspension (CS), and in some cases encapsulated granule (CG), granulate (GR) and suspension concentrate (SC).

The [redacted] is the manufacturer of two plant protection products, presented as example on this monograph, called LASSO EC and LASSO Microtech. The first of them is an emulsion concentrate formulation (EC) which contains 480 grams of active substance (Alachlor) per litre, and the second is a microencapsulated formulation (CS) which contains 480 grams of active substance (Alachlor) per litre.

The [redacted] is the manufacturer of the plant protection product called Alachlor (AHE 02), that is a microencapsulated formulation which contains 480 grams of active substance (Alachlor) per litre. **The applicant Shinung Corporation has not submitted any GAPs in EU countries the submitted GAP was in Taiwan, this information is essential for the inclusion of the active substance in the Annex I.**

[redacted] is the manufacturer of the plant protection product called Sanachlor 480 EC, which no code number was assigned. It is an emulsion concentrate formulation which contains 480 grams of active substance (Alachlor) per litre.

[redacted] is the manufacturer of the plant protection product called RENEUR, for which no code number was assigned. It is an emulsion concentrate formulation containing 480 grams of active substance (Alachlor) per litre.

[redacted] is the manufacturer of the plant protection product called ALANEX, for which no code number was assigned. It is an emulsion concentrate formulation containing 480 grams of active substance (Alachlor) per litre. **The applicant Makhteshim Agan International has not submitted any information on authorisations in EU Member states.**

2.2 Physical and chemical properties

The physical and chemical properties submitted by PHYTORUS were obtained from standard reference works. The test methods and materials were not generally identified. It is not known whether the tests were conducted in accordance with GLP. The results of these works should be considered with precaution .

Makhteshim Agan has not submitted any information about the physical and chemical properties of the active substance. Therefore Makhteshim Agan must submit information about the physical and chemical properties of the active substance.

Alachlor is a solid which is colourless as pure active substance. Alachlor (Pure Active substance) is more soluble in organic solvents than water; it is stable in pure water at a range of pH's and there is no evidence of photodegradation; it has a flash point>100ºC and is not considered to be flammable. Alachlor has a vapour pressure of 2.2×10^{-6} mm Hg at 25°C, Henry's Law constant (K_H) of 1.3×10^{-4}, and octanol: water partition coefficient (K_{OW}) of 1223, a water solubility of 242 mg/l at 25°C, is stable to UV radiation and is hydrolysed in water under strongly acid conditions. Alachlor is not classified for transport.

The physical and chemical properties of alachlor indicate a favourable environmental behaviour as far as vapour pressure, solubility and degradability are concerned, but the high octanol :water partition coefficient indicates bioaccumulation, and it is necessary to take it into account. Its flammability, explosive and oxidising properties should be taken into account during storage and use.

Data on the physical and chemical properties of the active substance are generally complete though details of the methodology and guidelines used were not described for most tests.

The formulated products included as a representative product in the Review are :

LASSO EC is an Emulsion Concentrate formulation containing 480 g/l of the active substance alachlor. Lasso EC is self emulsifying purple solution with a shelf life of two years at ambient temperature, **but additional data on the storage stability is required to justify the claim of stability for at least two years at ambient temperature. In addition data on the surface tension is required.**

LASSO MICROTECH is a suspension flowable formulation of microencapsulated alachlor. The formulation contains 480 g/l of the active substance alachlor. Lasso Microtech is a tan coloured liquid with a shelf life of two years minimum at ambient temperature. **But additional data on the storage stability to modern guidelines is required to justify the claim of stability for at least two years at ambient temperature. Data on the surface tension is required.**

ALACHLOR 480g/l CS is a capsule suspension. The formulation contains 480g/l of the active substance Alachlor. Alachlor 480g/l CS is brown crystalline, solid at room temperature with no oxidising and explosive properties; with a 2.54% decomposition at room temperature at 365 days, and

5.11% decomposition at 50ºC at 365 days. **However the pH value, viscosity and surface tension, persistent foaming, suspensability and suspension stability, dry sieve test and wet sieve test, emulsifiability, re-emulsifiability, emulsion stability have not been submitted by the applicant and all of those characteristics are necessary for the inclusion of the active substance in the Annex I. Furthermore additional data on the storage stability is required so as to calculate the shelf life at ambient temperature.**

SANACHLOR 480 EC presents an explosive risk as well as oxidising properties. Its pH is within the range that naturally occurs e.g. in soil. Its stability allows storage under practical and commercial conditions. Its technical properties indicate that no particular problems are to be expected, when it is used as recommended. Its flash point of 13.4ºC is the only property to be considered in the context of safety. **Additional data on Explosive properties is required to justify the high explosion risk of the product.**

RENEUR. The applicant (PHYTORUS) has not submitted the information required for the evaluation of the chemical and physical properties of the plant protection product. This information is necessary for the inclusion of the active substance in the Annex I.

ALANEX. Makhteshim Agan has not submitted any information about the physical and chemical properties of the plant protection product (ALANEX). This information is necessary for the inclusion of the active substance in the Annex I.

2.3 Details of uses and further information

Alachlor products are used in agriculture as herbicides to control annual grasses and small seeded broadleaf weed in maize, sweet corn, soyabean, sunflower and cotton. It is translocated throughout the plant with higher concentrations in vegetative than in reproductive parts. Once inside a susceptible plant, alachlor appears to disrupt the process of protein synthesis required to generate new cells and new tissue growth. Unable to produce the proteins necessary for continued growth the plant dies.

The following representative formulations containing Alachlor were submitted for the Ec Review :

MONSANTO

LASSO EC : Emulsion Concentrate formulation containing 480 g as/l. Monsanto Code Nº no available
LASSO MICROTECH : Microencapsulated formulation containing 480 g as/l ; Monsanto Code Nº MON 29840.

For cleaning application equipment it is necessary to carry out a triple rinse of empty containers, pour rinse water into spraytank and hand them to over public waste disposal services to be disposed of as hazardous waste, without re-using containers. Pre harvest intervals and re-entry periods or with holding periods to protect man and livestock are not required because alachlor containing products are applied to soil before plants emerge or at the 2-3 leaf stage. There is no possibility of neutralisation.

LASSO EC is sold in : 1L blow moulded bottle HDPE - Plug (39 mm) and cap ; 5L metal can with 2 lacquers Push in cap, pullout seal, screw cap ; 60L fluorinated plastic container ; 200L metal drum ; 380L shuttle.

LASSO MICROTECH is sold in : 1L blow moulded bottle HDPE - 63 mm induction seal cap ; 5L blow moulded bottle HDPE - 63 mm induction seal cap ; 10L blow moulded bottle HDPE ; 20L container HDPE ; 200L container HDPE ; 400L container HDPE.

Monsanto has submitted all data concerning recommended methods and precautions concerning handling, storage, transport or fire.

SHINUNG CORPORATION

ALACHLOR 480 g/l EC. **The applicant has not submitted any data concerning packaging and compatibility with packaging material, the procedures for cleaning application equipment, the re-entry periods and necessary waiting periods or other precautions to protect man, livestock and the environment.**

[redacted]

SANACHLOR is sold in :

1 litre bottle:	material:	HDPE-COEX/E/VAL
	shape/size:	round / 88.5 x 234
	opening:	42 mm diameter
	closure:	screwed on
	seal:	HF-seal, cardboard/wax/Al/PET/PE
5 litre container:	material:	HDPE/PA-COEX, PE-HD Lupolen or Hostalen
	shape/size:	square / 194 x 112 x 362, knob handle
	opening:	51.1 mm diameter
	closure:	screwed on
	seal:	HF-seal, cardboard/wax/Al/PET/PE

The equipment should be flushed out at the end of the spraying with water and detergents.

PHYTORUS

RENEUR is sold in 5 litres containers, but **the applicant has not submitted data concerning the packaging material and the design packaging.**

MAKHTESHIM AGAN

ALANEX. **The applicant has not submitted any data concerning packaging and compatibility with packaging materials, procedures for cleaning application equipment, re-entry periods,**

necessary waiting periods or other precautions to protect man, livestock and the environment. Procedures for destruction and decontamination.

2.4 Impact on human and animal health

2.4.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities contained in the active substance or to their transformation products

Summary of mammalian absorption, distribution, excretion and metabolism (Toxicokinetic).

Alachlor was rapidly absorbed after oral dosing, rapidly metabolised by a number of pathways and rapidly excreted in urine and faeces. The urine was the main route of excretion in the monkey, whit in rat, excretion was by both routes. Only a minimal amount was excreted as CO_2. In of the induction of the nasal tumours in the rat a comparative study was undertaken to examine metabolism by the nasal mucosa of the rat and the monkey. The later species was selected to aid extrapolation to man. The study confirmed that the rat had the greater potential to form the putative carcinogen, a quinone imine.

Based on Monsanto studies, Alachlor is absorbed more rapidly into the circulatory system via oral exposure than it is dermally in male, Long-Evans rats. Several metabolites of Alachlor are bound to the betasubutin of rat haemoglobin possibly through a reaction with cysteine on the subunit. A greater amount of ^{14}C-alachlor equivalents is bound to rat haemoglobin as compared to mouse, monkey and human. Alachlor forms a glutathione conjugate in red blood cells of mouse, monkey and human.

Radioactivity from the administered dose was found in blood and in the spleen, liver, kidney and the heart which may be a reflection of the amount of blood in those organs. In addition, a relatively high level of radioactivity was also found in the eyes, brain, stomach and ovaries.

Alachlor was rapidly metabolised and eliminated as conjugates of mercapturic acid, glucuronic acid and sulphate in urine and faeces (approximately 50:50). Elimination in CO_2 was minimal. Approximately 89 % of a single oral dose to rats was eliminated during the first 10 days with most of the elimination occurring during the first 48 h.

According to the metabolic pathways identified, the shemes can be subdivided into 5 metabolic compartments:

C1: Is dominated by cytochrome p-450 mediated oxidative metabolism.
C2: Glutathiione conjugation with subsequent metabolism towards lower molecular weight S-conjugates.
C3: Further transformation of biliary metabolites in the GIT before they are excreted or reabsorbed.
C4: Further metabolism of the thiol metabolites which are reabsorbed from the GIT.
C5: Further metabolism of liver metabolites in the olfactory epithelium.

In vitro incubation of liver and nasal turbinate tissues from rats, mice and squirrel monkeys showed that Alachlor is metabolised through secondary chloramide or methylsulphide to 2,6-diethyl aniline which is further oxidised to a reactive quinone imine. The enzymatic activity in rat nasal tissue was shown to be 144 times more active than the same tissue in squirrel monkeys.

Phytorus S.A, [redacted] and Shinung Co. had included information on absorption, distribution, excretion and metabolism of Alachlor in their reports. However, few (Phytorus S.A) or no ([redacted]and Shinung Co) experimental works had been included, and those submitted did no apport the essential data.

Summary of mammalian toxicology

In general terms, alachlor is well absorbed by all routes in all the animals tested: rat, rabbit, mouse, and monkey. Absorption is nearly 100 % from gut, in male and female rats. Percutaneous absorption is only 50 % of the applied dose in monkeys. Autoradiography studies have shown up an accumulation in blood and gastrointestinal (GIT) tract of all species. The binding of alachlor to haemoglobin is stronger in rats than in other species. Similarly, alachlor accumulates in the nasal turbinates of the rat but not of mouse, hamster and monkey. There is also an important accumulation in spleen, liver, kidney and heart. In addition to these organs there is a relatively high accumulation in eyes, brain stomach and ovaries. The high enterohepatic circulation of alachlor accounts for both the high intestinal tract concentration (which is in turn the causation of its subsequent binding to faecal material) and the low excretion of the metabolites in the urine.

The metabolism of alachlor consists essentially of 4 metabolic pathways: P-450 mediated oxidation, glutathione conjugation, C-S cleavage of glutathione metabolites in the GIT and further metabolism of thiol metabolites after reabsorption from the GIT. These metabolic pathways lead to the production of substrates for further metabolism in nasal tissue. These precursors to nasal metabolism are believed to be secondary amide methylsulfide (metabolite **31**) and secondary chloroacetamide (metabolite 13). Through hydrolysis of the amide bond combined with para-hydroxylation 4-amino-3,5-diethyl-phenol (metabolite **86***) is formed. This metabolite is either conjugated with sulphate (metabolite **20**) or converted by oxidation into 3,5-diethyliminoquinone (DEIQ) which immediately reacts with intracellular glutathione and protein to form adducts. The existence of this pathway was confirmed by the demonstration of accumulation of radioactivity and the presence of DEIQ-protein adducts as cysteine adducts in nasal tissue of rats following administration of alachlor and the precursor metabolite secondary amide methylsulfide (**31**). DEIQ-protein adducts as cysteine adducts were not found in nasal tissue of mice after administration of alachlor. Quantitative comparison of metabolism between gender, dose, route of administration and species shows that there are differences between rat, mouse and monkey. The important difference in metabolic profiles and urinary excretion rates between rat and monkey is thought to be primarily due to the different molecular weights required for liver metabolites to be excreted via the bile.

The molecular weight required in the monkey is greater (500) than that for the rat (325) whereby more glutathione conjugate metabolites in the monkey are excreted in urine than in bile. The influence of

gender, dose and route of administration is relatively small. The difference observed in urinary metabolic profiles in the monkey following oral and intravenous administration are thought to be due to glutathione conjugation of alachlor in the intestinal epithelium and subsequent metabolism by gut microflora prior to reabsorption. No difference was found between the percent of distribution of metabolites between the dermal and the intra-muscular routes of administration and corresponds well with the results from the intravenous study.

Although differences could be demonstrated in metabolic profiles between species through detection of metabolites in excreta, differences in ability to form DEIQ provide much more information on species susceptibility to form protein adducts in nasal tissue. In-vitro tests where the enzymatic activity to hydrolyse the amide bond and hydroxylate the aniline moiety of secondary amide methylsulfide (**31**) and diethylaniline (DEA) (**19***) respectively was compared between rat, mouse, monkey and man revealed very important differences. The ability of rat nasal tissue to form the 4-amino-3,5-diethylphenol (**86***) was found to be 38, 30 and 751 times greater than in mouse, monkey and man respectively.

These differences contribute to the explanation why nasal turbinate tumours were found only in rats and not in mice.

As far as acute toxicity of alachlor is concerned, a concise summary of the results is presented in table 2.4.1-1

Table 2.4.1-1: Summary of acute toxicity

Route	Species	Result		
Oral				
	Rat	LD_{50}	2598	mg/kg
	Rat	LD_{50}	1350	mg/kg
	Rat	LD_{50}	2182.2	mg/kg
Percutaneous				
	Rabbit	LD_{50}	=13300	mg/kg
	Rabbit	LD_{50}	> 2000	mg/kg
	Rat	LD_{50}	> 4982.3	
Inhalation (4 hour)	Rat	LC_{50}	1.04	mg/l
	Rat	LC_{50}	> 4.67	mg/l
Skin - irritation	Rabbit		Mild irritant	
Eye - irritation	Rabbit		mild irritant	
Skin - sensitisation	Guinea pig		Sensitiser	

According to acute exposure, **Alachlor** might be considered **harmful if swallowed**, **non-hazardous by dermal and inhalation routes, mild-irritant to skin and to eyes and a moderate sensitizer for guinea pigs and classified as harmful if swallowed Xn R22 and sensitizing agent R43.**

A summary of the results from short-term toxicity, genotoxicity, long term toxicity, oncogenicity, reproductive toxicity and immunotoxicity tests including different NOEL levels is presented in Table 2.4.1.2

The NOEL found in the rat was 2.5 mg/kg/day in one study (24 months), in another study was 0.5 mg/kg/day (25 months). In the dog was 1.0 mg/kg/day based on hemosiderosis of the liver, kidney and spleen.

The alachlor genotoxicity data base has been prepared using the documentation submitted by Monsanto, Shinung Corporation and Phytorus in support of the application. The two remaining applicants (Sanachem and Makteshim Agan) did not submit the required documentation. The genotoxic potential of alachlor has been investigated in a comprehensive range of *in vitro* and *in vivo* assays. Besides, the potential mutagenic of urine and bile from alachlor treated rats as well as of different alachlor metabolites has been evaluated using the Ames test. The major features of the alachlor derived from genotoxicity data base are the following:

1. Alachlor does not induce gene mutations in bacterial or mammalian cells.
2. The information given by the *in vitro* mammalian chromosome aberration studies precludes any conclusion on the *in vitro* clastogenicity of Monsanto-produced alachlor.
3. Alachlor induces DNA damage (SCEs) in cultured mammalian cells.
4. Monsanto-produced alachlor appears to be non-clastogenic in mammalian somatic cells *in vivo.* However, although a reported positive chromosomal aberration assay, with alachlor administered to rats i. p., could not be relevant to Monsanto-produced alachlor, this result should be confirmed. Nevertheless, in the only study carried out with Monsanto-produced alachlor where chromosomal aberration nor micronucleus was the endpoint, the test compound was administered by oral gavage and the number of metaphases analysed per animal was smaller than the recommended by OECD.
5. Taking into account the information given by the *in vivo* studies on DNA effects, it cannot be refused the possibility that alachlor induces DNA damage (UDS) acting as a weak genotoxic agent. Nevertheless, results suggest that there may be animal-to-animal variations in the metabolism of alachlor to a genotoxic form in liver.
6. It cannot be concluded that alachlor is not clastogenic for germ cells because negative results from the only study carried out cannot be considered acceptable because the purity of the test substance was not reported.
7. It can be said that there was no excretion of mutagenic metabolites in the urine or in the bile of rats treated with alachlor.
8. Most of metabolites of alachlor showed no mutagenic potential in the Ames test.
9. CP97230 is suggested to be a very weak mutagenic agent.
10. Marginal or equivocal activity was observed for DMTA.
11. DEA is suggested to be a very weak mutagenic agent in the Ames test but did not induce DNA damage in the *in vivo* alkaline elution assay.

12. CP101394 and CP101384 were the only clear mutagenic metabolites in the Ames test but their potency values are among the lowest observed in this assay.
13. The mutagenic activity observed for some alachlor metabolites does not appear to be biologically significant.
14. Alachlor does not cause nasal turbinate tumours in rat by a genotoxic (gene mutation) mode of action.

In summary, the overall weight of evidence from the *in vitro* and *in vivo* studies is that alachlor does not induce gene mutation. The mutagenic activity observed for some alachlor metabolites does not appear to be biologically significant. Specific examination of nasal turbinate activation indicates that alachlor is not causing nasal turbinate tumours in rats by a genotoxic (gene mutation) mode of action. Nevertheless, it should be noted that the potential clastogenicity of alachlor is still questionable. Therefore, more studies are required in order to give a definitive conclusion about its mutagenicity

Tumours of the nasal turbinates were seen at 15 mg/kg/day or higher in both males and females. These tumours are considered to be species-specific as they did not occur in either of the mouse 18-month studies or in the one-year dog study. Metabolism studies confirmed the species difference, by showing that the rat was likely to form much higher amounts of the putative carcinogen - a quinone imine- than the monkey, as it has already been commented in relation to enzymatic activity of the different species.

Changes in the stomach and thyroid were seen at the MTD only. The stomach tumours are believed to be the result of an active metabolite transferring from the nasal turbinates to the stomach. They are therefore probably species-specific, like the nasal turbinate tumours. Thyroid tumours are not uncommon in rats receiving high doses of xenobiotics, and are probably not of significance.

The oncogenicity studies in mice did not elicit treatment-related tumours of the nasal turbinates, stomach or thyroid. However, there was an increase in bronchoalveolar tumours among females receiving the highest dosage of 260 mg/kg/day. These tumours are not uncommon in untreated female mice and are probably not indicative of a carcinogenic response.

A multigeneration study and a teratology study were performed in the rat. A rabbit teratology study was also performed, but was not considered adequate to assess teratogenicity. No teratogenicity or effects on reproduction were observed. The multigeneration study only showed some evidence of renal effects in the F2 adult males and F3 pups at the highest dosage of 30 mg/kg/day, whilst the rat teratology study only had effects at the very high dosage of 400 mg/kg/day. The No Observed Effect Level for these reproductive and developmental toxicity studies was 10 mg/kg/day.

Table 2.4.1 -2: Summary of short-term toxicity, genotoxicity, long term toxicity, oncogenicity, reproductive toxicity and immunotoxicity tests.

Type of study	Species	Result with most sensitive species
Short term toxicity		

Type of study	Species	Result with most sensitive species
Oral, 90 days	Rat,	NOAEL 9.8 mg/kg/day
Oral, 6 months	Dog	NOAEL 5 mg/kg/day
Genotoxicity tests of parent		
In vitro studies	Bacteria, yeast and mammalian cells	Negative results in *Salmonella typhimurium, Escherichia coli* and CHO cells (± S9) for gene mutation induction. Positive and negative results for chromosomal aberration induction. Positive results in CHO cells (± S9) and human lymphocytes (-S9) for non-Monsanto-produced alachlor (CA), and negative non-consistent results (-S9) in human lymphocytes for Monsanto-produced alachlor (CA & MN). Negative and positive results for DNA effects. The only positive consistent results correspond to sister chromatid exchanges induction in human lymphocytes (-S9).
In vivo studies	Mammalian somatic cells.	Positive and negative results for chromosomal aberration induction. Positive results in rat bone marrow cells for non-Monsanto-produced alachlor (CA), and negative but inconclusive results in rat or mouse bone marrow cells for Monsanto-produced alachlor (CA & MN). Negative and positive results for DNA effects. The only weakly positive consistent results correspond to UDS induction in primary rat liver cell cultures.
	Mammalian germ cells.	Negative but inconclusive result for the mouse dominant lethal assay.
Additional assays	Plants	Negative for gene mutation in *Zea mays* gametophytes, and positive for chromosomal aberration in *Vicia faba* root meristematic cells. Non-consistent results.
Genotoxicity tests of meta-bolites		

Type of study	Species	Result with most sensitive species
In vitro studies	Bacteria	There are no excretion of mutagenic metabolites in the urine or in the bile of rats treated with alachlor in the Ames test. Most of alachlor metabolites (CP108267, CP108064, CP51214, CP108065, CP76095, CP76096, CP76097, CP91431, CP91432) show no mutagenic potential in the Ames test. CP97230 appears to be mutagenic only towards TA100 in the presence of S9 although data did not meet the criterion for a clear positive response. Marginal or equivocal mutagenic activity was observed for DMTA only towards TA1535 in the presence and absence of nasal turbinate S9 preparations from rats, mice or monkeys as metabolic activation system. Marginal or equivocal mutagenic activity in the Ames test was observed for DEA only towards TA100 and TA1535, being the more consistent activity observed in the presence of nasal turbinate S9 preparations. There was no apparent differences between rat, mouse or monkey nasal turbinate S9. CP101394 and CP101384 are clearly mutagenic in the Ames test.
In vivo studies	Mammalian somatic cells.	DEA does not induce single strand breaks in rat liver as determined by the alkaline elution assay.
Long term toxicity		
Oral, 6-12 months	Dog	NOEL 1.0 mg/kg/day
Oral, 2 years	Rat	NOEL 2.5 mg/kg/day
Oral, 25 months	Rat	NOEL 0.5 mg/kg/day (all effects)
Oral, 18 months	Mouse	NOEL: 16.6 (males) and 23.7 (females) mg/kg/day NOEL: 20.2 mg/kg/day for combined sexes
Oncogenicity		
Oral, 18-24 months	Rat, mouse	Positive (Rat)
Reproductive toxicity		
Oral, multigeneration	Rat	NOEL 30 mg/kg/day
Oral, developmental	Rat, Rabbit [1]	NOEL 100 mg/kg/day (maternal toxicity) NOEL 150 mg/kg/day (developmental toxicity)

Immunotoxicity		
In vitro	Human mononuclear cells	Negative

[1] Publication states that this rabbit study was not suitable for the assessment of teratogenic potential

Summary Chronic Toxicity and Carcinogenicity

In a one-year study in beagle dogs, alachlor technical (94. 1 % a.i.; was given by capsule at doses of 0 (control), 1.0, 3.0, or 10 mg/kg/day. Systemic toxicity was noted at the 3 mg/kg/day dose as hemosiderosis in the kidney of one male dog and in the spleen of another male dog; and at the high dose as hemosiderosis and hemolytic anemia in the liver of males (3/6). The systemic toxicity NOEL is 1.0 mg/kg/day. The systemic toxicity LOEL is 3 mg/kg/day based upon signs of hemosiderosis and hemolytic anemia ([redacted], 1984).

In a two-year feeding study, Long-Evans rats received doses of 0, 100, 300, or 1000 ppm (approximately 0 (control), 14, 42, or 126 mg/kg/day) technical alachlor in the diet for approximately 1l7 weeks in males and l06 weeks in females. It should be noted that the test substance used for the first 11 months of the study was stabilized with 0.5% epichlorohydrin while the test substance used for the remaining 16 months of the study was stabilized with epoxidized soybean oil. Epichlorohydrin is carcinogenic for male Wistar rats and Sprague-Dawley rats. When given in drinking water epichlorohydrin has been found to cause forestomach tumors (squamous cell papillomas and carcinomas) in male Wistar rats (Konishi et al. Gann 71:922-923, 1980). By the inhalation route epichlorohydrin has been found to cause squamous carcinomas of the nasal cavity (Laskin, et al. J. Natl. Cancer Inst. 65:751-757, 1980). The effect of epichlorohydrin on tumor formation in this study is not known.

Systemic toxicity was noted at 14 mg/kg/day and above as ocular lesions in the form of uveal degeneration syndrome, and as increased thyroid weights in both sexes; and as increased liver weight in the high dose groups. These observations were correlated with degenerative liver changes at all dose levels. There were decreased body weights in the mid and high dose males and the high dose females during the second year of the study. Statistical evaluation of mortality indicated an increasing trend for male and female rats with increasing doses. Male rats had an increased incidence of nasal respiratory epithelium adenomas, and adenomas and/or adenocarcinomas combined at 42 and 126 mg/kg/day ($p < 0.01$ and significant trends). Also, there was increased incidence in malignant mixed gastric tumors and gastric adenocarcinomas and/or malignant mixed gastric tumors combined at 126 mg/kg ($p < 0.01$ and significant trends). There were increased incidences in thyroid follicular cell adenomas and adenomas and/or carcinomas combined at 126 mg/kg ($p < 0.01$ and significant trends). There were increased incidences in the 126 mg/kg/day dose group for stomach osteosarcomas, and thyroid follicular cell carcinomas (both at $p < 0.05$). There were increased incidences of brain oligodendrogliomas of the hypothalamus, stomach osteosarcomas, and thyroid follicular cell carcinomas (all at $p < 0.01$) and significant trends. For female rats there was increased incidence of nasal turbinate adenomas, and adenomas and/or adenocarcinomas combined at 42 ($p < 0.05$) and 126 ($p < 0.01$) mg/kg/day and significant trends for these tumor types. There was also an increased incidence of malignant mixed gastric tumors, and gastric adenocarcinomas and/or malignant mixed gastric tumors combined ($p < 0.01$) at 126 mg/kg/day, as well as significant trends for these tumor types. Also, increased incidence at 14 and 126 mg/kg/day of mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas

combined ($p < 0.05$). There were significant increasing trends in liver adenomas, stomach osteosarcomas, and thyroid follicular cell adenomas and/or adenocarcinomas combined (all at $p < 0.01$). Of all the tumors listed above, the increasing trend observed in brain oligodendrogliomas of the hypothalamus, and the significant trend in brain ependymomas and ependymomas and/or malignant ependymomas combined in male rats and the significant pair-wise comparisons for mammary gland adenofibromas, adenofibromas and/or fibroadenomas combined, and adenofibromas, fibroadenomas, and papillary adenocarcinomas combined and liver adenomas in female rats were considered to have occurred at excessively toxic doses, and only the tumors of the nasal epithelium, stomach, and thyroid were treatment related and are the basis for considering alachlor to be carcinogenic in the rat. The systemic toxicity **NOEL could not be determined** but would be less than 14 mg/kg/day. The systemic toxicity LOEL is equal to or less than 14 mg/kg/day based on ocular lesions (uveal degeneration syndrome) and hepatic toxicity ([redacted], 1981).

In a second long-term study, Long-Evans rats were fed doses of 0, 0.5, 2.5 or 15 mg/kg/day technical alachlor (94.13%; stabilized with 1.28% epoxidized soybean oil) for 110 weeks (25 to 26 months). Systemic toxicity was noted at 15 mg/kg/day, highest dose tested (HDT), as molting of the retinal pigmentation (uveal degeneration syndrome), increased mortality rate (significant increasing trend) in females (no effect in males) and abnormal disseminated foci in male livers. Male rats had increased incidence of nasal respiratory epithelium adenomas at 15 mg/kg/day ($p < 0.01$ with significant trend). Female rats had an increased incidence of adrenal benign pheochromocytomas and nasal respiratory epithelium adenomas at the 15 mg/kg/day dose level ($p < 0.05$ and $p < 0.01$, respectively and significant trend). There was also increased incidence of thymus malignant lymphosarcomas at the 15 mg/kg/day dose level ($p < 0.05$); however, only the tumors of the nasal epithelium were treatment related and are the basis for considering alachlor to be carcinogenic in the rat. The systemic toxicity NOEL is 2.5 mg/kg/day and the systemic toxicity LOEL is 15 mg/kg/day, based on molting of retinal pigmentation and increased mortality in females, with abnormal disseminated foci of the liver in males ([redacted], 1984).

In a special two-year study, technical alachlor (94.13% a.i; stabilized with 1.28% epoxidized soybean oil) was administered in the diet at 126 mg/kg/day to Long-Evans rats for two years to assess ocular effects of the compound (uveal degeneration syndrome). It was observed that females were more sensitive than males, and that once the uveal degeneration syndrome was observed, it was irreversible (a group exposed to alachlor for the first 5 to 6 months). The nasal, thyroid and gastric tumors observed in earlier investigations were observed. The nasal tumors were noted at the end of the study (2 years) in the group that was exposed to alachlor for the first 5 to 6 months ([redacted], 1984).

In a carcinogenicity study, technical (alachlor; 92.6%.) stabilized with epichlorohydrin at the start of the study (for 11 months) and then with a lot stabilized with epoxidozed soybean oil was given to CD- 1 albino mice in the diet for 18 months at doses of 0 (control), 26, 78 or 260 mg/kg/day. Systemic toxicity was noted in the mid and high dose groups as increased liver weights, increased kidney weight in the mid and high dose males, and in the high dose females as reduced survival (statistical evaluation of mortality showed no significant incremental changes with increasing doses of alachlor in male mice while female mice showed a significant increasing trend in mortality with increasing doses of alachlor)

and body weight gains (10%), males were not similarly affected. Thyroid follicular atrophy was noted in the mid and high dose males and the high dose females. There was an increase in water consumption in the high dose groups. Males had a significant increasing trend in bronchioalveolar adenomas at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the male dosed groups with the controls. Female mice had significant increasing trends, in addition to significant differences in the pair-wise comparisons of the 260 mg/kg/day dose group with the controls, for bronchioalveolar adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$ ([redacted], 1981).

In a second carcinogenicity study, CD-1 albino mice (60 animals/sex/dose) received 0 (control), 100, 400 or 1600 ppm (male: 0, 16.64, 65.42, or 262.40 mg/kg/day; and female: 0, 23.73, 90.34, or 399.22 mg/kg/day respectively, calculated directly from food consumption data) of alachlor (94.64% a.i), in the diet for 18 months. Ten animals/ sex/ dose were sacrificed at 12 months. Systemic toxicity was noted in high dose males as lower body weight gains for the period ending on day 91; high dose males and females with lower body weight gains for the period ending on day 372 and high dose females with lower body weight gains to the end of the study. There were no decreases in food consumption, rather there were increases in high dose females. No treatment related effects on food efficiency were noted in the treated males; however, the high dose females had a dose related decrease in food efficiency at 12 and 18 months.

There was a statistically significant increase in absolute liver weights of the low and high dose females and liver weights relative to brain weights in high dose females at 12 months. Also, there was an increase in relative liver weights in high dose females at 18 months. The high dose males showed a statistically significant increase in absolute and relative liver weights at 18 months. There was a statistically significant decrease in kidney weights relative to body weights in high dose females at 12 months and a decrease in absolute kidney weight in high dose females at 18 months. The males at 18 months had a significant increase in absolute kidney weights in all dose groups, increased kidney weights relative to body weights in the low and high dose groups and increased kidney weights relative to brain weight in the mid and high dose groups.

Non-neoplastic observations included slight increases in tubular epithelium hyperplasia/ regeneration in the kidney(s) of high dose males, an increase in centrilobular hepatocellular hypertrophy in mid and high dose males along with an increase in high dose females of fibrous osteodystrophy of the sternum. Neoplastic observations included an increase in bronchoalveolar adenomas in all treated groups in males (7, 18, 27, and 22%, for the control, low, mid and high dose groups, respectively) and females (5, 14, 10, and 17% for the control, low, mid and high dose groups, respectively), statistical significance was achieved in mid dose males. The combined incidence of bronchoalveolar adenomas/carcinomas was increased in all treated groups in males (7, 18, 32, and 22% for the control, low, mid and high dose groups, respectively). Only the mid dose males were statistically significantly different from the controls.

These data indicate that CD-1 mice showed evidence of bronchoalveolar adenomas (mostly) and/or carcinomas in the lung, but the data were considered to be inconclusive in terms of the relationship to alachlor treatment especially when both mouse carcinogenicity studies are considered together.
The systemic toxicity NOEL for males is 16.6 mg/kg/day for males and 23.7 mg/kg/day for females (20.2 mg/kg/day for combined sexes). ([redacted], 1994).

Developmental Toxicity

Developmental studies are designed to identify possible adverse effects on the developing organism which may result from the mother's exposure to the pesticide during pre-natal development.

In a developmental toxicity (teratology) study, Charles River rats were given 0 (control), 50, 150 or 400 mg/kg/day of alachlor (92.19% a.i); by gavage on gestation days 6 through 19, inclusive. Maternal systemic toxicity was noted at the high dose as maternal deaths, and increased incidence of soft stools, red matter around the nose and mouth and anogenital staining and reduced body weight gains. Developmental toxicity was noted at the high dose as a slight increase in the mean number of early and late resorptions with related increased post-implantation loss and a slight reduction in the mean number of viable fetuses. The maternal toxicity NOEL is 150 mg/kg/day. The maternal toxicity LOEL is 400 mg/kg/day based on increased mortality, increased incidence of clinical signs and reduced body weight gains. The developmental toxicity NOEL is 150 mg/kg/day. The developmental toxicity LOEL is 400 mg/kg/day based on increased resorptions and decreased litter size ([redacted], 1980).

In a developmental toxicity study, New Zealand white rabbits received doses of 0 (control), 50, 100 or 150 mg/kg/day alachlor (94.7%) by gavage on days 7 through 19, inclusive. Maternal systemic toxicity was noted at the high dose as decreased body weight gain during the dosing period followed by a rebound in body weight gain during the period following dosing. No developmental toxicity was noted in the parameters measured. The maternal toxicity NOEL is 100 mg/kg/day. The maternal toxicity LOEL is 150 mg/kg/day based upon a reduction in body weight gains. The developmental toxicity NOEL is equal to or greater than 150 mg/kg/day (highest dose tested) and the developmental toxicity LOEL is greater than 150 mg/kg/day ([redacted], 1988).

Reproductive Toxicity

A reproduction study is designed to provide general information concerning the effects of a test substance on mating behaviour, conception, parturition, lactation, weaning, and growth and development of the offspring.

In a three-generation reproduction study, Sprague Dawley CD rats received either 0 (control), 3, 1 0, or 3 0 mg/kg/day technical alachlor (92.6%) in the diet. Parental/ Offspring systemic toxicity was noted at the high dose in the form of discoloration of the kidney and reduced kidney weights (especially in F2 parents and F3b pups). Histopathology revealed chronic nephritis in the high dose males. The high dose females of each parental generation and the F3b females had lower ovary weights (this decrease was maximal (17%) and significant in the F. generation, and was also associated with 17% decrease in

the ovaries to body weight ratio). No microscopic changes were reported in the ovaries and no effect was noted an reproductive parameters.

The no adverse effects of treatment were evident for the following parameters: maternal body weights or weight gains during gestation or lactation, the ratio of live to dead pups at birth, pup sex distribution, litter survival indices or pup body weights.

No adverse effects were noted on any reproductive parameter following continuous alachlor exposure over three successive generations. Therefore, the No Observed Effect Level (NOEL) is > 30 mg/kg/day, the highest dose tested (HDT). The reproductive toxicity NOEL is equal to or greater than 30 mg/kg/day (HDT)

Epidemiological studies

Three epidemiology studies were recently conducted on these workers to assess the following parameters: ocular effects for the period 1968-1990, mortality (1968-1990; 1968-1993), and cancer incidence (1968-1990; 1968-1993). Mortality and cancer incidence for the period 1968/9-1993 is the most recent update on the previous study for the period 1968-1990. Further updates on these studies are planned.

Exposure assessment

There was insufficient information on plant conditions to estimate alachlor exposures quantitatively over the study period. Therefore, a qualitative exposure estimation scheme was developed. Qualitative occupational exposure estimates for workers were based on work history information, industrial hygiene judgement, and, to a lesser extent, recent exposure monitoring data.

The first step in the exposure estimating process was the creation of a department/job title dictionary which included all work location and job assignments found in workers' personnel records. Jobs with similar exposure potential were consolidated by the plant industrial hygienist into occupational exposure categories (OEC). The plant hygienist then assigned each OEC a high, medium, low, or negligible qualitative exposure ranking for alachlor as well as for other specific chemicals.

The qualitative exposure rankings were based primarily on the opportunity for dermal contact with alachlor. Inhalation exposures were judged to be a minor component of total exposure to liquid alachlor due to alachlor's extremely low vapor pressure (1.6×10^{-5} mm Hg at 25°C). Current and historical airborne measurements have averaged less than 10 parts per billion, confirming the minor potential for airborne exposures. The more recent granular and water dispersible alachlor formulations create the possibility of airborne exposure (in the manufacturing plant) via dust, but even in these operations airborne measurements have averaged much less than 100 parts per billion.

A source of exposure of uncertain magnitude and duration was contamination of the plant drinking water. The contamination was discovered incidentally in June 1975 by evaluating a 'control' sample from the plant's drinking water while developing a method for measuring alachlor concentrations in

water. The resulting single measurement showed an alachlor concentration of 2 mg/l (i.e. 2 parts per million). Plant management immediately notified workers and brought in bottled drinking water to eliminate exposure. Soon thereafter, the plant's water supply was switched to other wells at the plant. Subsequent alachlor measurements from the new wells averaged 8 mg/l through 1980. At that time, a carbon filtration system was completed which reduced alachlor in the water supply to below the minimum detection level of 0.03 mg/l.

Worker exposure to alachlor from drinking water would depend on the duration of the water contamination at the plant and the amount of water consumed on a daily basis. Both aspects of exposure are unknown. However, if we assume that the well water concentration of alachlor was 2 mg/l and that workers drank 1 liter of water daily, exposure from drinking water would be approximately equivalent to exposure in jobs classified as having high exposure.

An analysis based only on occupational exposures was conducted because of the uncertainty of assumptions about exposure via plant drinking water. A relatively small number of workers had exposure only via drinking water and excluding these workers from the analysis of alachlor-exposed workers did not appreciably affect the results.

Ocular Study

In designing a study of ocular effects among workers, we began by reviewing the toxicological studies in Long-Evans rats with a veterinary ophthalmologist. The ophthalmologist concluded that the primary effect from alachlor ingestion at 126 mg/kg/day was to the uveal tract including the iris, ciliary body, and choroid. Long-Evans rats, like humans, have pigmented eyes. Degenerative uveal tract changes were characterised by pigment disruption and dispersion, inflammation, and atrophy. This resulted in secondary lesions involving adjacent structures - mainly lens-iris adhesions, cataracts and degeneration of the retina.

In consultation with the clinical ophthalmologist, it was decided that an early human lesion analogous to the uveal effects in Long-Evans rats is Pigment Dispersion Syndrome (PDS). PDS is defined as the loss of pigment from the mid-posterior iris with deposition of the pigment on the cornea, trabecular meshwork, lens and iris. Attention was focused on changes within the human iris because such changes would likely be early lesions of a hypothesised alachlor affect and because the iris is readily examined and the clinical syndrome is specifically described for the iris.

A broad range of ocular effects that might occur subsequent to pigment dispersion were also evaluated, both to evaluate the possibility of progression of the initiating lesion and the overall ocular health of the participants.

One unexposed study participant has eye defects meeting the study criteria of Pigment Dispersion Syndrome, while there were no cases of PDS among the exposed participants, (Relative Risk (RR) = 0, 95% Cl 0-24.3). For eye abnormalities other than PDS, prevalence rates were similar for exposed and unexposed study participants. Overall, ocular health is very similar in the two groups; however, minor

lens opacities which did not affect visual acuity were diagnosed slightly less often among exposed participants.

This study included the highest exposed workers at the plant with at least 17 (average 20) years of latency. The lack of any alachlor-related ocular effect in these workers makes it unlikely that the eye lesions seen in Long-Evans rats will occur among humans exposed to alachlor either in manufacturing or agricultural use.

Mortality Study

1036 workers met the criteria for inclusion in the mortality analysis and had potential alachlor exposure in manufacturing jobs or via drinking water. Mortality from all causes combined for these workers was lower than Iowa rates both for the total cohort (27 observed, 40.1 expected, Standardised Mortality Ratio (SMR) - 0.7, 95% Confidence Interval (CI) 0.4-1.0) and for those with 5 or more years exposure and 15 years since first exposure (4 observed, 11.1 expected, SMR = 0.4, 95% CI 0.1-0.9). Mortality from cancer was similar to Iowa rates (8 observed, 9.3 expected, SMR = 0.9, 95% CI 0.4-1.7) and there were slight to moderate deficits of cancer mortality for workers with 5 or more years exposure (3 observed, 4.8 expected, SMR = 0.6, 95% CI 0.1-1.8) and 15 or more years since first exposure (1 observed, 5.1 expected, SMR = 0.2, 95% CI 0-1.1). There were no deaths due to stomach, thyroid, and nasal cancer - the three cancers seen in excess in the chronic feeding studies of laboratory rats - versus very small expected values. The 8 observed cancer deaths were distributed among 8 different cancer sites and there were no noteworthy findings for specific cancers. Ischemic heart disease mortality was less than expected overall and for longer-term workers. Mortality from accidents was slightly elevated for the total exposed cohort, but not for those with 5 or more years exposure and 15 or more years since first exposure.

The result of low mortality for workers from major causes of death can be quantified approximately by comparing workers‘ life expectancy at age 20 with that of the Iowa general population. Alachlor workers have an approximate 5 year increase in life expectancy compared with Iowa residents. This projected survival advantage is due largely to markedly lower mortality for workers at ages 45 and above. Some of the age specific rates for alachlor workers are based on no deaths which increase the variability of the life expectancy calculation.

Cancer Incidence Study

1025 white males and females met the criteria for the cancer incidence analyses and were estimated to have potential exposure to alachlor either in their jobs or via drinking water. Linkage with State Health Registry of Iowa (SHRI) identified 37 cancers during the study period, 13 of which were in situ carcinomas, mostly cervical (n = 9) and skin (n = 2), and 24 were invasive cancers in 23 individuals. In situ cancers were not included in this study because incidence rates are routinely based on invasive cancers (with the exception of bladder cancer) and because population-based ascertainment of in situ cancers is questionable, especially for the cervix and skin melanoma.

Over the 1969-1993 study period, cancer incidence was slightly higher for alachlor workers than for the Iowa general population (24 observed, 17.1 expected, Standardised Incidence Ratio (SIR) - 1.4, 95% CI 0.9-2.1) SIRs were similarly slightly elevated for workers during active employ-ment (14 observed, 11.1 expected, SIR = 1.3, 95% CI 0.7-2.1) and after employment termination (10 observed, 6.0 expected, SIR = 1.7, 95% CI 0.8-3.0) suggesting that employment status was not a factor affecting cancer ascertainment. The cancer SIR varied by duration of exposure and time since first exposure. The SIR was elevated for workers with less than 5 years employment and less than 15 years since first exposure (10 observed, 5.1 expected, SIR = 2.0, 95% CI 0.9-3.6). The 10 cancers were varied including: 1 salivary gland, 1 rectum, 1 female breast, 1 cervix, 1 uterus, 1 testis, 1 melanoma, 2 Hodgkin's disease, and 1 chronic myeloid leukemia (CML). Workers with 5 or more years of exposure (13 observed, 10.2 expected, SIR = 1.3, 95% CI 0.7-2.2) and workers with 15 or more years since first exposure (9 observed, 7.8 expected, SIR = 1.2, 95% CI 0.5-2.2) had cancer incidence similar to expected values. During the 1991-1993 update period, there were 6 observed and 5.3 expected cancers (SIR = 1.1, 95% CI 0.4-2.5).

There were no cases of nasal, stomach or thyroid cancer versus small expected numbers of 0.04, 0.3, and 0.5, respectively. For most major cancer sites there were either no observed cases or 1 case and worker rates were similar to rates for the Iowa general population. There were slight elevations of observed to expected cases for a few cancer sites, but incidence for these sites was not appreciably elevated or depressed for workers with 5 or more years exposure and 15 or more years since first exposure. The SIR for colorectal cancer reflects 0 observed and 0.6 expected during the 1991-1993 update period.

701 of the 1025 alachlor workers (68%) were classified as having the potential for high exposure. The high exposures included occupational exposures and presumed drinking water exposures during the 1968-1975 period. Cancer incidence was fairly similar for these workers and the Iowa population (18 observed, 14.6 expected, SIR = 1.2, 95% CI 0.7-2.0). Analyses which considered only 1974-75 as the period of drinking water exposure gave similar results (17 observed, 12.9 expected, SIR = 1.3, 95% CI 0.8-2.1). Workers exposed 5 or more years with at least 15 years since first exposure had 4 observed and 4.2 expected cancers (SIR = 1.0, 95% CI 0.3-2.4).

Results for specific cancers show no observed cases or 1 case for most sites and slight elevations for colorectal cancer, chronic myeloid leukemia (CML), Hodgkin's disease, and melanoma based on 3, 2,

2, and 2 cases, respectively. There were, however, no cases of CML or Hodgkin's disease among workers with 5 or more years exposure and 15 or more years since first exposure. One of the CML cases was diagnosed soon after first employment at the plant, which, given the course of CML, indicates etiologic factors prior to employment at the Muscatine plant. Colorectal cancer incidence for workers with 5 or more years exposure and a least 15 years since first exposure showed 2 observed and 0.6 expected (SIR = 3.6, 95% CI 0.4-12.9).

A further analysis of cancer, colorectal cancer, and CML focusing on 412 alachlor production (only) workers. Many of these workers were employed in formulation and packaging operations where there was continuous potential for high exposure on a daily basis. Among workers with less than 5 years exposure there were 7 observed versus 4.5 expected cancers (SIR = 1.0, 95% CI 0.6-3.2), and for workers with 5 or more years exposure there were 2 observed versus 2.1 expected cancers (SIR = 1.0, 95% CI 0.1-3.4). There were no observed cases of colorectal cancer versus 0.6 expected and 1 case of CML versus 0.1 expected.

The major goals of the update 1990-1993 were to follow-up on the slight colorectal cancer excess seen in the initial incidence study (MSL 13819) and to continue to monitor patterns of cancer incidence for alachlor workers, especially for cancer sites seen in chronic feeding studies of rats. We did not see a relationship between cancer incidence and years of alachlor exposure or time since first exposure, and there were no cancers of the thyroid, stomach, or nose and nasal sinuses among exposed workers. The numbers of observed and expected cases were very small for most cancer sites which makes the SMRs and SIRs very imprecise and precludes informative exposure response analyses for individual cancer sites.

There were no new colorectal cancer cases during the update period versus 0.6 expected, lessening the observed/expected ratio previously reported. This observation, in conjunction with the lack of any cases among workers in formulation and finishing and the minor contribution of the large bowel to alachlor metabolism and excretion, tends to support a non-causal interpretation of the colorectal cancer findings for this cohort.

Conclusion

The major limitation of this study is the small numbers of incident cancers and cancer deaths.

In terms of power, the study had more than 80% power to detect a relative risk of 2.0 fol all cancers, but the power for major individual cancer sites would exceed 80% only for relative risks of 5 or higher

A second limitation is the possibility of exposure misclassification due to difficulty in estimating dermal occupational exposure, and exposure from plant drinking water.

Despite the limitations of this study, the findings are useful for assessing potential alachlor-related health risks.

At present, however, the available data from manufacturing workers do not indicate an appreciable hazard during the study period related to alachlor exposure.

2.4.2 ADI

The ADI is calculated on the basis of chronic feeding studies in the dog, rat, mouse, and reproductive toxicity in rat and rabbit. It is also assumed that nasal tumours in rats are not formed via a genotoxic mechanism for which exists a threshold, and that these tumours are not relevant to humans. With all these premises, an acceptable daily intake (ADI) should be based on the lowest No Observed Effect Level in rodents which is 0.5 mg/kg/day for rat. If a safety factor of 100 is applied to the calculation, an **ADI of 0.005 mg/kg/day.**

2.4.3 AOEL

The calculation of an AOEL is based on the results of subchronic toxicity tests in dog.

Dog	NOEL	6 month oral toxicity 5 mg/kg/day Mortality at and above 25 mg/kg/day. Liver toxicity at 25, 50 and 75 mg/kg/day by increased in serum enzyme levels and the occurrence of microscopic lesion. Anemia in animals given doses of 25 mg/kg/day and above.

The proposed AOEL should be established on the basis of the 6 month dog oral toxicity study: NOAEL of 5 mg/kg/day.

A safety factor of 10 rather than 100 is proposed given that:

1) In the absence of a 90 day non rodent study, on which the AOEL is routinely based the more conservative NOEL from the 6 month non rodent study (dog) has been used. Therefore there is a hidden safety factor in this calculation.
2) The increase in liver weight at 5 mg/kg in the 6 month dog study appears to be an adaptive change. No liver toxicity was observed at 3 & 10 mg/kg/day in a 1 year dog study.
3) No cases of tumours observed in rat oncogenic study were found among alachlor manufacturing workers with up to 22 years follow-up.
4) Operator exposure would be 2-3 weeks maximum for contract sprayers per year.

A safety factor of 10 is proposed, giving an **AOEL for man of 0.5 mg/kg/day**.

2.4.4 Drinking water limit.

On the basis that exposure through drinking water should not account for more than 10% of the ADI (0.005 mg/kg bw/day), assuming an average consumption of 2 litres of water per person per day and body weight of 60 kg, a maximum acceptable concentration of 15 mg/l is proposed.

In October 1992 the World Health Organisation (WHO) revised the Maximum Acceptable concentration of alachlor in drinking water from 0.3 µg/l to 20 µg/l (based on 10^{-5} life time cancer risk).

2.4.5 Impact on human or animal health arising from exposure to the active substance or to impurities contained in it

MONSANTO

Lasso NF-79-WB has been tested for acute toxicity (oral and dermal), primary irritation and sensitisation potential (material test for this study is LASSO ME. All studies include a QA statement, but do not comply with GLP.

In rats the acute oral median lethal dose (LD_{50}) was considered to be greater than 5000 mg/kg.

During the percutaneous study , there was a slight evidence of toxicity signs, and the acute dermal median lethal dose (LD_{50}) was considered to be greater than 2000 mg/kg in rabbit.

Inhalation study has been not conducted.

Material test (Lasso NF-79-WB) is slightly irritant to rabbit skin. No corrosive effects were noted.

The results of the acute eye irritation/corrosion test with the tested sample of LASSO NF-79-WB showed to be non-irritant to rabbit eye.

A skin sensitisation study showed that the compound LASSO ME was considered a sensitizer to guinea pig

Lasso EC has moderately acute oral toxicity and low acute dermal toxicity. Lasso Microtech has low acute oral and dermal toxicity. The skin and eye irritant properties of the formulations are taken in account in the recommendations for the use of protective clothing during use.

SHINUNG CORPORATION

Tradiachlor has been tested for acute oral toxicity and primary irritation (skin and eye). All studies were undertaken followed procedures of the OCDE using GLP.

In rats, the acute oral median lethal dose (LD_{50}) is 1.2 g/kg (males and females combined).

Material test (Tradiachlor) was considered moderate irritant to rabbit skin.

The results of the acute eye irritation/corrosion test with Tradiachlor showed to be irritant to rabbit eyes.

Its percutaneous study, inhalation toxicity and sensitising potential has not been examined.

In conclusion, Tradiachlor is hazardous by oral route, moderate irritant to rabbit skin and irritant to rabbit eye.

[redacted]

Sanachlor 480 EC has been thoroughly tested for acute toxicity, primary irritation and sensitisation potential. All studies were undertaken with a single batch of formulation (7-7-94), and followed procedures of the OCDE.

In male rats the acute oral median dose (LD_{50}) was considered to be 2094 mg/kg.

During the percutaneous study, there was a slight evidence of toxicity signs, and the acute dermal median lethal dose was considered to be > 4032 mg/kg in the rabbit

Inhalation (nose only) at the mean concentration of a 5.44 ± 0.12 g/m^3 gave rise a evidence of toxicity signs; since one male and one female rat died at this limit concentration, which was less than 50% of the rats, it was not necessary to perform a full study. It was concluded that the 4-hour LC_{50} values of Sanachlor 480 EC was higher than 5.44 g/m^3.

Material test (Sanachlor 480 EC) is very slightly irritant to rabbit skin. No corrosive effects were noted.

The results of the acute eye irritation/corrosion test with the tested sample of Sanachlor 480 EC showed to be very irritant to rabbit eyes.

A skin sensitisation study in guinea pig using the Magnusson and Kligman Maximisation Test demonstrated a lack of skin sensitisation potential.

In conclusion, Sanachlor 480 EC is harmful by the oral route, non-hazardous by the dermal and inhalation route, very slightly irritant to rabbit skin, very irritant to rabbit eyes and the result of a study of skin sensitisation in guinea pigs were negative.

PHYTORUS

Reneur has been tested for acute toxicity (oral and dermal) and primary irritation.. All studies were undertaken with a single batch of formulation (89.319), followed procedures of the OCDE using GLP.

In rats the material test had low oral toxicity and the acute oral median dose (LD_{50}) was considered to be greater than 2000 mg/kg.

During the percutaneous study, there was a slight evidence of toxicity signs, and the acute dermal median lethal dose (LD_{50}) was considered to be greater than 2000 mg/kg in the rat.

Material test (Reneur) was irritant to rabbit skin. No corrosive effects were noted.

The results of the acute eye irritation/corrosion test with Reneur showed to be irritant to rabbit eyes.

Its inhalation toxicity and sensitising potential has not been examined.

In conclusion, Reneur is non-hazardous by oral and dermal route, irritant to rabbit skin and irritant to rabbit eye.

MAKHTESHIM AGAN

No data was submitted. This information will be required.

2.5 Methods of analysis

2.5.1 Analytical methods for the analysis of the active substance as manufactured

MONSANTO

Adequate methodology has been provided for the analysis of the content of active substance and impurities in technical grade material as manufactured.

SHINUNG CORPORATION

Although an analytical method has been provided for the analysis of the active substance, no data for Alachlor technical and no methodology for impurity analysis have been provided.

[redacted]

Although an analytical method has been provided for the analysis of the active substance, no data for Alachlor technical and no methodology for impurity analysis have been provided.

PHYTORUS

An analytical method has been provided for the analysis of the active substance, but no data for Alachlor technical have been provided. Main impurities of the technical active substance have been determined, although the analytical method has not been provided.

MAKHTESHIM AGAN ICC

No methodology has been provided.

2.5.2 Analytical methods for formulation analysis

MONSANTO

Adequate methodology has been provided for the analysis of the content of active substance in the EC formulations. **Validation should be provided for CS formulations.**

SHINUNG CORPORATION

Analytical methodology has been provided for the determination of the content of active substance in formulations. **Validation of the methodology should be provided for CS formulations.**

[redacted]

Adequate methodology has been provided.

PHYTORUS

Adequate methodology has been provided.

MAKHTESHIM AGAN ICC

No methodology has been provided.

2.5.3 Analytical methods for residues analysis

MONSANTO

Adequate methodology has been provided for residues analysis in plant material and animal products, based on the definition of the Alachlor residue in these matrices that should be: Alachlor derived metabolites as the sum of the common chemophores DEA and HEEA, expressed as parent Alachlor.

Adequate methodology has been provided for residues analysis in soil, water, air, human urine and dermal gauze patches however **analytical methodology for the determination of Alachlor metabolites in water and validation of the method for determining Alachlor in air should be provided.**

An assessment of the inclusion of Alachlor in standard multi-residue methods is required.

SHINUNG CORPORATION

Incomplete methodology has been provided for residues analysis. Several published methods have been provided for the analysis of Alachlor in plant material, soil and water, however **no methodology has been provided for Alachlor metabolites in plant material, animal products and soil. No methodology has been provided for Alachlor residues analysis in air and human urine.**

[redacted]

Incomplete methodology has been provided for residues analysis. Several published methods have been provided for the analysis of Alachlor in plant material, soil and water, however **no methodology has been provided for Alachlor metabolites in plant material, animal products and soil. No methodology has been provided for Alachlor residues analysis in air and human urine.**

PHYTORUS

Incomplete methodology has been provided for residue analysis. Several published methods have been provided for the analysis of Alachlor in plant material, **but no methodology has been provided for Alachlor metabolites. No methodology has been provided for Alachlor residues in animal products, soil, water, air and human urine.**

MAKHTESHIM AGAN ICC

No methodology has been provided.

2.6 Definition of the residues

2.6.1 Definition of the residues relevant to MRLs

Similar metabolites have been identified in animal and plant studies. Alachlor is metabolised in both plants and animals via initial displacement of chlorine by nucleophiles such as glutathione, hydroxylation on the ethyl side chain, and sugar conjugation.

The definition of the alachlor residue in plant and animal tissues should be: alachlor and derived metabolites, containing the intact aniline moiety or the 1-ethyl hydroxilated aniline moiety, detected as the sum of the common chemophores DEA (2,6-diethylanuline) and EA (2-Ethylaniline) when acid hydrolysis is employed or DEA (2,6-diethylanuline) and 1'-HEEA (2-(1'-hydroxyethyl)-6-ethylaniline) upon base hydrolysis, expressed as parent alachlor.

The equivalence between 1'HEEA and EA with base and acid hydrolysis respectively is supported in the study IIA, 6.2 "The metabolism of two carbon –^{14} labelled Alachlor metabolites in laying hens"

2.6.2 Definition of the residues relevant to the environment

The parent compound has been identified as a significant residue in all compartments.

In addition, metabolites 65, 70 and 78 are significant in soils, waters, and water/sediment systems.

Other metabolites can be significant in some specific routes. Particularly, metabolites 54 (soil), 51 and 52 (water/sediment).

For ground waters leaching only the parent compound is considered relevant.

2.7 Residues

2.7.1 Residues relevant to consumer safety

Plant metabolite studies were conducted to identify the major metabolites in corn foliage, corn grain, soybean foliage and soybean grain.

The test substance used in these studies consisted of a mixture of [^{12}C]-, [^{13}C]-, and [^{14}C]- alachlor which was uniformly labelled with ^{14}C in the phenyl ring and with ^{13}C in the methylene carbon adjacent to the carbonyl.

Alachlor is extensively and rapidly metabolised in plants via initial displacement of chlorine by nucleophiles such as glutathione, hydroxylation on the ethyl side chain and sugar conjugation giving rise to numerous low level metabolites.

The metabolites are hardly translocated to grain, which is contradictory with the posterior studies in rotational crops, in no case parent Alachlor was found in the plant.

In corn five alachlor-derived metabolites constituting 19% of the foliage-contained activity were identified. Over 80% (eleven metabolites, constituting 0.08 to 2.2% of foliage activity) of the numerous, low-level residues in foliage and grain were characterised by chromatography, high voltage electrophoresis, and strong acid hydrolysis to either 2,6-diethylaniline (DEA) or 2-ethylaniline (EA).

The metabolism in soybean is very complex, originating a great amount of metabolites, in some cases very difficult to identify. Three important metabolic attack points leading to the observed alachlor metabolites. Displacement of the alachlor chlorine atom by oxygen or sulphur nucleophiles proceeded to the degree that no alachlor nor chlorine containing metabolites were detected in the foliage or beans. Hydroxylation at the benzyl position was quite important as 88% of the identified alachlor foliage metabolite radioactivity was due to metabolites oxygenated at the benzyl position. Thirdly, sugar conjugation was important since 53% of the identified alachlor foliage metabolite radioactivity was due to sugar conjugates. The identified alachlor metabolites from foliage all fell into one of three compound classes: neutral sugar conjugates, 26.1%, oxanilic acids, 13.9%; sulfonic acids, 8.8%. In the case of bean metabolites, all of those which were identified were oxanilic acids, and strong evidence was developed to indicate a major presence of sugar conjugates. Experimental evidence indicated an almost complete absence of sulfonic acid metabolites in the beans.

According to the directive 96/86/CE of the Commission it will be necessary to carry out metabolism studies on three category crops unless the impossibility of a distinct metabolism is justified. Only studies in corn and soyabeans were submitted therefore only two crop categories were covered (cereals and oilseeds). In addition the registration for cabbage, cauliflower and peas is solicited, and taking into account the complex metabolism of alachlor in corn and soya beans. It will be convenient to supply studies of metabolism and distribution in leafy crops.

Animal metabolism studies were conducted to identify metabolites in milk and edible tissues in goats and eggs and edible tissues in chickens.

As alachlor itself is not a component of the plant residue on which animals feed Monsanto and EPA agreed on the selection of two representative structures 2,6-diethylphenyl-N-methoxymethyl-2-hydroxyacetamide (t-hydroxyalachlor (39)) and 2-ethyl-6-(1-hydroxyethyl)phenyl)-N-methoxy-methyl-2-(methylsulfonyl)acetamide (t-hydroxysulfone (27)). T-hydroxyalachlor (39) represents DEA class of metabolites and t-hydroxysulfone represents 1-HEEA class of metabolites detected in plant metabolism studies.

The test substances were uniformly labelled with ^{14}C in phenyl ring and with ^{13}C in the methylene carbon adjacent to carbonyl.

Excretion was the primary route of elimination of the administered radioactivity. As in plants the test substances were modified via hydroxylation on the ethyl side chain and sugar conjugation.

Alachlor derived residues have been quantified in GLP residue trials to support the registered uses of Lasso EC and Lasso Microtech in cotton, maize, sweet corn (soft corn), soybean and sunflower in the European Union.

In the evaluation of the residue trials no distinction has been considered between the Northern zone and the Southern zone, due to the great number of trials carried out, it has been difficult to situate conditions for one or another zone, due to the fact that trials were carried out in the USA. On the other hand the solicited GAP, basically refers to Southern countries.

In cotton, 7 trials have been carried out, in the USA with EC 480 g/l with doses in some cases superior to those recommended for Europe. In all the cases the residues found in seed were lower than 0.02 mg/kg. It will be convenient to carry out 2 trials with the CS 480g/l formulation on the doses of 2.40 kg a.s/ha, given that this formulation has not been tested, it is solicited in the GAP and it is necessary to complete the 8 trials according to the Directive 96/68/CE.

In corn/maize, 38 trials have been carried out with the CS formulation and 11 trials with EC, with dosis corresponding to the GAP or higher. Only doses much higher than recommended, in the order of triple, in the residue content there appear concentrations of 0.02-0.03 mg/kg in grain. The number of trials carried out on residue in corn/maize are considered sufficient.

In sweet corn, 10 trials have been carried out with CS formulation and another ten with the EC formulation with doses corresponding to the GAP or higher. The studies carried out are considered sufficient, since the conditions of use and the number of the same are adjusted to the GAP in all the cases the residues were lower than 0.05 mg/kg.

In soybean 28 trials have been carried out (15 with a CS formulation an 13 with a EC formulation) with doses corresponding to the GAP or higher. The information presented concerning residue data is considered sufficient.

In sunflower 10 trials have been carried out, only with EC formulation. Nevertheless in the GAP CS formulation is represented, taking into account the irregularity existing in the results obtained in the EC formulation it is considered convenient to carry out 4 trials with CS formulation.

Regarding peanut cabbage, cauliflower and peas, no experimental data has been supplied, it will be necessary to carry out trials as established in the Directive 96/86 CE, so as to be able to admit the GAP proposed for this crops. Likewise for maize and soybean in conditions of application distinct to the proposed by Monsanto (See Level 4).

Corn is primarily grown in the European Union as a feed grain for animals. A small amount is processed via wet milling to give food grade corn starch and via dry milling to give a food grade oil which is derived from crude oil which is then refined, bleached and deodorised.

Alachlor derived residues do not concentrate in the processed fraction food grade corn oil. Alachlor derived residue levels in food grade corn oil are below limit of determination ie < 0.02 mg/kg.

Alachlor derived residue levels in corn starch were below or slightly above the limit of determination (< 0.02-0.025) using exaggerated application rates of 17.9 and 44.8 kg ai/ha. Alachlor derived residues do not concentrate in food grade corn starch and residue levels are expected to be < 0.02 (LOD) when normal application rates are used.

A small amount of soft corn (sweet corn) is grown in the European Union for direct human consumption alachlor-derived residues levels are below the limit of determination ie < 0.02 mg/kg.

Food grade soybean oil is derived from crude oil which is then refined, bleached and deodorised. Soybean oil is the primary human consumable product derived from soybeans. Soybean protein products are used to a small extent in a variety of products suitable for human consumption. Alachlor derived residues do not concentrate in soybean oil and residue levels are usually < 0.04 mg/kg (LOD) in refined oil and always below 0.04 mg/kg in deodorised soybean oil.

Food grade sunflower oil is derived from crude oil when is then refined, bleached and deodorised. Deodorised sunflower oil is the primary human consumable product derived from sunflower.

Alachlor derived residues do not concentrate in sunflower oil and residue levels are below the limit of determination ie <0.02 mg/kg in deodorised sunflower oil

In the different process stages in obtaining oil a lowering of Alachlor residue is observed. The process alkali-refining produces a notable reduction of Alachlor residues (70-80%).

Alachlor livestock feeding studies were conducted in dairy cattle, pig and chickens.

Alachlor livestock feeding studies were conducted using a synthetic mixture of five compounds representative of corn and soybean metabolites, 40 wt% t-hydroxyethyl methylsulfone; 15 wt% hydroxyalachlor 15 wt% t-oxanilic acid; 15 wt% t-sulfonic acid, 15 wt% t-sulfinyl lactic acid.

Animals were dosed for 28 days at approximately 4, 12 and 40 ppm (nominal total diet alachlor equivalent). After dosing for 28 days animals were sacrificed and edible tissue collected, the remaining animals were sacrificed after a 28-day withdrawal period.

The 4 ppm dose level represents the maximum residue level in a dairy cattle diet and is 20 times greater than the maximum residue level in chicken or pig feed.

The maximum alachlor derived residue found in the milk and edible tissues of dairy cattle, edible tissues of pigs and eggs and edible tissues of chicken at the feeding level of 4 ppm are less than 0.01 mg/kg. **The dosing used in the study was overestimated and represented the 1X, 3X and 10X times the estimated residue according to "Harris, L.E., Guide for estimating toxic residues in animal feeds or diets " U.S. NTIS PB Rep. PB-243, 748, January 1975." This dosing does not represent a real residue intake but taking into account that this was overestimated and the residue level in the different analysed portion was very low, this study is considered acceptable and the MRL can be established as the detection limit.**

In the study carried out on rotational crops, the presence of significant quantities of Alachlor in wheat, lettuce and radish was observed at 90, 120 and 365 DAT, which is contradictory to the metabolism studies carried out in plants where translocation did not occur. Given that it is dealing with a residual herbicide, it is considered that more information with reference to the presented data is needed.

The potential for exposure to alachlor in the diet is very low. In the European Union, alachlor is used primarily in crops such as maize and soybeans, which are either grown for animal feed or are processed before entering the human food chain. Alachlor is also used on sweet corn which is grown for direct human consumption. No more than 20% of the hectares grown in any country are treated with alachlor. Therefore, more than 80% of all these crops cannot contain any alachlor derived residue.

If dietary exposure is calculated using worst case assumptions (*e.g.* the entire diet consisted of food containing alachlor derived residues at the limit of detection), human consumption would be less than 3% of ADI, considering the ADI proposed by the Rapporteur.

2.7.2 Residues relevant to worker safety

Since products containing Alachlor are applied pre-emergence or early post-emergence is not necessary to re-enter fields shortly after spraying. The half life of Alachlor soil is approximately 5-16 days and 2.54 hours in air therefore it is not necessary to determine a re-entry time to workers.

2.7.3 Compliance with existing MRLs and/or proposed MRLs

The MRLs proposed by the applicant are considered acceptable.

Of the proposed MRL's only soybean grain and sunflower seed are above the limit of determination. These MRL's are not of significance for dietary intake calculations, as these crops are primarily processed into oil where it has been demonstrated that residues are below the limit of determination.

Table 2.7.3-1: Proposed EU MRLs

Error! Bookmark not defined.Commodity	**Proposed Pre-harvest Interval**	**Proposed MRL (mg/kg)**
Cotton (seed)	NR	0.02*
Maize (grain)	NR	0.02*
Sweet corn (grain)	NR	0.05*
Soybean (grain)	NR	0.2
Sunflower (seed)	NR	1.0
Sunflower (Straw)	NR	2.0
Foodstuffs of animal origin	NR	0.01*

* Limit of determination
NR Not required.

Pre-harvest intervals are not required as alachlor formulations are applied when the edible portion of the plant is not formed. Residue trials confirm that there are no residues above limit of detection.

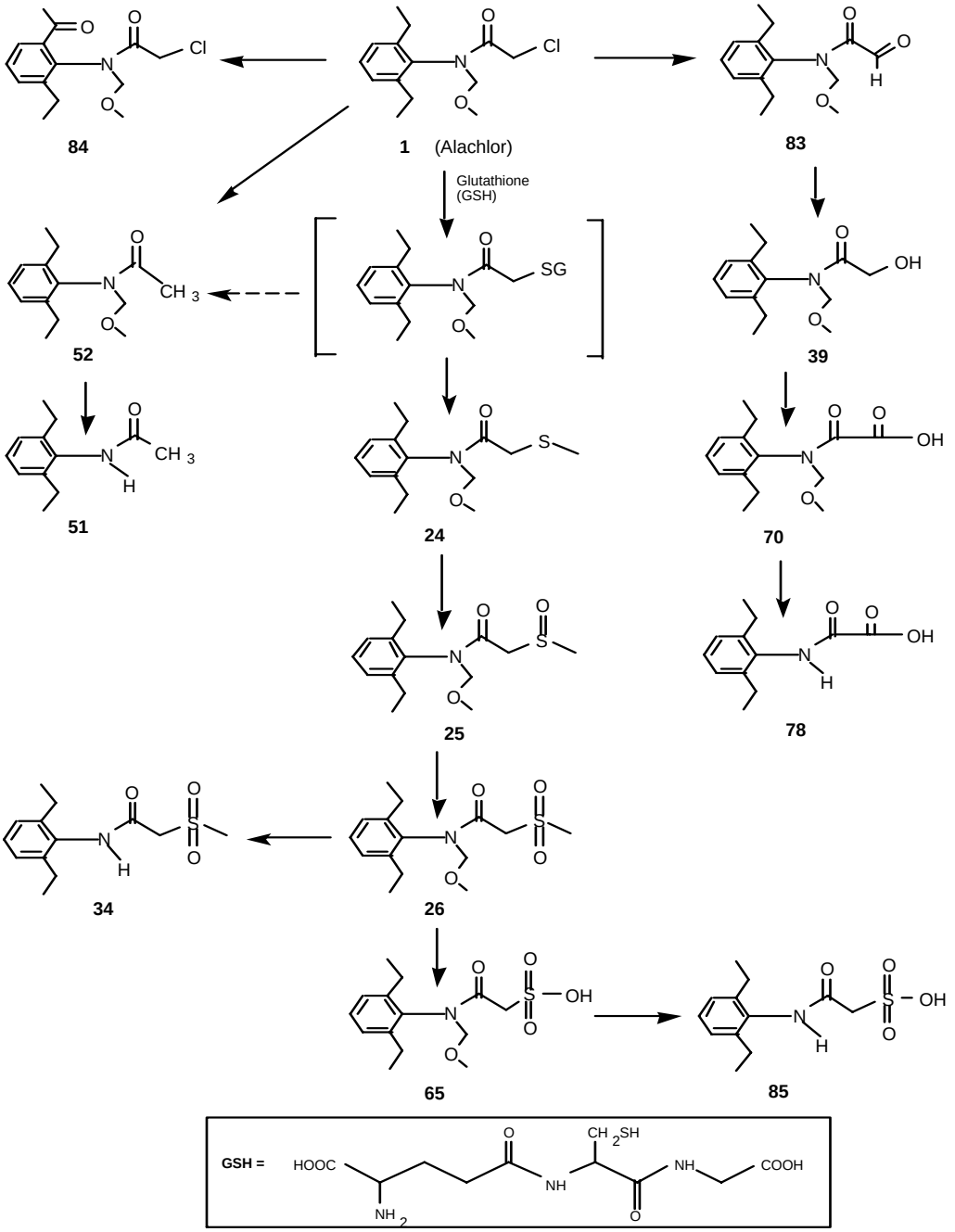
2.8 Fate and behaviour in the environment

2.8.1 Fate and behaviour in soil

Most degradation studies indicate that alachlor disappears relatively rapid in soils. The DT_{50} under aerobic conditions is, in general, less than 30 days. In some laboratory studies a higher persistence was observed, 98% remaining after 66 days, and although no information is given a reduction in microbial activity must be suggested as a feasible explanation. Field studies confirmed the rapid degradation of this pesticide.

The available information confirms the data reviewed by Chesters *et al*. (1989). Microbial degradation must be considered as the main contributor to the disappearance of alachlor from the soil under aerobic conditions. The disappearance rate decreases with the temperature.

The postulated metabolic pathway for alachlor during aerobic soil incubation is presented below. Several steps may be involved in each conversion.



In addition to the proposed degradation pathway presented above, the following Table summarises the metabolites of alachlor identified in different environments.

Table 2.8.1-1: Metabolites of alachlor (AO) identified in various environments

Code no.	Chemical name	Identified in
A0	2-Chloro-2´,6´-diethyl-N-(methoxymethyl) acetanilide	Parent compound
A1	2-Chloro-2´,6´-diethylacetanilide	Soils, photolytic and microbial (lab); culture, fungus
A2	2,6-Diethylaniline	Soils, photolytic (lab); culture, fungus; soil (flooded), microbial (lab); rat liver
A3	2´,6´-Diethylacetanilide	Soils, photolytic (lab); soils (flooded) microbial (lab)
A4	Chloroacetic acid	Soils, photolytic (lab)
A5	2´,6´-Diethyl-N-methoxymethylaniline	Soils, photolytic (lab); culture, fungus
A6	1-Chloroacetyl-2,3-dihydro-7-ethylindole	Soils, photolytic and microbial (lab); culture, fungus
A7	8-Ethyl-2-hydroxy-1-methoxymethyl-1,2,3,4-tetrahydroquinoline	Soils (upland), microbial (lab)
A8	7-Ethyl-1-hydroyacetyl-2,3-dihydroindole	Soils (upland), microbial (lab)
A9	2´,6´-Diethyl-N-2-hydroxy(methoxymethyl) acetanilide	Soils (upland and flooded), microbial (lab); culture-actinomycete, bacteria
A10	9-Ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2-(3H)-one	Soils (upland), microbial (lab)
A11	3-Dihydro-1-formyl-7-ethylindole	Soils (flooded), microbial (lab)
A12	N-(2,6-Diethylphenyl) formamide	Soils (flooded), microbial (lab)-intermediate
A13	2´,6´-Diethyl-N-(methoxymethyl)acetanilide	Soils (flooded), microbial (lab)
A14	2-Chloro-2´-ethyl,6´-etheneacetanilide	Culture, fungus-intermediate
A15	2-Hydroxy-2´,6´-diethylacetanilide	Culture, fungus-intermediate

No conclusive evidence on the rate of degradation under anaerobic conditions or by photolysis can be reached, due to the discrepancy observed in the limited information presented by the applicants.

Biodegradation will be the most important process by which alachlor will be lost from most soils. Although biotransformation of alachlor from soil was rapid, there was little mineralisation. This indicates that biotransformation proceeds via cometabolism. Some loss of alachlor from soil will occur as a result of photolysis by sunlight, but photolysis will not be competitive with biodegradation. Several metabolites have been identified in both, biodegradation and photodegradation studies. A tentative route for the degradation of alachlor has been proposed. In addition, several micro-organisms are able

to degrade alachlor under laboratory conditions. The metabolites identified in these studies have been included in an additional table.

Alachlor does not persist in the soil - the DT_{50} values from most field studies were 4-24 days. Most values for laboratory studies were within this range. However, a very low degradation rate was reported in some studies. As a conclusion, an average DT_{50} in soils of 15 days, and a realistic worst-case DT_{50} of 30 days are considered appropriated values for the disappearance of alachlor in soils. These figures are expected to cover all European conditions except those with a very low microbial activity. The DT_{90} is expected to be lower than one year.

Alachlor has a high to medium mobility in soil. Field studies show the potential for low concentrations of alachlor to leach beyond the root zone. Mobility decreases with increasing organic carbon content. Correlation between organic matter content, soil adsorption coefficient and mobility in soil have been observed. The presence of continuous pores or channels in soil will increase the mobility of alachlor in soil. Column leaching studies indicates that alachlor must be considered the main residue in the leachate for soils with low to medium organic carbon content. Several metabolites, were detected in leachates from soils with high organic content, however, due to the low mobility and rapid degradation of this herbicide, these metabolites only represented a very low percent of the applied dose. Thus, it is concluded that under those realistic conditions in which the mobility of alachlor is relevant, the parent compound must be considered as the major residue.

Field studies confirmed the mobility of alachlor under certain conditions. Although most analysis were below the detection level, relatively high concentration of alachlor in soil water were detected when rainfall events were produced shortly after application. Thus, a potential for contamination of groundwater must be expected. This potential has been confirmed by model predictions and monitoring data.

Similarly, these events also produced a significant run-off of alachlor which must be considered when estimating the PECs for surface water.

Predicted Environmental Concentrations in Soils (PEC)

The Predicted Initial Concentration ($PIEC_S$) in soil has been calculated for the maximum application rate 3.36 kg a.i./ha authorised in Greece for soils with an organic matter content higher than 4%, the maximum application rate without restrictions 2.9 kg a.i./ha authorised in Spain for maize, and the most common application rate of 2.4 kg a.i./ha.

The estimate follows:

Table 2.8.1-2: Predicted alachlor initial concentrations in soils.

Crop	Soil coverage (%)	Application rate (a.s.)		Portion of a.s. reaching soil		PEC_S (mg a.s./kg soil) initial - 5 cm soil depth
		kg/ha	mg/m^2	kg/ha	mg/m^2	
None	0	3.36	336	2.36	336	4.48
None	0	2.9	290	2.90	290	3.9
None	0	2.4	240	2.4	240	3.2

1 Assumes soil density is 1.5g/ml

2.8.2 Fate and behaviour in water

Non coherent data have been reported for hydrolysis and photolysis. From the available information it can be concluded that these processes do not play a significant role in the disappearance of alachlor from water, which is mainly due to biodegradation. This conclusion is confirmed by data showing that the degradation under sterile conditions is much lower than in non-sterile media. Volatilisation is not a significant cause of losses.

Alachlor is not ready biodegradable and DT_{50} values in the range of 200-500 days in river water have been determined. The DT_{50} values decreased by the addition of soil or sediment, reaching values of 23-206 days. In a water/sediment study using two different sediments, the DT_{50} were also significantly lower than those observed for natural water. DT_{50} values of 18-37 days were observed. These values are similar to those reported for soil samples. Norchloroalachlor was the main metabolite. In all cases, the level of mineralisation was very low.

Although a rapid degradation of alachlor under anaerobic laboratory conditions was reported, most data suggest that the rate of anaerobic degradation of alachlor would be very low. The disappearance of alachlor in groundwater free of aquifer materials (e.g., sand) was very slow and the half-life was in the range 808-1518 days. The potential of alachlor to contaminate ground-waters has been identified. Data suggest that alachlor can appear in groundwaters several months after application. Leaching and accumulation in the subsoil has been considered as the most likely explanation. Dilution has been considered the main cause for the gradual decrease of alachlor concentrations in contaminated groundwaters.

Predicted environmental concentrations (PEC) in water.

Predicted environmental concentrations in groundwaters (PECgw).

Alachlor has a high to moderate mobility in soils. This mobility is inversely related to the organic matter content and the adsorption coefficient of the soil. A potential for groundwater contamination has been identified, particularly for soils with low organic matter content. The presence of continuous pores or channels in soil will increase the mobility of alachlor in soil.

Three different models have been used to predict the impact of alachlor at the top of groundwater bodies. The predictions have been established for maize cultivation in France and Italy. Models indicate that the concentrations of alachlor should be below 0.01 µg/l for the average scenarios, but can reach detectable levels under reasonable worst-case. The application rate employed by the modeller was 2.4 mg a.i./ha. The rate of 3.36 kg a.i./ha is restricted to soils with more than 4% of organic matter where alachlor mobility is expected to be very low. Thus the application rate of 2.4 is considered appropriate for this assessment.

Table 2.8.2-1: Predicted concentrations of alachlor impacting at the top of the groundwater body for the worst-case scenario.

Error! Bookmark not defined.	**LEACHP**	**PRZM-2**	**MACRO**
Normal irrigation			
Predicted peak concentration (µg/l)	0.01	<0.01	0.19
Predicted mean annual concentration (µg/l)	<0.01	<0.01	0.01
Early irrigation			
Predicted peak concentration (µg/l)	0.08	0.02	2.30
Predicted mean annual concentration (µg/l)	0.02	<0.01	0.30

Field studies and monitoring data confirmed this potential. Although some monitoring data showed maximum values in the range of 10µg/l, it is considered that the MACRO prediction, 2.30µg/l, without additional dilution factors, represents an acceptable realistic worst-case condition under normal agricultural practice in Europe.

This concentration can be reached immediately or several months after the herbicide application, depending on the rainfall events.

Considering the inverse relationship between alachlor mobility and organic matter content, as well as the relatively rapid degradation of the pesticide under aerobic conditions, groundwater contamination is only expected for soils with low organic matter content and when rain-fall events occurs after the treatment. Therefore, in most cases the use of alachlor will not be associated to groundwater pollution if good agricultural practices are followed and the soil has a medium to high content of organic matter.

Predicted environmental concentrations in surface waters (PECsw)

Taking into account the relative mobility of alachlor both drift and surface run-off are considered relevant for the estimation of PECs for surface waters. Both possibilities have been confirmed by field and monitoring data. Although discharges *via* drains has been observed in some circumstances, in general it is considered of low relevance when compared to drift and run-off.

Spray drift is expected to be the most important cause of surface water contamination immediately after application of alachlor. The prediction of the concentration of alachlor in surface waters should be done for three application rates:

3.36 kg a.i./ha the highest authorised but restricted to soils with more than 4% of organic matter

2.9 kg a.i./ha the highest application rate

2.4 kg a.i./ha the most common application rate

The spray drift obviously depends on the distance between the treated field and the adjacent surface waters. The Initial Predicted Environmental Concentrations have been estimated on the base of spray drift values established under practical conditions for water ponds of 0.3 m of depth.

Table 2.8.2-2: Initial Predicted Concentrations of alachlor due to spray drift after the application at the rate of 3.36 mg a.i./kg.

Crop	**Application rate**		**Distance**	**Drift**	**Drift**	**Initial**
	(kg/ha)	**(mg/m^2)**	**m**	**%**	**(mg/m^2)**	**PEC (µg/l)**
All crops	3.36	336	1	4.0	13.4	44.3
			2	1.6	5.44	17.7
			3	1.0	3.35	11
			5	0.6	2	6.6
			10	0.3	1	3.3

Table 2.8.2-3: Initial Predicted Concentrations of alachlor due to spray drift after the application at the rate of 2.9 mg a.i./kg.

Crop	**Application rate**		**Distance**	**Drift**	**Drift**	**Initial**
	(kg/ha)	**(mg/m^2)**	**m**	**%**	**(mg/m^2)**	**PEC (µg/l)**
All crops	2.9	299	1	4.0	11.5	38.1
			2	1.6	4.7	15.3
			3	1.0	2.9	9.5
			5	0.6	1.7	5.7
			10	0.3	0.9	2.8

Table 2.8.2-4: Initial Predicted Concentrations of alachlor due to spray drift after the application at the rate of 2.4 mg a.i./kg.

Crop	Application rate (kg/ha)	Application rate (mg/m^2)	Distance m	Drift %	Drift (mg/m^2)	Initial PEC (µg/l)
All crops	2.4	240	1	4.0	9.5	31.6
			2	1.6	3.9	12.6
			3	1.0	2.4	7.9
			5	0.6	1.4	4.7
			10	0.3	0.7	2.4

Model calculations and field studies can be used to estimate the Predicted Environmental Concentration of alachlor due to run-off. The summary of MACRO simulations appears in Table 2.8.2-5. Run-off values from a field study have been presented in Table 7.1.4-6.

Table 2.8.2-5: MACRO simulation results for concentrations of alachlor in runoff for average- and worst-case scenarios for movement to surface waters in France and normal and early irrigation in Italy

Error! Bookmark not defined.	Average-case scenario	Worst-case scenario	Normal irrigation	Early irrigation
Predicted peak concentration in runoff (mg/l)	<0.05	26.5	19.0	19.0
Predicted mean annual concentration in runoff (µg/l)	<0.05	7.10	4.16	5.19

Model estimations for the worst-case and irrigation scenarios fall well within the range observed in the field study, excluding some very high values which are considered of limited relevance. Thus, the predicted peak concentration in runoff, applying a dilution factor of 0.1 as prescribed by EPPO, has been used for the estimation of PECsw due to runoff. Estimations appears in Table 2.8.2-6.

Table 2.8.2.-6: Initial PECsw of alachlor due to runoff.

Application rate	Concentration in runoff (µg/l)	Dilution factor	Initial PECsw (µg/l)
2.4 kg a.i./ha	26.5	0.1	2.65

2.8.3 Fate and behaviour in air

Results summarised under the physical-chemical properties section indicate moderate stability in air t1/2 2.544 hours.

From its vapour pressure 2.9 mPa at 25 degrees C, alachlor is expected to be present partially in the vapour phase and partially in the particulate form in air.

Additional information can be found in the review of Chesters *et al*. (1989). Based on estimation methods, the rate constant for the reaction of alachlor in the atmosphere with hydroxyl radicals is 1.85×10^{-10} cu cm/molecule-sec. Based on a daily average concentration of hydroxyl radicals in the atmosphere of 5×10^{5} radicals/cu cm, the half-life of this reaction would be 2.1 hours. Based on an estimation method, gas phase alachlor may be removed from the atmosphere with a half-life of 2.1 hours due to reaction with photochemically produced hydroxyl radicals. Partial removal of particulate alachlor from the air may occur by dry deposition. The fact that alachlor has been detected in rainwater suggests it will be removed from the atmosphere by wet deposition as well.

Predicted environmental concentrations in air

Because of the low volatility the occurrence of alachlor in air is not considered significant.

2.9 Effects on non-target species

The available information, in general, is sufficient to assess the potential effects of Alachlor on non target organisms. However, some ongoing reports have been indicated, particularly for alachlor formulations, the applicants are requested to present this information as soon as available.

The available data set includes information for several formulated products, no relevant differences between the active ingredient and the studied formulations have been observed. Information on GR formulations has not been presented, the specific risk of these formulation for birds cannot be assessed.

2.9.1 Effects on birds

The acute toxicity data on alachlor on birds presented by the applicants indicates that this herbicide is not particularly toxic for birds. Acute oral LD_{50} values are in the range of 1000 mg/kg bw, while all acute dietary LC_{50} values are greater than 5620 ppm.

An initial risk assessment of alachlor can be done considering the available information. No chronic toxicity data is available.

Birds may be exposed to alachlor by the consumption of contaminated feed. Depending on species this may be insects, grass, grain or fish. In order to consider the worst case conditions it is assumed that birds feed exclusively on contaminated material and that large grass eating birds have a daily demand of 25% of their body weight, small granivorous birds 30% of their body weight, and small insectivorous birds 35% of their body weight. Two application rates are used, the absolute maximum,

3.36 kg a.i./ha, and the most common rate, 2.4 kg a.i./ha.. The exposure must be compared to the acute oral toxicity for chickens, 916 mg/kg.

Table 2.9.1-1: TER estimations for acute oral toxicity studies of alachlor.

Feed	Application rate (kg a.i./ha)	Estimated initial residue (mg/kg)	Relative feed Demand (%)	Maximum daily intake (mg/kg bw)	Acute toxicity (mg/kg bw)	TER
Grass	3.36	377	25	94	916	9.75
Grain	3.36	9	30	2.7	916	339
Small insects	3.36	97	35	34	916	27
Grass	2.4	269	25	67	916	13.7
Grain	2.4	6.5	30	2	916	458
Small insects	2.4	70	35	24.5	916	37

Additionally, a similar approach has been applied for the comparison with the acute dietary toxicity studies. The toxicity of the active substance to all tested species is very low, with all LC_{50} values greater than 5620 ppm. This figure will be used for the estimations.

Table 2.9.1-2: TER estimations for the acute dietary toxicity studies of alachlor.

Feed	Application rate (kg a.i./ha)	Estimated initial residue (mg/kg)	Acute toxicity (mg/kg bw)	TER
Grass	3.36	377	>5620	>14.9
Grain	3.36	9	>5620	>624
Small insects	3.36	97	>5620	>58
Grass	2.4	269	>5620	>20.9
Grain	2.4	6.5	>5620	>864
Small insects	2.4	70	>5620	>80

The maximum concentration of alachlor in fish, obtained by applying a BCF of *c.a*. 500 to the highest Initial PECsw is expected to be lower than 25 mg/kg. Thus, consumption of contaminated fish does not represent a significant risk for birds.

The estimated TERs are above the trigger value of annex VI with the only exception of the TERa for grass eating birds for the absolute maximum dose, which is only slightly lower than the recommended value. Alachlor is mainly applied on the bare soil, the calculations represent a very worst-case scenario and the TERa estimated for the quail should be 16, and all TERst are higher than 10. Therefore, it is concluded that alachlor does not represent a significant risk for birds.

No information on the chronic toxicity of alachlor technical on birds has been presented.

No information on the granulate formulations has been presented. The risk of GR formulations to birds cannot be assessed.

2.9.2 Effects on aquatic organisms

A significant amount of information on the toxicity of alachlor to aquatic organisms has been presented by the applicants. According to the EU classification system, alachlor can be considered highly toxic for algae, toxic for fish and harmful for aquatic invertebrates. The validated data are included in the following tables.

Table 2.9.2-1: Summary of acute toxicity data on fish.

Chemical	Test organisms	Test conditions	Toxicity endpoint	Result (mg a.i./l)
Technical alachlor	Bluegill sunfish	static/nominal	96 hours LC_{50}	2.8
Technical alachlor	Rainbow trout	static/nominal	96 hours LC_{50}	1.8
Technical alachlor	Channel catfish	static/nominal	96 hours LC_{50}	2.1
Technical alachlor	Bluegill sunfish	flow-through/measured	96 hours LC_{50}	5.5
Technical alachlor	Rainbow trout	flow-through/measured	96 hours LC_{50}	5.3
Technical alachlor	Fathead minnow	flow-through/measured	96 hours LC_{50}	5.0
Technical alachlor	Singui	static/nominal	96 hours LC_{50}	3.7
Lasso M	Rainbow trout	static/nominal	96 hours LC_{50}	1.5
Lasso M	Bluegill sunfish	static/nominal	96 hours LC_{50}	3.2
Lasso EC	Rainbow trout	static/nominal	96 hours LC_{50}	1.8
Lasso EC	Bluegill sunfish	static/nominal	96 hours LC_{50}	2.8
Sanachlor 480 EC	Zebra fish	static/measured	96 hours LC_{50}	1.8
Sanachlor 480 EC	Rainbow trout	static/measured	96 hours LC_{50}	>2
Metabolite 65	Rainbow trout	static/measured	96 hours LC_{50}	>104 mg/l
Metabolite 70	Rainbow trout	static/measured	96 hours LC_{50}	>100 mg/l

Table 2.9.2-2: Summary of acute toxicity data on aquatic invertebrates.

Chemical	Test organisms	Test conditions	Toxicity endpoint	Result (mg a.i./l)
Technical alachlor	Daphnia magna	flow-through/measured	48 hours EC_{50}	13
Technical alachlor	Daphnia magna	static/nominal	48 hours EC_{50}	10
Technical alachlor	Daphnia magna	static/nominal	48 hours EC_{50}	26
Technical alachlor	Crayfish	static/nominal	96 hours LC_{50}	>320
Lasso EC	Daphnia magna	static/nominal	48 hours EC_{50}	15.1

Chemical	Test organisms	Test conditions	Toxicity endpoint	Result (mg a.i./l)
Lasso	Daphnia pulex	static/nominal	48 hours EC_{50}	9.0
Sanachlor 480 EC	Daphnia magna	static/measured	48 hours EC_{50}	10.8
Metabolite 65	Daphnia magna	static/measured	48 hours EC_{50}	>104 mg/l
Metabolite 70	Daphnia magna	static/measured	48 hours EC_{50}	>95 mg/l

Table 2.9.2-3: Summary of chronic toxicity data on fish and aquatic invertebrates.

Chemical	Test organisms	Test conditions	Toxicity endpoint	Result (mg a.i./l)
Technical alachlor	Rainbow trout	flow-through/measured	96 days NOEC	0.19
Technical alachlor	Fathead minnow	flow-through/measured	60 days NOEC	0.52
Technical alachlor	Daphnia magna	flow-through/measured	21 days NOEC	0.23
Technical alachlor	Mud crab	static/nominal	NOEC developm.	14
Lasso	Mud crab	static/nominal	NOEC developm	10
Sanachlor 480 EC	Rainbow trout	flow-through/measured	14 days NOEC	0.25
Sanachlor 480 EC	Daphnia magna	flow-through /measured	21 days NOEC	0.23

Table 2.9.2-4: Summary of toxicity data on algae and aquatic plants.

Chemical	Test organisms	Toxicity endpoint	Result (mg a.i./l)
Technical alachlor	*Selenastrum capricornutum*	72 hours EC_{50}	0.0012
Technical alachlor	*Selenastrum capricornutum*	120 hours NOEC	0.00035
Technical alachlor	*Selenastrum capricornutum*	72 hours EC_{50}	0.00115
Technical alachlor	*Selenastrum capricornutum*	72 hours NOEC	0.006
Technical alachlor	*Selenastrum capricornutum*	96 hours EC_{50}	0.062
Technical alachlor	*Selenastrum capricornutum*	96 hours NOEC	0.0056
Lasso EC	*Selenastrum capricornutum*	72 hours EC_{50}	0.0082
Lasso EC	*Selenastrum capricornutum*	72 hours NOEC	0.0043
Sanachlor 480 EC	*Selenastrum capricornutum*	72 hours EC_{50}	<0.005
Lasso	*Lemna minor*	48 hours EC_{50}	0.01
Alachlor 48 %	*Chlorella pyrenoidosa*	96 hours EC_{50}	0.096
Alachlor 48 %	*Chlorella pyrenoidosa*	96 hours NOEC	0.02
Alachlor 63.3 %	*Chlorella pyrenoidosa*	96 hours EC_{50}	0.126
Alachlor 63.3 %	*Chlorella pyrenoidosa*	96 hours NOEC	0.05

The toxicity of the technical product and the different formulations falls within the same range, thus the lowest data for technical alachlor will be used in the assessment.

TER values have been calculated for different application rates and buffer zones, using the initial PECs. Results are summarised in the following table.

Table 2.9.2-5: Summary of TER estimations for aquatic organisms, based on initial PECsw.

Application rate (kg a.i./ha)	Drift Buffer Zone (m) or run-off	TER
	Fish acute	
3.36	1	41
3.36	2	102
2.4	1	58
2.4	2	143
2.4	- run-off -	679
	Daphnia acute	
3.36	1	225
2.4	1	316
2.4	- run-off -	3773
	Fish chronic	
3.36	1	4.3
3.36	2	10.7
2.4	1	6.0
2.4	2	15.1
2.4	- run-off -	71.7
	Daphnia chronic	
3.36	2	13
2.4	2	18
2.4	- run-off -	87

The TERa for fish and the TERlt for fish and Daphnia are higher than the trigger values when the buffer zone is lower that 2 m. These results indicates that for fish and daphnia the risk of alachlor can be controlled by appropriate risk management measures. A buffer zone of only 2 m is enough to achieve acceptable TER values for fish and daphnia even considering initial PECs.

All TER estimations for algae and other aquatic plants (even for the lowest application rates and buffer zones of 10 m) are below 1, indicating that alachlor posses a very high risk for algae and aquatic plants. This is not surprising considering that alachlor is a herbicide. This risk cannot be reduced by risk management measures as far as both, drift and run-off can be responsible for TER values below 1. A mesocosm study is required to assess the real consequences of this risk on plant communities.

No conclusive evidence on the bioaccumulation potential of alachlor can be established. Some studies suggest a rapid metabolisms of the pesticide in fish which would reduce the bioaccumulation potential estimated from the Kow. The real BCF must be equal to or lower than the estimation *c.a.* 500. Considering the Precautionary principle, this BCF value has been used to assess the potential risk for fish eating birds.

The two tested metabolites, 65 and 70, were not toxic for fish and daphnia. The toxicity of these metabolites to algae, and the toxicity of the other identified significant metabolites for the three taxonomic group is required to perform a final assessment.

2.9.3 Effects on terrestrial vertebrates other than birds

No specific information on this item has been presented by the applicants. However, the available toxicity data on mammals, presented in the toxicity section, indicates that alachlor does not represent a significant risk. Alachlor is of low toxicity to mammals as indicated by rat acute oral LD_{50} of 1350 mg/kg. The no observed effect level (NOEL) in the 90 day mouse study was 195 mg/kg/day. Acute and long-term TERs for small mammals are expected to be similar to those estimated for birds. The realistic long-term TERs are expected to be above 5 which is a level of concern identified in Annex VI.

2.9.4 Effects on bees and other arthropods

Honey bees were tested with different alachlor formulations using the oral and contact routes and a spray simulation. Alachlor did not produce significant effects at the highest tested concentrations. It is concluded that alachlor is not toxic to honey bees. Alachlor and its formulations are expected to pose minimal risk to honey bees.

The alachlor formulations have been tested on three species of terrestrial insects, in addition to the honey bee. These laboratory tests are conducted at the maximum recommended application rate. Alachlor was found to be relatively harmless to both carabid beetles and green lacewings.

2.9.5 Effects on earthworms

The estimated TERs for earthworms using Initial PECsoil are 86, 99 and 120 for application rates of 3.36, 2.9 and 2.4 kg a.i./ha respectively. All TERs are expected to be higher than 100 for time-weighted PECsoil. It is concluded that the risk of alachlor to earthworms is low.

2.9.6 Effects on soil micro-organisms

Effects of alachlor on soil micro-organisms have been identified in some cases but not in others. Some of these effects were not dose related effects, therefore, the interpretation is not easy. The weight of evidence suggest that alachlor does not have an specific risk for soil micro-organisms, although some populations and activities can be temporarily affected.

2.9.7 Effects on other non-target organisms (Flora and fauna) believed to be a risk

No specific information has been presented.

2.9.8 Effects on biological methods for sewage treatment

No significant risk of alachlor for the biological treatments of sewage plants is expected. This conclusion is supported by the available information on the effects of alachlor in degradation and soil effect studies. In addition, no significant contamination of sewage treatment plants is expected to arise from normal agricultural uses.

2.10 Classification and labelling

2.10.1 Alachlor

The Applicant Makhteshim-Agan has not submitted any data concerning classification and labelling.

Proposal

Hazard symbol:	Xn - Harmful
	N - Dangerous for the environment.
Risk phrases:	R 22 - Harmful if swallowed
	R 40 - Possible risk of irreversible effects
	R 43 - May cause sensitisation by skin contact
	R 50/53 - Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.
Safety phrases:	S 2 - Keep out of children's reach.
	S 13 - Keep away from food, drink and animal feeding stuffs.
	S 24 - Avoid contact with skin
	S 37 - Wear suitable gloves.
	S 46 - If swallowed, seek medical advice immediately and show this container or label
	S 56 - Dispose of this material and its container in a hazardous or special waste collection point
	S 57 - Use appropriate container to avoid environmental contamination.

2.10.2 Plant Protection products

Lasso EC (Monsanto):

Specific proposal cannot be made pending completion of all of the relevant studies required in Level 4 of this monograph.

Lasso Microtech (Monsanto):

Proposal

Hazard symbol:	Xn - Harmful
	Xi - Irritant
	N- Dangerous for the environment.
Risk phrases:	R 40 - Possible risk of irreversible effects
	R 43 - May cause sensitisation by skin contact
	R 50/53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.
Safety phrases:	S 2 - Keep out of children's reach.
	S 13 - Keep away from food, drink and animal feeding stuffs.
	S 24 - Avoid contact with skin
	S 36/37/39 - Wear appropriate clothes and gloves and protection for eyes and face.
	S 46 - If swallowed, seek medical advice immediately and show this container or label.
	S 56 - Dispose of this material and its container in a hazardous or special waste collection point
	S 57 - Use appropriate container to avoid environmental contamination.

ALACHLOR 480 g/l CS ([redacted]):

Specific proposal cannot be made pending completion and evaluation of all of the relevant studies, required in Level 4. However a previous proposal is presented.

When the toxicological information will be complete this proposal of classification and labelling will be justified correctly.

Proposal

Hazard symbol:	Xn - Harmful
	Xi - Irritant
	N - Dangerous for the environment.
Risk phrases:	R 40 - Possible risk of irreversible effects
	R 41 - Risk of serious damage to eyes
	R 43 - May cause sensitisation by skin contact
	R50/53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.
Safety phrases:	S 2 - Keep out of children's reach
	S 13 - Keep away from food, drink and animal feeding stuffs.
	S 24 - Avoid contact with skin
	S 36/37/39 - Wear appropriate clothes, gloves and eyes and face protection.
	S 46 - If swallowed, seek medical advice immediately and show this container or label.

S 56 - Dispose of this material and its container in a hazardous or special waste collection point

S 57 - Use appropriate container to avoid environmental contamination.

SANACHLOR 480 EC ([redacted]):

The explosive properties of the manufactured plant protection product should be justified by the applicant.

Proposal

Hazard symbol: E - Explosive

Xn - Harmful

Xi - Irritant

N- Dangerous for the environment.

Risk phrases: R 2 -Risk of explosion by means of impact, friction, fire or other source of ignition.

R 10 - Flammable

R 40 - Possible risk of irreversible effects.

R 41 - Risk of serious damage to eyes.

R 50/53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.

Safety phrases: S 2 - Keep out of children's reach.

S 13 - Keep away from feed drink and animal feeding stuffs

S 24/25 - Avoid contact with eyes and skin

S 36 - In the case of eye contact, wash immediately with abundant water and seek medical advice.

S 36/37/39 - Wear appropriate clothes, gloves and eyes and face protection.

S 46 - If swallowed, seek medical advice immediately and show this container or label.

S 56 - Dispose of this material and its container in a hazardous or special waste collection point

S 57 - Use appropriate container to avoid environmental contamination.

RENEUR (Phytorus):

Proposal

Hazard symbol: Xn - Harmful

Xi - Irritant

N- Dangerous for the environment.

Risk phrases	R 10 - flammable R 40 - Possible risk of irreversible effects. R 41 - Risk of serious damage to eyes. R43 - May cause sensitisation by skin contact R 50/53 Very toxic to aquatic organisms. May cause long-term adverse effects in the aquatic environment.
Safety phrases:	S 2 - Keep out of children's reach. S 13 - Keep away from feed drink and animal feeding stuffs S 24 - Avoid contact with eyes. S 36/37/39 - Wear appropriate clothes, gloves and eyes and face protection. S 46 - If swallowed, seek medical advice immediately and show this container or label. S 56 - Dispose of this material and its container in a hazardous or special waste collection point S 57 - Use appropriate container to avoid environmental contamination.

ALANEX (Maktheshim-agan):

Specific proposal cannot be made pending completion and evaluation of all relevant studies required in Level 4 of this monograph.

The applicant has not submitted any proposal of classification and labelling of the plant protection product ALANEX. This information is necessary for the inclusion of the active substance in Annex I.

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