Evaluation of the Plant Protection Product

Simplex – aminopyralid + fluroxypyr

Regarding application for authorisation

The Norwegian Food Safety Authority, National Registration Section

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For the Norwegian Scientific Committee on Food Safety
October 2010

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1. Summary

Simplex is a new product containing two active substances (aminopyralid and fluroxypyr), of which one of the substances (aminopyralid) is a new active substance in Norway. The intended use is as a herbicide in established grassland for forage, established ley and pasture and in grass at the first year of sowing. The Norwegian Institute for Agricultural and Environmental Research does not recommend use in the first year of sowing because of lack of efficacy data from the northern zone.

The Standardized Area Dose is 200 g product per decare (or 6 g aminopyralid and 20 g fluroxypyr per decare). Simplex will be used either before the first cutting (May/June) or between the first cutting and the second cutting (July/August). The application will be with tractor mounted sprayer, but in some cases it can also be relevant to use hand-held sprayer (spot spraying).

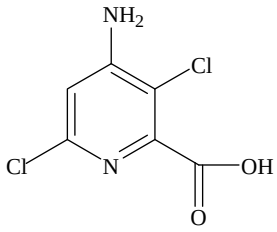
Simplex has effect against broad-leaf weeds, and will be especially important for use against creeping thistle (*Cirsium arvence*), spear thistle (*Cirsium vulgare*) and dock (*Rumex longifolius*).

Both substances in Simplex belong to the chemical group of pyridine karboxyl-acid. Because of infrequently use and influence on several action sites in the plants, there will be a low risk of development of resistance.

1.1 Identity and physical/chemical data

Product name	Simplex
Active substances	Aminopyralid and fluroxypyr
Formulation	Emulsion, water in oil.
Concentration of active substance	30 g aminopyralid/l and 100 g fluroxypyr/l.

Aminopyralid

IUPAC-name	4-amino-3,6-dichloropyridine-2-carboxylic acid
CAS number	150114-71-9
Structural formula	

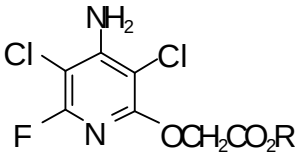
Aminopyralid

Molecular mass		207.026 g/mole
Solubility in water	Very high	2480 mg/l (20°C, pH 7)
Vapour pressure	Low	9.52×10^{-9} Pa (20°C)
Henrys law constant	Low	9.61×10^{-12} Pa m^3/mol (20°C, pH 7)
log Pow	Low	-2.87 (19°C, pH 7)
pKa		2,56

Fluroxypyr meptyl

IUPAC-name	1-methylheptyl [(4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetate
CAS number	81406-37-3

Structural formula



Molecular mass		367.2 g/mole
Solubility in water	Low	0.09 mg/l (20°C)
Vapour pressure	High	1.349 x 10^{-3} Pa (20°C)
Henrys law constant	Medium	1.06 x 10^{-3} Pa m^3/mol
log Pow	High	4.53
pKa		

1.2 Mammalian toxicology

Aminopyralid

Toxicokinetics

Absorption:
Aminopyralid was readily absorbed in rats and rabbits.
Rat: ca. 60% absorption within 7 days, based on urinary excretion and levels in tissues/carcass in repeated low-dose groups.
Rabbit (non-pregnant): 77% absorption within 3 days based on urinary excretion.
Rabbit (pregnant): 83-86% within 3 days based on urinary excretion.

Distribution:
Rat: Limited number of tissues was investigated. The highest concentrations were observed in skin and carcass. Low concentrations were measured in kidneys, liver and spleen.
Rabbit: Limited number of tissues was investigated. Detectable levels of the test compound were only measured in GI tract and spleen.
Rats and rabbits did not show any evidence of accumulation of the test substance.

Metabolism:
Aminopyralid was excreted unchanged indicating an absence of metabolism in rats and rabbits.

Excretion:
Rat: Aminopyralid was effectively cleared through the urine and faeces, whereas bile was not investigated.
Rabbit: Most of the administrated dose was excreted within 24 h mainly through the urine. The bile was not investigated in this study.

Acute toxicity
Aminopyralid is of low acute toxicity to the rat by the oral, dermal and inhalation routes of exposure.

Irritation and sensitisation

Aminopyralid is characterized as extremely irritating to the eye based on persisting irritation in the eyes of two of three rabbits (The criteria for classification as **Xi; R41 Risk of serious damage to eyes** are fulfilled), whereas it was not found to be neither a skin irritant nor a skin sensitiser.

Genotoxicity
All genotoxicity studies were negative apart from a weak positive result in an *in vitro* cytogenicity assay. The positive result at cytotoxic concentrations is not considered to be of concern because there was a clear negative result in an *in vivo* bone marrow micronucleus assay. Overall, aminopyralid does not present a genotoxic concern according to the tests performed.

Sub-chronic toxicity
In these studies (dietary studies), the main target organs were the caecum in rats and the stomach (inflammation) and the liver (hyperthropy) in dogs. No adverse effects were seen in mice. The dermal sub-chronic study in rats showed evidence of slight dermal irritation (hyperplasia) in males. The most sensitive species in sub-chronic studies is the dog.

Chronic toxicity
In chronic toxicity studies the critical effects were slight body weight decrease (males), enlarged caecum with slight mucosa hyperplasia, and urinary changes in rats, whereas the critical effect observed in female mice was increased mortality. No progression in severity of the caecal effects was observed in the chronic study in rats compared to sub-chronic studies.

Carcinogenicity
Aminopyralid is not a carcinogen in animal-studies.

Reproductive toxicity
No adverse effects on reproduction parameters and no histological findings indicative of reproductive toxicity were observed.

Teratology
In high-dose rabbits, incoordinated gait was accompanied by decreased amounts of faeces, significant reductions in body weight; body weight losses, decreased body weight gains, and decreased feed consumption were observed. Those animals that died had pale kidneys, watery, dark cecal contents, erosions/ulcers of the stomach (glandular mucosa) and hairballs. No adverse effects were seen in rats.

Neurotoxicity
Aminopyralid did not result in any neurotoxic effects.

Special studies in rabbits
Transient incoordination is a consistent finding in rabbit studies with aminopyralid and aminopyralid TIPA. This finding has mostly been investigated in pregnant rabbits. Clinical observation of the behaviour of pregnant rabbits treated with high doses of aminopyralid TIPA revealed the primary abnormal finding to be incoordination of all 4 limbs. The ADME study suggests that the bioavailability of aminopyralid would be greater in late-stage pregnant rabbits than non-pregnant and early-stage pregnant rabbits. The cause of the incoordination in rabbits is not known. In the absence of clear evidence as to the mode of action it is not possible to comment on the relevance of this hazard for humans. The precautionary approach of assuming that this effect is relevant for human hazard assessment is therefore appropriate (The criteria for classification as **Xn; R48/22 Danger of serious damage to health by prolonged exposure if swallowed** are fulfilled).

Medical data
No data reported.

Simplex

Co-formulants
The product does contain a co-formulant above the limit; 30% aromatic hydrocarbon solvent, and hence meet the criteria for R66; Repeated exposure may cause skin dryness or cracking (classification not needed, covered by R38) and **R67; Vapours may cause drowsiness and dizziness.**

Acute toxicity
Simplex was not harmful by swallowing, skin contact or by inhalation. Hence, no classification for acute oral, dermal and inhalation toxicity is required.

Irritation and sensitisation
Simplex is characterized as extremely irritating to the eye based on the irritation persisting in the eyes of two of three rabbits (The criteria for classification as **Xi; R41 Risk of serious damage to eyes** are fulfilled).

Simplex is also found irritating to the skin (the product should be classified with **Xi; R38 Irritating to skin**) based on the persistence of desquamation in all 3 animals, whereas it was not found to be a dermal sensitiser.

Dermal absorption
No studies on dermal absorption have been conducted for either aminopyralid or fluroxypyr. The dermal absorption is not expected to be higher than the oral absorption. The higher oral absorption in the rabbit (~80%) compared to the rat (50-60%) should not be disregarded. As a surrogate for a real dermal absorption value the dermal absorption is set to 80% for both the concentration and the diluted solution.

Operator, worker and bystander exposure

Operator exposure
UK POEM model estimate of exposure suggests that levels of exposure will be within acceptable levels for operators without PPE for application using a boom sprayer. For application with knapsack sprayers the UK POEM estimate of exposure requires gloves to be worn when handling the undiluted product during mixing and loading and during application of the diluted spray solution. However, as a result of the hazard classification, a face-shield and gloves are necessary PPE to be worn during mixing and loading operations, due to the risk of causing serious damage to eyes (**R41**) and as the product is irritating to skin (**R38**).

Worker and bystander exposure
For bystanders or for workers re-entering treated crops, predicted exposure to aminopyralid/aminopyralid potassium is less than 10% of the active substance's AOEL. The risk to bystanders and workers from exposure to aminopyralid/aminopyralid potassium is acceptable.

1.3 Residues in food and feed

Residues in food and feed are not discussed in this report.

1.4 Environmental fate and ecotoxicological effects

Environmental fate and behaviour

Degradation in soil
Aminopyralid was steadily degraded in soil under aerobic conditions. The only metabolite observed was CO_2 indicating that the phenyl ring of aminopyralid is mineralised. The aerobic degradation rate is medium, DT50: 26-147 days, geometric mean 56 days. DT90: 88-488 days. The degradation rate is dependent on temperature. Bound residue and mineralization accounted for 10-23 and 24-69 % of AR (Applied radioactivity) respectively. At 10 °C the degradation rate is slow. DT50: 402 days. DT90: 1335 days. Bound residue and mineralization accounted for 6 and 9 % of AR respectively. Some DT50 and DT90 values are extrapolated beyond the duration of the degradation studies and should be regarded as uncertain. Aminopyralid was very stable under anaerobic conditions and no degradation rate was estimated. Only minor metabolites were observed. Photolysis can be regarded as an important route of degradation for aminopyralid in soil as the substance degrades much more rapid when irradiated than under dark conditions. The field dissipation of aminopyralid in soil was found to be medium to high with DT50: 4-22 days, geometric mean 12 days, and DT90: 13-72 days.

Sorption/mobility
The sorption of aminopyralid to soil can be classified as low with Kf: 0.01-0.73 (arithmetic mean 0.17) and Koc: 0.31-21.7 (arithmetic mean 8.3). The Freundlich exponent (1/n) ranged from 0.32 to 1.52 (arithmetic mean 0.85) indicating some non-proportional dependence of adsorption on the concentration. There is evidence that the sorption is stronger at low pH (acidic soils).

Degradation in water
Aminopyralid was completely stable to hydrolysis under all tested conditions. Photolysis is an important degradation pathway for aminopyralid if one compares half lives from the irradiated samples with half lives from the dark controls. Two major photoproducts were seen in the irradiated samples, oxamic acid and malonamic acid. Aminopyralid is classified as 'not readily biodegradeable'. The degradation for a whole water/sediment system was recalculated by RMS using the biphasic Hockey Stick approach and the degradation can be classified as moderate with DT50: 83-104 days when assessing the first phase. Geometric mean was 93 days. When assessing the second phase DT50 was 829-1495 days. Geometric

mean was 1113 days. Bound residue constitutes between 3 and 15 % of AR after 101 days. The mineralization was low, reaching between 1 and 3 % of AR after 101 days in the different systems. Only minor unknown metabolites < 5 % were observed at any time point.

Fate in air
No significant volatilisation (0.2 % AR) of 14C-phenyl-aminopyralid was observed from plant surfaces. The estimated atmospheric half-life was 6.4 days.

Exposure

Soil
According to a simple model recommended by the EU working group FOCUS, the expected PIEC (Predicted Initial Environmental Concentration) in soil is 0.04 mg a.s./kg soil after the application of 60 g a.s./ha with 50 % interception (plant cover). Aminopyralid concentrations were calculated assuming a worst case DT50 (lab, 20 °C) of 147 days. With an interception of 90 %, the PIEC is 0.008 mg a.s./kg soil.

Groundwater
The leaching behaviour of aminopyralid was estimated using all the nine FOCUS groundwater scenarios and the two recommended models, FOCUS-PELMO (version 3.3.2) and FOCUSPEARL (version 2.2.2). The use considered was Simplex in pasture grass according to the representative GAP, i.e. a single application of 60 g a.s./ha. The cropping scenario chosen for these simulations was "grass/alfalfa", the recommended interception (plant cover) is 90 %, thus the application rate used for all scenarios was 60 g a.s./ha x 10%, or 6 g a.s./ha. These modelling assessments indicate that there are situations where contamination of groundwater is likely to occur. The trigger of 0.1 µg/L is exceeded in 6 of the 9 FOCUS scenarios. The highest concentration estimated in these scenarios was 0.31µg/L with the Jokioinen scenario.

The leaching behaviour of aminopyralid was also estimated using the Norwegian (Heia) and Swedish (Önnestad and Krusenberg) groundwater scenarios with the FOCUS crop grass/alfalfa, using the model MACRO (4.4.2). The same input values for the active substance were used, except more realistic application dates. Application in June using a geo mean half life from laboratory studies and an interception of 50 % gave the highest PEC values (80^{th} percentile) with levels between 1.6 and 2.1 µg/l for all three scenarios. Application in late May with only 50 % interception and the same half life, gave PEC values between 1.4 and 2.0 µg/l. Applications in late May and in August also gave values well above the threshold of 0.1µg/l, even with an interception of 90 %. Even applying half the dose with 90 % interception in addition gave values well above the threshold for all three scenarios.

Surface water
FOCUS SWASH was used to estimate the exposure of surface water and sediment. The highest predicted concentration in water from the assumed most relevant scenario, D1-Lanna, was 5.7 µg a.s./l. For the same scenario, global max in sediment was 1.8 µg a.s./kg dw. The highest estimated concentration in water overall was predicted from the D2-Brimstone scenario, with 22.6 µg a.s./L. The respective concentration in sediment was 3.3 µg a.s./kg dw.

Terrestrial organisms

Mammals
Aminopyralid showed low acute (LD50: >5000 mg a.s./kg bw) and reproductive (NOEL: 1000 mg a.s./kg bw/d) toxicity. Calculated TER values (>600 and 298, respectively) are above the relevant triggers (Acute: <10, Chronic: <5). Similar calculations based on low acute toxicity (LD50 >5000 mg/kg bw) of Simplex resulted in an acute TER value (>13) above the trigger (<10).

For the short-term and long-term scenarios it is assumed that the two active substances are unlikely to co-exist in the same proportions as in the original formulation. Hence it can be argued that since fluroxypyr has Annex I listing for use at rates up to 40 g/decare, that the lower rate of use in Simplex (20 g/decare) should not pose unacceptable short or long term risks to mammals.

Birds
Aminopyralid showed low acute (>2250 mg a.s./kg bw), dietary (>5620 mg a.s./kg diet) and reproductive (NOEC: 2700 mg a.s./kg diet) toxicity. All calculated TER values (>600, >726 and 103-175, respectively) are

above the relevant triggers (Acute: <10, Chronic: <5). Similar calculations based on low acute toxicity (LD50: >2250 mg/kg bw) of Simplex resulted in acute TER values (>18) above the trigger (<10).

For the short-term and long-term scenarios it is assumed that the two active substances are unlikely to co-exist in the same proportions as in the original formulation. Hence it can be argued that since fluroxypyr has Annex I listing for use at rates up to 40 g/decare, that the lower rate of use in Simplex (20 g/decare) should not pose unacceptable short or long term risks to birds.

Bees
Aminopyralid showed low contact toxicity to bees (LD50: >100 µg/bee) and low oral toxicity to bees (LD50: >120 µg/bee). Hazard quotients for contact (Qhc) and oral exposure (Qho) are estimated to be 0.6 and 0.5, respectively. These do not exceed the trigger value (>50).

Simplex showed low contact toxicity to bees (LD50: >200 µg/bee) and low oral toxicity to bees (LD50: >100 µg/bee). Hazard quotients for contact (Qhc) and oral exposure (Qho) are estimated to be 10 and 20, respectively. These do not exceed the trigger value (>50).

Non-target arthropods
In Tier 1 laboratory acute contact toxicity studies, aminopyralid showed low effects on predatory mites (*T. pyri*) and parasitoids (*A. rhopalosiphi*). Although precise LR50 values can not be calculated, it can be seen that LR50 values for both species are >6 g/decare. Hazard quotients are below the trigger (>2) both in-field and off-field. Simplex was tested in extended lab studies with *A. rhopalosiphi* and *T. pyri*, and in a first tier study with A. carnea. The tests did not show effects above the trigger effect level of 50 %.

Earthworms
Aminopyralid showed low acute toxicity (LC50: >1000 mg/kg d.w. soil), and TER is estimated to be >25000. Simplex showed moderate acute toxicity (LC50: 710 mg/kg d.w. soil), and TER is estimated to be 534. These values do not exceed the trigger (<10). Since the log Pow for aminopyralid is <2.0 the toxicity is not corrected to take account of the relatively high organic matter content of the artificial test soils. A 50 % crop interception is used in the calculations.

Microorganisms
Soils treated with up to 100 times the normal application rate of aminopyralid did deviate less than 25 % (trigger) from untreated controls with respect to carbon mineralisation/respiration and nitrogen mineralisation. Soils treated with up to 4.2 times the normal application rate of Simplex showed negligible deviation (<2 %) from untreated controls with respect to carbon mineralisation/respiration and nitrogen mineralisation.

Aquatic organisms

The TER calculations for aminopyralid below are based on maximum PEC-values from FOCUS surface water modelling Step 1 and the lowest acute (LC50 or EC50) or chronic (NOEC) values for the different organism groups. All calculations are based on an application rate of 6 g a.s./decare.

For Simplex, it should be safe to assume that the runoff and drain flow will not move the intact formulation into the aquatic environment, due to the varying adsorption and degradation properties of the formulation's constituents. Spray drift is the only realistic source of contamination of the aquatic environment by intact Simplex. TER values are calculated based on spray drift estimations with 1 meter buffer zone according to Rautmann et al. (2001).

Fish
Aminopyralid showed low acute toxicity (LC50: >100 mg a.s./L) and low toxicity (NOEC: 1,3 mg a.s./L) in an early life stage test. Calculated TER values are >4902 and 64, respectively. These do not exceed the relevant triggers (Acute: <100, Chronic: <10).

Simplex was toxic to rainbow trout (LC50: 7.6 mg/L), but the TER (412) was above the relevant trigger (<100).

Invertebrates
Aminopyralid showed low acute toxicity (EC50: >100 mg a.s./L) and low reproductive toxicity (NOEC: 100 mg a.s./L). Calculated TER values are >4902 and 4902, respectively. These do not exceed the relevant triggers (Acute: <10, Chronic: <5).

Simplex showed moderate toxicity to *Daphnia magna* (EC50: 35 mg/L), but the TER (1895) was above the relevant trigger (<100).

Sediment dwelling organisms
Aminopyralid showed low chronic toxicity to *C. riparius* larvae (NOEC: 130 mg a.s./L, NOEC: 46.7 mg a.s./kg sediment). Calculated TER values are 6373 and 58375, respectively. These do not exceed the relevant trigger (<5).

Aquatic plants
Aminopyralid showed low toxicity to duckweed (EC50: >88 mg a.s./L). Calculated TER is >4313, which do not exceed the relevant trigger (<10).

Algae
Aminopyralid showed moderate toxicity (EC50: 18->100 mg a.s./l). Calculated TER values are ≥882, which do not exceed the relevant trigger (<10).

Simplex was toxic to the freshwater diatom *N. pelliculosa* (EC50: 1.5-2.0 mg/l), but the TER (≥82) was above the relevant trigger (<10).

Microorganisms
Aminopyralid showed low toxicity to respiration of activated sewage sludge (EC50: >1000 mg a.s./l).

Bioconcentration
The log Pow for aminopyralid is given as -2.87 in pH 7 buffered solution. Bioconcentration studies are therefore not submitted, and aminopyralid is not expected to bioaccumulate.

1.5 Dossier quality and completeness

The dossier is complete and is adequate as a basis for an evaluation of the active substance, metabolites and product.

2. Product status

Our reference	06/10459
Active substance	Aminopyralid + fluroxypyr
Product name	Simplex
Applicant	Dow AgroSciences
Importer	Felleskjøpet Agri BA
Concentration of active substances	Aminopyralid: 30 g/l Fluroxypyr: 100 g/l
Formulation	Emulsion, water in oil
Packaging	1 litre
Function	Herbicide
Application background	A new product containing two active substances, where aminopyralid is new in Norway and fluroxypyr already is approved in several products.
Application date	30.08.2007
Sales data	Fluroxypyr has been on the Norwegian market since 1991. Average sales in the period 2005-2009 is 15 103 kg.
Status i the EU	Aminopyralid is notified in the EU and is assessed. Fluroxypyr was included on Annex 1 in 2000.

3. Agronomi

Teksten i dette kapitlet er hentet fra Bioforsk Plantehelse sin agronomiske vurdering samt etikettforslag fra importør.

3.1 Bruk/virkning

Søkt bruksområde

Gras til fôr (ikke frøproduksjon) og varig eng og beite uten kløver, samt gjenlegg uten kløver og uten dekkvekst.

Virkeområde

Tofrøbladede ugras. Meget god virkning på byhøymole, krushøymole, vanlig høymole, stornesle, løvetann, åkertistel, veitistel, krypsoleie, vassarve og balderbrå. Svak virkning mot orientveronika.

Virkemåte

Begge virkestoffene (aminopyralid og fluroksypyr) hører til kjemisk hovedgruppe auxiner, og kjemisk undergruppe pyridin karboksylsyrer. Stoffene tas raskt opp i planten og transporteres og konsentreres i vekstpunktene. Transport til røttene tar inntil en uke etter sprøyting. Det tar minst 4 uker til 2-4 måneder før en ser full virkning av Simplex på ugraset.

Virkemekanisme

I følsomme planter medfører Simplex en auxineffekt som sees som unormal vekst, bla. abnorm strekningsvekst. Etter hvert blir plantene nekrotiske og dør.

Resistens

Resistens for aminopyralid og fluroksypyr er ikke kjent i Norge. Det er rapportert om noen tilfeller av resistens mot syntetiske auxiner i åkertistel, og balderbrå. Effekten av Simplex er langvarig og det ikke vil være behov for årlig sprøyting. Dette sammen med at preparatet virker på flere virkeseter i plantene, medfører at det vil være liten risiko for utvikling av resistens. Det vurderes ikke som noe behov for å påkreve advarselsetninger og resistensstrategi-anbefalinger på etiketten.

Simplex vil i tillegg kunne fungere som en resistensbryter for ALS-hemmerne (sulfonylurea-preparater).

3.2 Behandlingsmåte og dosering

Dosering:
Anbefalt dosering på etiketten er 200 ml/daa. Norske forsøk har vist at effekten av halv dose i de fleste tilfellene har gitt nesten like bra virkning som full dose på bl.a. løvetann, høymole og krypsoleie (i Finland var man oppe i 75% av full dose for å få tilsvarende bra effekt). Dersom det skulle være nødvendig å redusere dosen kan en trolig klare seg med 150 ml prepart per daa, men den langvarige virkningen kan da i enkelte tilfeller bli noe dårligere.

I grasmark vil sprøyting på store rosetter av flerårig ugras utføres før 1. slått (mai/juni) eller mellom 1. og 2. slått (juli/august). For god virkning bør en vente 1 uke med slått/beiting (dette er også behandlingsfristen). Det vil være lite behov for årlig sprøyting mot de flerårige ugrasene.

I gjenlegg uten dekkvekst er oppgitt behandlingstidspunkt på framspirt ugras etter at graset har spirt og ikke før tidligst 8 uker etter såing. Graset kan såes til ulik tid, og behandlingstidspunktet kan derfor variere fra juli til oktober. Bioforsk Plantehelse vil imidlertid ikke anbefale denne bruken på grunn av manglede effektdata.

Bruksbegrensninger:
Det vil være begrensing i bruk av etterkultur ved bruk av Simplex. Hvete kan såes 4 uker etter bruk, mens kløver eller andre belgvekster ikke skal såes før etter 4 måneder. Poteter, gulrot eller andre skjermplaner eller salat skal ikke dyrkes påfølgende år etter behandling av preparatet. I tillegg kan også rester av Simplex i plantemateriale eller husdyrgjødsel gi skade på følsomme kulturer. Det anbefales derfor dyp pløying før planting/såing av følsomme vekster.

NAD

Med bakgrunn i preparatets dosering i grasmark til fôr (beite og slått) fastsettes normert arealdose (NAD) til 200 ml preparat per dekar. Dette tilsvarer 6 g aminopyralid og 20 g fluroksypyr per daa.

Spredeutstyr

Det er mest vanlig å påføre preparatet med åkersprøyte, men i forbindelse med flekksprøyting kan det være aktuelt å bruke ryggsprøyte.

3.3 Anbefaling fra Bioforsk Plantehelse

Simplex er et ugrasmiddel med god og langvarig virkning mot tistel og høymole i grasmark, noe som er etterspurt i markedet. En godkjenning vil trolig redusere behovet for bruk av andre preparater i eng uten belgvekster til slått og beite, og det vil ta lenger tid før ny behandling er nødvendig. Dersom preparatet ikke skulle bli godkjent vil det være behov for hyppigere bekjemping av ugraset med andre preparater og en vil fortsatt ha problemer med tistel.

Ulempen med preparatet er begrensning i etterkultur og at husdyrgjødsel og planterester ikke kan brukes til kompost.

Det vil ikke være noe risiko for utvikling av resistens mot Simplex i forbindelse med bekjempelse av ugras i grasmark. Preparatet kan virke som en resistensbryter f.eks. mot sulfonylurearesistens i vassarve og balderbrå.

Bioforsk Plantehelse anbefaler godkjenning av preparatet til bruk i eng og beite uten belgvekster til fôr.

Bruk i grasgjenlegg uten dekkvekst anbefales ikke godkjent, på grunn av manglende forsøksdata.

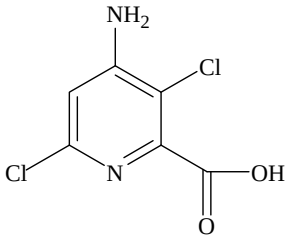
4. Identity and physical/chemical data

Aminopyralid

IUPAC-navn 4-amino-3,6-dichloropyridine-2-carboxylic acid

CAS number 150114-71-9

Struktural formula



Aminopyralid

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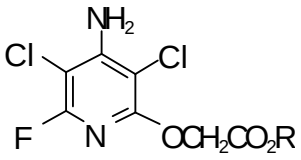
log Pow	Low	-2.87 (19°C, pH 7)
pKa		2,56

Fluroxypyr meptyl

IUPAC-navn 1-methylheptyl [(4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetate

CAS number 81406-37-3

Struktural formula



Molecular mass		367.2 g/mole
Solubility in water	Low	0.09 mg/l (20°C)
Vapour pressure	High	1.349 x 10^{-3} Pa (20°C)
Henrys law constant	Medium	1.06 x 10^{-3} Pa m^3/mol
log Pow	High	4.53
pKa		

5. Mammalian Toxicology

This assessment is based on documentation submitted by the notifier (Dow AgroSciences, 2006), and the EU Draft Assessment Report of the United Kingdom (DAR, August 2006 Volume 3, Annex B.6) (Annex T1).

There is an ongoing evaluation of aminopyralid in EFSA.

5.1 Aminopyralid

5.1.1 Toxicokinetics

Rat, single and repeated dose oral

Study design: Three groups of 10 weeks of age male Fischer 344 rats (4 /group) were administrated 50 and 1000 mg/kg bw single and 50 mg/kg bw repeated (14 days) oral doses [14C]aminopyralid by gavage (see table 5.1. below). OECD guideline 417/GLP/QA.

Table 5.1: Outline study design (ADME study)

Group	Dose (mg/kg bw)	No. of animals	Time of sacrifice (hr) after last dose
Single low dose	50	4	Sacrifice at 168 h post-dosing (7 days)
Repeated dose group - 14 days of non-labelled test material followed by a single dose of radiolabelled test material	50	4	Sacrifice at 168 h post-dosing (7 days)
Single high dose	1000	4	Sacrifice at 168 h post-dosing (7 days)

Results: Absorption: Single and repeated dosages of radiolabelled aminopyralid demonstrated a rapid absorption through the oral route. Urinary excretion and tissue plus carcass levels indicate that 50, 59 and 42% of the administered dose of [^{14}C]aminopyralid is absorbed in the single low, repeated low, and single high dose groups, respectively (Annex T1,Table B.6.2). The amount of absorbed test material eliminated in the faeces was not directly determined in this study. Furthermore, bile cannulated animals should have been employed in this study.

Distribution: The radiolabelled test compound was rapidly depleted from the body after oral dose administration. A limited number of tissues were investigated, due to the identification of caecum as the only target organ of toxicological concern in 90-days dietary study. Both caecum and ileum were identified as target organ in rats, whereas stomach and liver categorized as target organ in dogs (Annex T1, Section B.6.3.6). Most probably aminopyralid does not persist in the organ/tissues not examined, relating to the low levels of the test compound present in the skin and remaining carcass (containing 0.03 – 0.45% and 0.04 – 0.26% of administrated dose, respectively) investigated after 7-day depletion. Less than 0.01 % of the administrated dose was detected in the kidneys, liver and spleen. The high-dose group had relatively high mean value in the skin, but it was variable between individuals and the values were not reproducible in other treatment groups.

Metabolism: Unchanged aminopyralid represented 100% and ≥96% of the radioactivity detected via HPLC-radiochromatography in the faecal extracts and the urine, respectively. Three unknown components (≤4%) found in urine were also detected in similar quantities in the analysis of the dose formulation, which indicate a possibility that they are trace impurities in the radiolabelled test material. Summarized, these data indicate an absence of metabolism of aminopyralid in the rat.

Excretion: Aminopyralid was effectively cleared through the urine (T½α of 3-4 h for high and low single dosages and repeated low dosage) and faeces. Most of the administered dose (67-91%) was excreted within 24h. Less than 0.1% of the administered dose was detected in the expired air (Annex T1, Table B.6.2). Of the dose administered, 41-59% was excreted in urine and 33-43% in faeces 7 days after the end of dosing. Excretion mostly occurred within the first 24h after the end of dosing: 37-58% of the

administered dose in urine, 29-42% of the administered dose in faeces. The excretory pattern was quite similar for all three dose groups, with exception of urinary excretion in the repeated low dose group that was slightly higher.

Accumulation: The rapid absorption and excretion, and absence of metabolism (for a substance of high water solubility and low Log Pow at physiological pH) indicate a low potential for accumulation (Liu 2004, Anon 2004).

Recovery:
Overall recovery (up to 168 h) for all dose groups was greater than 95% of the administrated dose (Liu, 2004).

Rabbit, single and repeated dose oral

Study design: This ADME study has been conducted in pregnant and non-pregnant rabbits as part of further investigations into the occurrence of incoordination in rabbits exposed to aminopyralid (see the rabbit-studies under Section 5.1.10 Special studies in this assessment). Three groups of 5-6 months of age female New Zealand White rabbits (3/group) were administrated oral doses of [^{14}C]aminopyralid by gavage. Non-pregnant rabbits (Group 1) and pregnant rabbits (Group 2) were administered a single oral dose of 371 and 362 mg [^{14}C]-aminopyralid/kg bw at GD 7, respectively. A repeat dose experiment was also conducted in which pregnant rabbits (Group 3) were given daily doses of 279 mg unlabelled aminopyralid/kg bw from GD1 to GD21, followed by 279 mg [^{14}C]- aminopyralid/kg bw on GD 22. No specific guideline applicable to this study/GLP.

Results: Absorption: The test material showed extensive absorption based on the amount in urine and tissues; 77% in non-pregnant rabbits and 83-86% in pregnant rabbits.

Distribution: At 72 h post-dosing, the GI tract was the only tissue with detectable amounts of radioactivity for all study animals. These amounts represents 0.11-0.15% of the administered dose. The spleen from two of the three animals in group 3 had detectable amounts of radioactivity of <0.005% of the administered dose. These findings indicate that aminopyralid-derived radiolabel is unlikely to accumulate in tissues.

Metabolism: Orally administrated aminopyralid was not metabolized by rabbits.

Excretion: Urine was the principal route of elimination with 77, 83, and 86% of the administrated dose eliminated 72 h post-dosing, for groups 1, 2, 3, respectively. The vast majority (93-97%) of the urinary total were eliminated by 24 h post-dosing. Faeces accounted for 20, 16, and 8% of the administered dose for groups 1, 2, and 3 at 72 h post-dosing, with the vast majority (95-97%) being eliminated in the first 24 h.

Aminopyralid seems to be more systemically available to the pregnant (GD 22) rabbits than to the rabbits in the other 2 groups. Urine and faeces accounted for at least 99% of the recovered radioactivity for all three groups.

Bioavailability: Relative bioavailability for the animals in group 3 was higher than for group 1 (by 75%) and for group 2 (by 115%). It is not clear if this increase in bioavailability in group 3 animals was due to repeat dosing or to animals being at a late stage of pregnancy.

Repeat-dose pregnant rabbits appeared to have a significant increase in bioavailability of test material as indicated by increased plasma levels of aminopyralid and a higher percent of the dose eliminated in urine. It is considered that the evidence indicates that the bioavailability of aminopyralid is greater in late-stage pregnant rabbits than in non-pregnant or early-stage pregnant rabbits.

Reduced binding of aminopyralid to plasma proteins, and hence an increase in the amount of free aminopyralid in plasma would also increase the bioavailability of aminopyralid in these late-stage pregnant rabbits compared with the other tested groups of rabbits and rats.

Summarized, aminopyralid was rapidly absorbed and eliminated unchanged (primarily in urine) in all groups, and additionally it was not metabolised. Taken together, the findings indicated that aminopyralid is unlikely to accumulate in tissues.

The main differences between rats and rabbits were that in rabbits there was evidence of more extensive absorption following oral dosing, with excretion primarily in urine. In addition, plasma protein binding of aminopyralid *in vitro* was slightly greater in non-pregnant rats than in non-pregnant rabbits, which in turn showed greater binding than pregnant rabbits (the lowest binding was in late-stage pregnant rabbits) (Hansen, Mendrala, Markham and Saghir 2005).

Summary (toxicokinetics):

Aminopyralid is not metabolised in rats or rabbits. The absorption and excretion patterns of the [14C] moiety were similar among all groups.

Absorption:
Aminopyralid was readily absorbed in rats and rabbits.
Rat: c. 60% absorption within 7 days, based on urinary excretion and levels in tissues/carcass in repeated low dose-groups.
Rabbit (non-pregnant): 77% absorption within 3 days based on urinary excretion.
Rabbit (pregnant): 83-86% within 3 days based on urinary excretion.

Distribution:
Rat: Limited tissues were investigated. At 168 h the highest concentration were observed in skin (0.03-0.45% of dose) and carcass (0.04-0.26% of dose). Less than 0.01% of dose was measured in kidneys, liver and spleen.
Rabbit: Limited tissues were investigated. Detectable levels of the test compound were only measured in GI tract and spleen.
Rats and rabbits did not show any evidence of accumulation of the test substance.

Metabolism:
Aminopyralid was excreted uncharged indicating an absence of metabolism in rats and rabbits.

Excretion:
Rat: 67-91% of the administrated dose was excreted within 24 h. Aminopyralid was effectively cleared through the urine (37-58% of the administrated dose) and faeces (29 – 42%), whereas bile was not investigated. Approximately, 41 – 59% and 33 – 43% of radioactivity was recovered in urine and faeces, respectively, during 7-day depletion.
Rabbit: 90-92% of administrated dose were excreted within 24 h (72-83% of the administrated dose were cleared through the urine, whereas the remaining part were excreted via faeces). The bile was not investigated neither in this study.

The compound of toxicologically significant interest was the parent compound.

5.1.2 Acute Toxicity

Acute Oral

Acute oral LD50 in Fischer 344 rats was found to be > 5000 mg/kg bw for males and females (GLP/QA/OECD 401). A single male rat died on test day 3. In relation to the finding of incoordination in some pregnant rabbits dosed by gavage with aminopyralid (Annex T1, Section B.6.6.3.b), it is notable that in this rat study incoordination was seen only in the rat that died. Decreased muscle tone was noted in three rats (including the one that died). The male rat that died had gross findings consisting of haemolysed blood and gas in the gastrointestinal tract and perineal soiling (Brooks and Yano, 2001).

Acute Dermal

Acute dermal LD50 in Fischer 344 rats was found to be > 5000 mg/kg bw for males and females (GLP/QA/OECD 402) (Brooks and Yano, 2001).

Acute Inhalation

Acute LC50 after exposure (nose-only) by inhalation to the test article as a dust aerosol for 4 h in albino Fischer 344 rats (GLP/QA/OECD 403) was found to be >5.5 mg/L air for males and females. The exposure aerosol was characterized by a mass median aerodynamic diameter (MMAD) of 2.5 µm with a geometric standard deviation of 2.45 (Kiplinger, 2001).

Summary (acute toxicity):

Aminopyralid is of low acute toxicity to the rat by the oral, dermal and inhalation routes of exposure.

5.1.3 Irritation and sensitisation

Skin irritancy

Mean values (24, 48 and 72 h) were found to be 0 for erythema and 0 for edema. The compound is not a skin irritant in three New Zealand White Rabbits (GLP/QA/OECD 404) (Brooks, 2001).

Eye irritation

Mean values (24, 48 and 72 h) were 2.53 for conjunctival redness, 2.2 for corneal chemosis, 1.77 for corneal opacity and 0.89 for iritis in three New Zealand White Rabbits (**Xi; R41**) (GLP/QA/OECD 405). In addition to the eye irritation responses mentioned above, conjunctival discharge was seen in all rabbits up to days 14 or 21. Two rabbits had ocular irritation that continued through study termination. Although the mean 24-72 h readings for chemosis, iritis and conjunctival redness indicates that the substance should be classified with R36, the persistence of irritation in three rabbits for at least 21 days (and in two rabbits for 35 days) reveals that the substance should be classified as **Xi; R41 Risk of serious damage to eyes** (Brooks, 2001).

Skin sensitisation

Aminopyralid was not considered to be a contact sensitiser in Hartley albino Guinea Pigs. The study was conducted using the Maximization Design (GLP/QA/OECD 406). No dermal reaction was observed (Wilson, 2001).

Summary (irritation and sensitisation):

Aminopyralid is characterized as extremely irritating to the eye based on the persistence irritation in the eyes of two of the three rabbits (The criteria for classification as **Xi; R41 Risk of serious damage to eyes** are fulfilled), whereas it was not found to be neither a skin irritant nor a skin sensitiser.

Table 5.2: Summary of acute toxicity, irritancy and sensitisation studies with aminopyralid.

Test	**Species**	**Result**	**Classification** (93/21/EEC)	**Reference**
Acute oral	Rat	LD50>5000 mg/kg bw	None	Brooks and Yano 2001
Acute dermal	Rat	LD50>5000 mg/kg bw	None	Brooks and Yano 2001
Acute inhalation	Rat	LC50>5.5 mg/l	None	Kiplinger 2001
Skin irritation	Rabbit	No signs of irritation	None	Brooks, 2001
Eye irritation	Rabbit	Conjunctivitis (all rabbits) and corneal opacity (one rabbit) persisted to beyond 21 days	R41	Brooks, 2001
Skin sensitisation (maximisation test)	Guinea pig	No significant skin responses	None	Wilson, 2001

5.1.4 Genotoxicity

Table 5.3: Summary of the genotoxicity studies:

	Study	Test system	Concentration/ dose range tested	Result	Reference/ Guidelines/ GLP
In vitro:					
Point mutations in bacteria	Bacterial reverse mutation assay	*S.typhimurium* TA98, TA100, TA1535 & TA1537. *E.coli* WP2uvrA.	100, 333, 1000, 3330, and 5000 μg/plate (+/- S9)	Negative (+/- S9)	Mecchi, 2001/ OECD 471/ GLP/ QA
Cytogenetics assay in mammalian cells	Chromosomal aberration assay (Clastogenecity)	Rat lymphocytes	Dose levels of up to 1700 or 2070 µg/ml (- S9) investigated for cytotoxic effects Dose levels of up to 2070 µg/ml (+ S9) investigated for cytogenetic effects	-S9 = Weak positive +S9 = Negative	Linscombe, Jackson, Schisler and Beuthin, 2002/ OECD 473/ GLP/ QA
Gene mutation in mammalian cells	Forward mutation assay	Chinese hamster ovary cells (CHO/HGPRT)	Dose levels of up to 2070 µg/ml (+/- S9)	Negative (+/- S9)	Linscombe, Schisler and Beuthin, 2001/ OECD 476/ GLP / QA
In vivo:					
Chromossomal aberration	Micronucleus	Mouse bone marrow polychromatic erythrocytes Strain: CD-1 mice. Only males were evaluated in the main study. Test substance administered by gavage to groups of male CD-1 mice	0, 500, 1000 or 2000 mg/kg bw/day on 2 consecutive days	Negative	Spencer and Gorski 2002/ OECD 474/ GLP / QA

Summary (genotoxicity):

All genotoxicity studies were negative apart from a weak positive result in an *in vitro* cytogenicity assay (when tested without additional metabolic activation for 24 h) in the presence of significant toxicity. The positive result at cytotoxic concentrations is not considered to be of concern because there was a clear negative result in an *in vivo* bone marrow micronucleus assay tested up to a limit dose of 2000 mg/kg bw. Hence, aminopyralid is considered to be a weak clastogen in *in vitro* chromosomal aberration assay in the absence of S9. Aminopyralid induced a clastogenic response in rat lymphocyte cultures treated at cytotoxic concentrations continuously for 24 h, but not after 4 h exposure. It is possible that the positive cytogenetic response was related to cytotoxicity. Aminopyralid does not present a gentoxic concern according to the tests performed. However, there are limitations in the bacterial reverse mutation assay *in vitro* and an additional study *in vivo* should have been included (Further explanations from the RMS see Annex T1, Section B.6.4.3 page 131).

5.1.5 Sub-chronic toxicity

Rat, diet, 4-week

Study design: Fischer 344 rats (5/sex/group) were administrated the test material in the diet at concentrations calculated to be equivalent to target doses of 0, 10, 100, 500 or 1000 mg/kg bw/day for 4 weeks. OECD guideline 407/GLP/QA.

Results: There were no effects in mortality, body weights, feed consumption, ophthalmologic and clinical observations, organ weights or clinical pathology parameters. The only gross observation was increased size of the caecum, noted in three males and two females given 500 mg/kg bw/day, and in all animals at the top dose level. There were no histopathologic changes associated with the caecal alteration. Furthermore, urinalysis revealed slightly increased specific gravity in both sexes, a tendency to lower urinary pH (in males) and reduced protein content at the top dose level. In contrast to the 90-day (Dryzga & Stebbins, 2001) and chronic (Johnson & Dryzga, 2004) rat studies, no effects were noted on urinary ketone content.

NOAEL: 1000 mg/kg bw/day in both male and female rats (1126 and 1094 mg/kg bw/day respectively), based upon no adverse effects. Target organ in this study was the caecum (Stebbins and Day, 2000).

Rat, diet, 13-week

Study design: Fischer 344 rats (10/sex/group) were given test diets formulated to provide target doses of 0, 10, 100, 500, or 1000 mg aminopyralid/kg bw/day for 13 weeks to evaluate the potential for systemic toxicity. Recovery groups of Fischer 344 rats (10/sex) were fed 0 or 1000 mg/kg bw/day for 13 weeks and were given control feed for additional 4 weeks to evaluate the reversibility of potential effects induced during the 13 weeks of treatment. OECD guideline 408/GLP/QA.

Results: Males and females (in the main groups) given 500 mg/kg bw/day and above revealed urinary changes (increased volume; decreased concentration of proteins and ketones; decreased pH) (Table 5.4). These alterations were unaccompanied by histopathological changes. There was complete recovery of the urine pH, protein, and ketone alterations for both sexes given 1000 mg/kg bw/day for 13-weeks, followed by a 4-week recovery period.

Both sexes (in the main groups) given 500 or 1000 mg/kg bw/day for 13 weeks had increased absolute and relative full (including contents) and empty caecal weights (Table 5.5). Weights of full and empty caecum for males and females given 1000 mg/kg bw/day were still identified as increased, relative to recovery group controls, at the end of the 4-week recovery period (Table 5.6). However it is notable that mean empty weights of the caecum (absolute and relative) in recovery group high dose males were not increased compared to main group control males. Also, although mean empty weights of the caecum (absolute and relative) in recovery group high dose females were higher than main group control females, the range of individual values for relative empty weights for recovery group high dose females was within the range of values for main group control females.

The only gross observation noted was an increased size of the caecum of all animals given 1000 mg/kg bw/day for 13 weeks. Following the 4-week recovery period, rats (2/sex) given 1000 mg/kg bw/day still had increased size of the caecum.

Very slight and diffuse hyperplasia of the mucosal epithelium of the caecum and ileum (terminal ileum) were seen in all males given 1000 mg/kg bw/day (Table 5.7). There were no histopathological findings that correlated to the increased caecal weights of males given 500 mg/kg bw/day, and females given 500 or 1000 mg/kg bw/day. Following the 4-week recovery period, there was complete recovery of the epithelial hyperplasia of the caecum and ileum for all males given 1000 mg/kg bw/day.

Table 5.4: Some urinary parameters in 13-week rat dietary study (excluding recovery groups).

Target dose mg/kg bw/day	**0**	**10**	**100**	**500**	**1000**
Males					
pH	7.0 (3) 7.5 (5) 8.0 (2)	7.0 (4) 7.5 (4) 8.0 (2)	6.5 (1) 7.0 (4) 7.5 (4) 8.5 (1)	**6.0 (1)** **6.5 (3)** **7.0 (5)** **7.5 (1)**	**5.5 (1)** **6.0 (9)**
Protein (mg/dl)	+ (1) ++ (9)	++ (10)	++ (10)	+ (1) ++ (9)	**+ (8)** **++ (2)**
Ketones (mg/dl)	+ (10)	+ (10)	+ (10)	+ (10)	**Negative (1)** **Trace (9)**
Females					
pH	7.0 (4) 7.5 (4) 8.0 (2)	6.5 (1) 7.0 (3) 7.5 (5) 8.0 (1)	6.5 (1) 7.0 (6) 7.5 (3)	**6.0 (2)** **6.5 (6)** **7.0 (2)**	**5.0 (1)** **6.0 (7)** **6.5 (2)**
Protein (mg/dl)	+ (8) ++ (2)	+ (5) ++ (5)	Trace (2) + (4) ++ (3) +++ (1)	Trace (1) + (8) ++ (1)	**Negative (1)** **Trace (5)** **+ (4)**
Ketones (mg/dl)	Negative (3) Trace (6) + (1)	Negative (2) Trace (8)	Negative (4) Trace (5) + (1)	Negative (6) Trace (4)	**Negative (9)** **Trace (1)**

Bold indicates effects considered treatment related.
Data tabulated as the number of animals (N) with the stated value.
A: control data from 13-week study in 1999: mean vol for males = 5.4, females = 5.4

Table 5.5: Significant caecal weight alterations of rats given aminopyralid in the diet for 13 weeks.

Males				
Target dose (mg/kg bw/day)	**Absolute Full Caecum (g)**	**Relative Full Caecum (g/100g bw)**	**Absolute Empty Caecum (g)**	**Relative Empty Caecum (g/100g bw)**
0	4.720	1.532	1.831	0.590
10	4.542	1.476	1.754	0.570
100	4.953	1.603	1.780	0.576
500	**8.838$**	**2.899$**	**2.080***	**0.680***
1000	**13.289$**	**4.406$**	**2.361***	**0.781***
Females				
Target dose (mg/kg bw/day)	**Absolute Full Caecum (g)**	**Relative Full Caecum (g/100g bw)**	**Absolute Empty Caecum (g)**	**Relative Empty Caecum (g/100g bw)**
0	3.450	2.022	1.167	0.684
10	3.441	2.021	1.184	0.695
100	3.660	2.141	1.175	0.688
500	**4.801$**	**2.831$**	1.237	0.729
1000	**9.451$**	**5.594$**	**1.539***	**0.910***

** Statistically different from control mean by Dunnett's test, alpha = 0.05.*
$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.
Bold indicates effects considered treatment-related.

Table 5.6: Caecal weight of rats given aminopyralid in the diet for 13 weeks, followed by a 4-week recovery period.

Males				
Target dose (mg/kg bw/day)	**Absolute Full Caecum (g)**	**Relative Full Caecum (g/100g bw)**	**Absolute Empty Caecum (g)**	**Relative Empty Caecum (g/100g bw)**
0	4.062	1.244	1.510	0.463
1000	**6.418***	**2.028***	**1.815***	**0.573***
Females				
Target dose (mg/kg bw/day)	**Absolute Full Caecum (g)**	**Relative Full Caecum (g/100g bw)**	**Absolute Empty Caecum (g)**	**Relative Empty Caecum (g/100g bw)**
0	3.417	2.001	1.133	0.661
1000	**5.265***	**3.033***	**1.379***	**0.807***

** Statistically different from control mean by Dunnett's test, alpha = 0.05.*
Bold indicates effects considered treatment-related.

Table 5.7: Histopathological lesions of the caecum and ileum in male rats given aminopyralid in the diet for 13 weeks.

Dose (mg/kg bw/day)	0	10	100	500	1000
Caecum: hyperplasia, epithelium, diffuse, very slight	0	0	0	0	**10**
Ileum: hyperplasia, epithelium, diffuse, very slight	0	0	0	0	**10**

Bold indicates effects considered treatment related.

NOAEL: 500 mg/kg bw/day for males (actual calculated dose: 543 mg/kg bw/day), based upon increased caecal weights, decreased urine pH and histopathological changes in the caecum and ileum at the top-dose level. NOAEL for females is 1000 mg/kg bw/day (actual calculated dose: 1060 mg/kg bw/day) (Dryzga, Stebbins, 2001).

Mouse, diet, 4-week

Study design: CD-1 mice (5/sex/group) were given test diets formulated to supply 0, 10, 100, 500 or 1000 mg/kg bw/day for 4 weeks. OECD guideline 407/GLP/QA.

Results: Haematology revealed lower white blood cell counts in both sexes at the top dose level; the value for males is significantly lower than the concurrent control and lies outside the historical control range. Histopathological changes were limited to hepatocyte hypertrophy (hepatocyte size) with eosinophilia and decreased glycogen in two of five top-dose males (Annex T1, Table B.6.16).

NOAEL: 1000 mg/kg bw/day for male and female CD-1 mice (equal to 1038 and 1058 mg/kg bw/day respectively), based upon absence of adverse effects (Yano, Dryzga, 2000).

Mouse, diet, 13-week

Study design: CD-1 mice (10/sex/group) were given test diets formulated to supply 0, 10, 100, 500 or 1000 mg/kg bw/day for 13 weeks. OECD guideline 408/GLP/QA.

Results: In contrast to the findings of the 28-day study, no effect was seen on the total white blood cell count in top-dose males, however values were slightly lower in top-dose females. Differential count in this group showed a marginally higher proportion of neutrophils and a lower proportion of lymphocytes. Clinical chemistry revealed higher concentrations of sodium and chloride in treated groups of males, though without a dose-response relationship. Gross necropsy revealed an accentuated hepatic lobular pattern in the top dose male with the highest bodyweight. Microscopically, single incidences of very slight centrilobular hepatocyte hypertrophy and slightly increased centrilobular hepatocyte glycogen were also seen in top dose males; all pathological findings were seen in different animals (Annex T1, Table B.6.17).

NOAEL: 1000 mg/kg bw/day (equal to mean intake of 1020 mg/kg bw/day) can be determined for this study, in the absence of any adverse effects at the top dose level (Stebbins, Day, and Thomas, 2001).

Dog, diet, 4-week

Study design: Beagle dogs (2/sex/group) were administered test material in the diet at concentrations of 0, 0.15, 0.45 or 1.5% (equal to 0, 62, 193 and 543 mg/kg bw/day in males and 0, 62, 177 and 556 mg/kg bw/day in females) for 4 weeks. OECD guideline 409/GLP/QA.

Results: A very slight decrease in feed consumption (in high-dose males) and reduced weight gain (in both sexes) are notified, however this is not considered to be of relevance according to absence of these effects in 90-day dog study with more dogs (4/sex/dose level).

NOAEL: 1.5% in the diet (equal to 543 mg/kg bw/day in males and 556 mg/kg bw/day and in females) is proposed based on the absence of any adverse effects (Stebbins and Baker 2000).

*Only 4 animals have been employed per group (instead of eight as according to the guideline 409). Therefore, the study might be considered as supplemental more than acceptable.

Dog, diet, 13-week

Study design: Beagle dogs (4/sex/group) were administered test material in the diet at concentrations of 0, 0.15, 0.75, or 3.0 % (equal to 0, 54.5, 282 and 1070 mg/kg bw/day in males and 0, 52.7, 232 and 929 mg/kg bw/day in females) for 13 weeks to evaluate the potential for systemic toxicity. OECD guideline 409/GLP/QA.

Results: Microscopic effects in the stomachs were observed, characterized by slight, diffuse hyperplasia and hypertrophy of the mucosal epithelium, in all males and females given 3.0 % of the test substance. The mucosal hyperplasia was characterized by increased numbers of mucous cells and chief cells in the fundus of the stomach. Mucous cell hyperplasia was also noted in the pylorus of the stomach. Hypertrophy of mucous cells, characterized by increased cytoplasmic volume, was most prominent in mucous cells of the pylorus. There was no accompanying degeneration, necrosis or inflammation of the mucosa of the stomach.

Table 5.8: Incidence of histopathological effects on the stomach of dogs in 90-day dietary study

Dose (% in diet)	**0**	**0.15**	**0.75**	**3.0**
Stomach: hyperplasia, and hypertrophy, mucosa, diffuse, slight **(Males)**	0	0	0	**4**
Stomach: hyperplasia, and hypertrophy, mucosa, diffuse, slight **(Females)**	0	0	0	**4**

Bold indicates effects considered treatment-related.

High-dose animals had higher absolute and relative liver weights relative to controls, though within or near the historical control range of 13-week dietary studies recently conducted at this laboratory with

Beagle dogs. There were no alterations in liver-specific clinical chemistry parameters, and there were no corresponding microscopic alterations of the liver.

At 0.75 and 3% slight increases in relative weight of spleen were noted (17-18% in males, and 26-27% in females). However, the dose response is not very convincing in males and no abnormal pathology or haematological effects were seen in this study or in the one year dog study.

There were slight decreases in mean thymus weight of top dose females (15% in absolute weight, 21% in relative weight) and a similar finding was seen in the 13-week rat study. However the decrease in thymus weight was observed in the absence of histopathological findings in the thymus in this study nor in the 1-year dog study (thymus was not weighed in the 1-year study).

Table 5.9: Mean absolute and relative liver and spleen weight data in 90-day dietary study in dogs

Dose (%) **Liver**	**Absolute weight (males)** **g**	**Relative weight (males)** **g/100g bw**	**Absolute weight (females)** **g**	**Relative weight (females)** **g/100g bw**
0	236.15	2.477	192.43	2.415
Historical[1]	257.33 to 268.10	2.338 to 2.357	204.43 to 228.40	2.276 to 2.830
0.15	224.15	2.540	213.98	2.596
0.75	226.23	2.377	196.98	2.499
3.0*	266.35 (13% increase[2])	2.850 (15% increase[2])	236.00 (23% increase[2])	2.761 (14% increase[2])
Spleen				
0	150 ± 33	1.5 ± 0.1	119 ± 30	1.5 ± 0.2
0.15	126 ± 30	1.4 ± 0.2	144 ± 15	1.7 ± 0.1
0.75	174 ± 47	1.8 ± 0.3	148 ± 37	1.9 ± 0.4
3.0	169 ± 34	1.8 ± 0.4	161 ± 35	1.9 ± 0.3

**Significantly different from controls, averaged across both sexes, Dunnett's test, alpha = 0.05.*
[1]Historical control group mean range from two, 13-week dietary studies done since 1997 in the same laboratory.
[2] % increase compared to concurrent control

NOAEL: 0.75 % in the diet of female and male Beagle dogs (232 and 282 mg/kg bw/day respectively) based on histopathological changes in the stomach (and possibly the alterations in organ weights) at 3 % (females 929 mg/kg bw/day; males 1070 mg/kg bw/day) (Stebbins and Baker 2002).

Dog, diet, 52-week

Study design: Beagle dogs (4/sex/group) were fed diets containing 0, 0.03, 0.3, or 3% aminopyralid (equal to 0, 9.9, 99, and 967 mg/kg bw/day in males and 0, 9.2, 93 and 1038 mg/kg bw/day in females) for one year to evaluate the potential for systemic toxicity. OECD guideline 452/GLP/QA.

Results: High-dose females had lower final mean body weights (9 %) and decreased mean body weights gain (58 %). High-dose animals had higher mean relative liver weights (males 22 % and females 11 %). In addition, there was an increase in the mean absolute liver weight of top-dose males (21 %). These liver weight alterations corresponded to the very slight hypertrophy of hepatocytes seen at top dose level.

Table 5.10: Mean absolute and relative liver weights in 1-year dietary study in dogs

Dose (%)	Absolute liver weight (males) g	Relative liver weight (males) g/100g bw	Absolute liver weight (females) g	Relative liver weight (females) g/100g bw
0	253.98	2.443	236.78	2.914
Historical[1]	283.13	2.498	226.40	2.682
0.15	240.10	2.553	225.53	2.818
0.75	272.58 (9% increase[2])	2.643 (8% increase[2])	240.60	2.837
3.0	**307.28** (21% increase[2])	**2.970*** (22% increase[2])	241.25 (2% increase[2])	**3.224*** (11% increase[2])

Significantly different from controls, averaged across both sexes, Dunnett's test, alpha = 0.05. Bold indicates effects considered treatment-related.
[1]Historical control group mean data from one 1-year dietary study done in 2002.
[2] % increase compared with concurrent control.

Gross pathology investigation revealed diffuse thickening of the stomach mucosa in two high-dose females. Microscopic effects were noted in the stomachs of all high-dose animals. The stomach effects consisted of slight, diffuse mucosal hyperplasia and hypertrophy, slight chronic mucosal inflammation, and slight lymphoid hyperplasia of the gastric mucosa. The hyperplasia and hypertrophy were characterized by: increased numbers of mucous cells in the cardia, fundus, and pylorus of the stomach; and increased size of mucous cells, especially in the pylorus where the cytoplasm of affected cells was distended with mucus. The inflammatory effect consisted of lymphocytes and plasma cells accumulating in the lamina propria of the cardia, fundus, and/or pylorus. The lymphoid hyperplasia was characterized by numerous prominent lymphoid follicles with germinal centers, located in the cardia, fundus, and/or pylorus. The severity of the gastric mucosal hyperplasia and hypertrophy was comparable to the severity of these alterations in dogs from the 13-week dietary study, whereas the gastric mucosal inflammation and lymphoid hyperplasia were not present in dogs from the 13-week study. The only other histopathological effect was very slight hypertrophy of centrilobular to midzonal hepatocytes in two males and two females given 3.0% aminopyralid.

A detailed and helpful discussion of the stomach lesions is submitted by the study author (See Annex T1 page 242).

Unlike the response seen in rats, no gross or histopathological effects on the caecum were observed in any of the submitted dog studies.

Table 5.11: Notable lesions in 1-year dietary study in dogs (n= 4 dogs/sex/dose)

Sex	Lesion	Dose in diet (%)			
		0	**0.03**	**0.3**	**3**
Male	**Stomach**				
	Hyperplasia, lymphoid tissue, mucosa slight	0	0	0	**3**
	Hyperplasia and hypertrophy, mucosa, diffuse slight	0	0	0	**4**
	Inflammation, chronic, mucosa, diffuse				
	very slight	0	0	0	**2**
	slight	0	0	0	**2**
Female	**Stomach**				
	Hyperplasia, lymphoid tissue, mucosa slight	0	0	0	**4**
	Hyperplasia and hypertrophy, mucosa, diffuse slight	0	0	0	**4**
	Inflammation, chronic, mucosa, diffuse				
	very slight	0	0	0	**4**
	slight	0	0	0	0
Male	**Liver**				

	Hypertrophy, hepatocyte, centrilobular/midzonal, very slight	0	0	0	**2**
Female	**Liver**				
	Hypertrophy, hepatocyte, centrilobular/midzonal, very slight	0	0	0	**2**
Male	**Parathyroid gland**				
	Cyst, focal	0	1	1	2
	Cyst, multifocal	0	0	1	0
Female	**Parathyroid gland**				
	Cyst, focal	0	0	0	0
	Cyst, multifocal	0	2	0	1
Male	**Pituitary gland**				
	Cyst, pars distalis (=anterior pituitary)	1	1	2	3
	Cyst, pars nervosa (=posterior pituitary)	1	0	0	0
Female	**Pituitary gland**				
	Cyst, pars distalis	1	2	2	1
	Cyst, pars nervosa	0	0	0	0

Table 5.12: The Dow Chemical Co. TERC Laboratory Beagle Dog Historical Control Values for Parathyroid and Pituitary Cyst

Study/Report Date	1 Year/3-2002		90-Day/9-2002		90-Day/5-2003		1-Year/3-2004		1-Year/9-2004	
	M	F	M	F	M	F	M	F	M	F
Parathyroid cyst	0/4	2/4	0/4	2/4	0/4	1/4	1/4	2/4	1/4	2/4
Pituitary cyst pars distalis	1/4	2/4	2/4	1/4	4/4	0/4	0/4	2/4	1/4	3/4

M: male F: female

NOAEL: 0.3 % in the diet of female and male Beagle dogs (equal to 93 and 99 mg/kg bw/day respectively) based on decreased body weight gain in females, increased relative liver weight in males, slight hepatocyte hypertrophy and stomach lesions in both sexes after 3 % aminopyralid exposure (females 1038 mg/kg bw/day; males 967 mg/kg bw/day) (Stebbins and Day 2003, Anon, 2005).

Rat, dermal, 4-week

Study design: Fischer 344 rats (10/sex/group) were dermally exposed at a semi-occluded skin test site to 0, 100, 500, or 1000 mg aminopyralid, as a suspension in 0.5 % aqueous CMC /kg bw/day, 6 h/day, 7 days a week for 28 days. The dose volume was 4 ml/kg, with target concentrations of 0, 25, 125 and 250 mg/ml. OECD guideline 410/GLP/QA

Results: There were no gross signs of dermal irritation. One high-dose male had scabs on the dermal test site on day 28 of study, which may be associated with the clipping procedure. Males given 100 or 500 mg/kg bw/day had increases in absolute and relative full caecum weights (Annex T1,Table B.6.26). However, there was no dose-response relationship when data for the top dose are taken into account. Also there were no increases in empty caecum weight at any dose level.

A few males and females given 0 or 1000 mg/kg bw/day, and one male given 500 mg/kg bw/day, had mottling or necrosis of the papillary process of the liver (Annex T1, Table B.6.28). The only histopathological observation was slight epidermal hyperplasia at the dermal test site in two males given 500 and three high-dose males (Table 5.13). The epidermis of animals with slight hyperplasia was reported to be approximately twice as thick as the epidermis of unaffected control animals. The slight epidermal hyperplasia was indicative of minimal irritation in response to dermal application of the compound.

There was no microscopic evidence of systemic toxicity at any dose level.

Table 5.13: Treatment-Related Histopathology Findings at the Dermal Test Site of Rats Dermally Exposed to aminopyralid for 28 Days

	Males				Females			
Dose (mg/kg bw/day)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Skin-Dermal Test Site (no. examined)	10	10	10	10	10	10	10	10
Within normal limits	3	2	1	0	7	9	5	6
Hyperplasia, epidermis, very slight	7	8	7	7	3	1	5	4
Hyperplasia, epidermis, slight	0	0	**2**	**3**	0	0	0	0

Bold type indicates incidence of effects judged by the study author to be treatment-related (but reported as not statistically significant).

NOAEL (systemic): 1000 mg/kg bw/day for both sexes.

NOAEL (dermal) males: 100 mg/kg bw/day related to local effects on the treated skin of males based on the slight epidermal hyperplasia (indicative of slight irritant response) at 500 mg/kg bw/day and above.

NOAEL (dermal) females: 1000 mg/kg bw/day (highest dose tested) (Stebbins, Thomas and Day, 2002).

Summary (sub-chronic toxicity):

In the 90-day rat dietary study, high doses of aminopyralid caused an increase in both full and empty weight of the caecum. Very slight mucosal hyperplasia of the caecum and ileum was seen in male high-dose rats. Urinary changes were also observed after exposure to high doses of aminopyralid. The intestinal and urinary changes were shown to be partly or completely reversible following a 28-day recovery period.

In 90-day and 1-year dietary studies with dogs, histopathological changes in the stomach mucosa were seen in animals ingesting a high dose (1000 mg/kg bw/day). After 90 days the gastric changes were limited to an increased number and size of mucous secreting cells and increased number of pepsin (proteolytic enzyme) secreting chief cells. These changes were also seen at the end of the 1-year study, together with a slight chronic inflammatory response and slight lymphoid hyperplasia in the gastric mucosa. Slight liver effects (increased relative liver weight and/or slight hepatocyte hypertrophy) were also notable findings at 1000 mg/kg bw/day in the 90-day and 1-year dog studies.

In a 28-day dermal study in rats, evidence of slight dermal irritation (slight dermal hyperplasia) was seen in males at 500 mg/kg bw/day but no systemic effects were observed up to a limit dermal dose of 1000 mg/kg bw/day.

Critical effects/target organs:
In these studies (dietary studies), the main target organs were the caecum in rats and the stomach (inflammation) and the liver (hyperthropy) in dogs. No adverse effects were seen in mice.
The dermal sub-chronic study in rats showed evidence of slight dermal irritation (hyperplasia) in males at 500 mg/kg bw/day.

NOAEL-values:
Rats (oral):500 mg/kg bw/day for males (543 mg/kg bw/day), based upon increased caecal weights, decreased urine pH and histopathological changes in the caecum and ileum at the top-dose level. 1000 mg/kg bw/day for females (1060 mg/kg bw/day).
Rats (systemic): 1000 mg/kg bw/day.
Dogs: 0.3 % in the diet of female and male dogs (93 and 99 mg/kg bw/day), based on decreased body weight gain in females, increased relative liver weight in males, slight hepatocyte hypertrophy and stomach lesions in both sexes. The most sensitive species in sub-chronic studies is the dog.
Mice: 1000 mg/kg bw/day (1020 mg/kg bw/day).

Table 5.14: Summary of short-term toxicity studies with aminopyralid

Study	NOAEL (mg/kg bw/d)	Effects	Reference
Oral studies			
4-week rat, diet	M: 1126 mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins and Day 2000
	F: 1094 mg/kg bw/d	*Changes in both sexes in caecum (increased size) at≥ c.550 mg/kg bw/d, and urinalysis at c.1000 mg/kg bw/d*	
13-week rat, diet with 4-week recovery	M: 543 mg/kg bw/d	No adverse effects at highest dose tested.	Dryzga and Stebbins 2001
	F: 1060 mg/kg bw/d	*Changes in caecum and urinary parameters at c.540 mg/kg bw/day and above, and in ileum at 1090 mg/kg bw/d.*	
4-week mouse, diet	M: 1038 mg/kg bw/d	No adverse effects at highest dose tested.	Yano and Dryzga 2000
	F: 1058 mg/kg bw/d	*Histopathological liver changes in a few males at 1038 mg/kg bw/d considered to represent a minimal adaptive effect*	
13-week mouse, diet	M: 1020mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins, Day and Thomas 2001
	F: 1020 mg/kg bw/d	*Histopathological liver changes in 2 males at 1020 mg/kg bw/d considered to represent a minimal adaptive effect*	
4-week dog, diet	M: 543 mg/kg bw/d	No adverse effects at highest dose tested.	Stebbins and Baker 2000
	F:556 mg/kg bw/d		
13-week dog, diet	M: 282mg/kg bw/d	Stomach histopathology & altered organ weights	Stebbins and Baker 2002
	F: 232 mg/kg bw/d	Stomach histopathology & altered organ weights	
52-week dog, diet	M: 99 mg/kg bw/d	Stomach histopathology liver hypertrophy, increased liver weight	Stebbins and Day 2003a
	F: 93 mg/kg bw/d	Stomach histopathology, liver hypertrophy, decreased body weight gain	
Dermal study			
4-week rat, dermal	systemic effects M and F : 1000 mg/kg bw/d	No adverse systemic effects at the highest dose tested (limit dose)	Stebbins, Thomas and Day 2002
	Local effects on skin	Local effects on skin: M: Slight epidermal hyperplasia	

	M: 100 mg/kg bw/d F: 1000 mg/kg bw/d	F: no effects	

5.1.6 Chronic toxicity and carcinogenicity

Combined chronic toxicity/oncogenicity study 2-year feeding study in rats:

Study design: Five groups of Fischer 344 rats (65/sex/dose level) were fed diets containing 0, 5, 50, 500, or 1000 mg aminopyralid/kg bw/day for approximately 24 months to assess the potential chronic toxicity and oncogenicity of the substance. 10 rats/sex/dose level were necropsied after 12 months (chronic toxicity group), 5 rats/sex/dose level were also necropsied at the same time for chronic neurotoxicity investigation, while the remaining 50 rats/sex/dose level were maintained on their respective diets for up to 24 months. The chronic neurotoxicity section of this study is reported separately. OECD guideline 452/GLP/QA.

Results: Males given 500 or 1000 mg/kg bw/day and females given 1000 mg/kg bw/day had decreased body weights that developed gradually and were maintained throughout the study period. Body weight gain for high-dose males was lower than controls, and fulfilled the criteria > 10 % reduction, which is considered to be an adverse effect. Additionally, feed consumption was increased for males. Results from feed efficiency data for the first 13 weeks did not reveal any consistent pattern.

Table 5.15: Body weights and body weight gains in rat chronic toxicity/carcinogenicity study

	Dose Level (mg/kg bw/day)				
Test Day	**0**	**5**	**50**	**500**	**1000**
	Males (g)				
1	150.5	147.8	147.9	149.5	151.3
29	255.0	252.1	251.9	253.9	252.5
91	334.3	330.1	328.1	328.9	**325.9**
120	353.1	346.4	342.3*	**344.8***	**339.7***
176	385.1	379.5	375.8*	**374.9***	**370.1***
260	418.8	413.1	410.1	**407.3***	**401.1***
364	444.1	436.6	433.9	**430.1***	**421.4***
Gain 1-364	*293.6*	*288.8*	*286.0*	*280.5*	*270.9*
540	466.4	454.1	457.4	**444.3***	**436.2***
729	431.4	420.3	421.8	**409.1**	**412.9**
Gain 1-729	*280.1*	*273.1*	*274.5*	*258.4*	*262.4*
	Females (g)				
1	113.7	113.6	113.7	113.1	112.8
29	154.3	156.2	155.0	153.9	151.4
91	182.5	182.8	183.3	182.4	**178.0***
120	188.5	188.4	187.7	187.2	**183.7***
176	200.5	201.0	200.6	199.1	**196.3***
260	214.0	216.4	217.1	214.4	**210.8**
364	229.1	233.1	231.7	231.1	**224.9**
Gain 1-364	*115.4*	*119.5*	*118.0*	*118.0*	*112.2*
540	275.4	278.2	279.7	273.9	**264.2**
729	294.4	294.0	305.1	289.4	**283.5**
Gain 1-729	*180.7*	*180.7*	*191.1*	*177.3*	*170.7*

** Statistically Different from Control Mean by Dunnett's Test, alpha = 0.05.*
Bold type indicates effects considered treatment-related by study authors.

Some inconsistent changes in prothrombin time are observed in high-dose animals. These effects differed between sexes, prothrombin times were altered only the first part of the study, and the changes were small.

The only clinical pathology parameter affected was some degree of increase in AST levels of high-dose females which was present the first 12 months of the study period. Though, uncertainty is connected to the effect on the basis of: 1) AST level were not increased at 18 and 24 months; 2) similar elevation was not found in the 13-week study at the same dosages; 3) even though males seems to be a bit more sensitive for other effects attributed to treatment, they were not affected; 4) histopathological effects were not notified in any organ that could be correlated with the increased AST; 5) increased dosing duration did not give any clear progression.

Table 5.16: Mean (+SD) AST activity (U/L) of female rats in the chronic toxicity/carcinogenicity study:

Timepoint	Dose Level (mg/kg bw/day)				
	0	**5**	**50**	**500**	**1000**
3 months	102(± 10)	108(±18)	107(±9)	111(±19)	**133**(±26)*****
6 months	112(±28)	118(±27)	112(±29)	118(±23)	**180**(±74)**$**
12 months	111 (±28)	104(±20)	99(±18)	130(±23)	**162**(±77)
18 months	139(±51)	120(±36)	100(±22)	100(±18)	122(±24)
24 months	88(±15)	102(±14)	129(±70)	169(±113)	113(±27)

**Statistically different from control mean by Dunnett's test, alpha = 0.05*
$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05
Bold indicates effects considered treatment related by study authors .

Animals exposed for the two top-dose levels had distinct pattern of urinalysis changes, though in the absence of associated histopatological changes in the kidney. An increased urine volume and decreased urine specific gravity, pH, protein, and ketones were confirmed (Annex T1 Table B.6.35, B.6.36, B.6.37, B.6.38, B.6.39). Similar urinalysis effects were found in the 13-week oral rat study. It is worth noting that these effects are variable in a dose-response relationship manner, less definitive at 3 months and by the end of the study than at 6 months, and unaccompanied by renal histopathologic changes. In the absence of associated histopathological changes in the kidney, the urinary changes are considered adaptive to altered water balance resulting from caecal enlargement and renal excretion of aminopyralid.

The only target organ considered affected by aminopyralid was caecum, which had an increase in size, weight, and very slight, diffuse mucosal hyperplasia (Annex T1, Table B.6.40, B.6.41, B.6.42). However, it is noteworthy that the degree of increase was slightly less after 24 months than the effects found at 12 months. The effects were reversible together with absence of histopathology changes.

A detailed and helpful discussion of the caecum enlargement effect and urinalysis is submitted by the study author (See Annex T1 page 153-154 and 242).

Aminopyralid was non-carcinogenic to Fischer 344 rats under the condition of this study.

NOAEL: 50 and 500 mg/kg bw/day in males and females respectively, based on a slight body weight decrease after exposure to aminopyralid (Johnson and Dryzga, 2004).

Mouse, 18 months

Study design: CD-1 mice (50/sex/dose level) were fed diets containing 0, 50, 250, or 1000 mg aminopyralid/kg bw/day for approximately 18 months to evaluate the substance oncogenic potential. OECD guideline 451GLP/QA.

Results: There were no observed adverse effects in males at any dose level.
Mortality rates were higher in all treated females, with statistically significant differences being seen at 50 and 1000 mg/kg bw/day. Only the apparent increased mortality observed after exposure for 1000 mg/kg bw/day was prominent and of interest, related to the lack of evident dose-response relationships, and the absence of any treatment-related histopathological effects. The most common

cause of death of high-dose female mice was probably the incidence of nephropathy, but the evidence for a substance-related increase in nephropathy appeared to be equivocal.

Table 5.17: Total mortality (including animals killed in a morbid condition) for males and females in the mouse carcinogenicity study, and the commonest attributed causes of death/morbidity for females in this study

	Dose (mg/kg bw/day)			
	0	**50**	**250**	**1000**
Total mortality for **males** (%)	38	32	34	42
Total mortality for **females** (%)	16	34*	30	42*
Cause of Death/morbidity for **females** (%)				
Not determined	0	4	4	4
Kidneys – nephropathy	8	4	8	**22**
Hydrothorax	0	4	0	6
Lymphosarcoma	4	8	6	6
Inflammation of back and neck	0	0	0	6
Uterus – sarcoma	0	4	2	0

**Statistically significant difference from controls by Gehan-Wilcoxon test, experiment-wise alpha=0.05*
Note: mortality/morbidity was sometimes attributed to more than one effect

Table 5.18: Historical control values for total mortality in 18-month oncogenicity studies with CD-1 mice:

Report Date	December 2001		May 2004		October 2004	
	Male	Female	Male	Female	Male	Female
	26%	24%	26%	20%	22%	22%

- 18-36% female mortality: 7 studies at unnamed laboratories initiated 1988-92 (Gignis and Clifford, 2000).
- Studies from test laboratory within 2 years of the present study (Anon, 2005a).

See also Annex T1 Table B.6.45a.

Dermatitis was noted, but this alteration did not occur in a dose-related manner. Males from the control group had the highest incidence of dermatitis. Very slightly increase in feed consumption occurred on days 7-14 for high-dose males.

Females exposed to 50, 250 or 1000 mg/kg bw/day aminopyralid had increased incidence of dilatation of the ovarian bursa, however, this observation lacked a dose-response pattern and histopathological evidence (Annex T1, Table B.6.46, B.6.47). High-dose females had also increased incidence of pale kidneys, which may correspond to the observed nephronpathy (Annex T1, Table B.6.49) seen in these animals. Furthermore, decreased amount of body fat, presence of haemolysed blood in the gastrointestinal tract, pulmonary atelectasis and perineal soiling were also gross pathology seen in high-dose females (Annex T1, Table B.6.48).

There was no evidence of tumour development in any organ or tissue.

It is worth noting that neither any gross lesions nor histopathological observations were seen in the caecum, unlike the findings in the 2-year rat study.

NOAEL: 1000 mg/kg bw/day (males) and 250 mg/kg bw/day (females), based on an increase in mortality in high-dose females. The most common cause of death is nephronpathy, but the evidence is equivocal (Stebbins and Day, 2003, Anon, 2005).

Summary (chronic toxicity and cancer):

Critical effects/target organs:
In chronic toxicity studies the critical effects were slight body weight decrease (males), enlarged caecum with slight mucosa hyperplasia, and urinary changes in Fisher 344 rats, whereas the critical effect observed in female mice was increased mortality. No progression in severity of the caecal effects were observed in the chronic study in rats compared to sub-chronic studies.

NOAEL-values:
Rats: 50 mg/kg bw/day, based on reduced body weight in males.
Mice: 250 mg/kg bw/day based on increased mortality in females.

Cancer: Aminopyralid is not a carcinogen in animal-studies.

Table 5.19: Summary of chronic toxicity and carcinogenicity studies with aminopyralid

Study	NOAEL (mg/kg bw/d)	Effects	Reference
2- year chronic toxicity/carcinogenicity rat, diet	M: 50 F: 500	Slightly reduced body weight gain in males *Effects on the caecum and urinary parameters in both sexes at ≥500 mg/kg bw/day* *No oncogenic response in this study*	Johnson and Dryzga, 2004
18-month carcinogenicity mouse, diet	M: 1000 F: 250	Increased mortality (and equivocal evidence for substance-related nephropathy) in females *[No adverse effects in males* *No oncogenic response in this study]*	Stebbins and Day, 2003

5.1.7 Reproductive toxicity

Rat, two-generation study

Study design: CD rats (30/sex/dose level) were fed diets containing 0, 50, 250, and 1000 mg aminopyralid/kg bw/day for approximately 10 weeks prior to breeding, and continuing through breeding (2 weeks), gestation (3 weeks) and lactation (3 weeks) for each of two generations to evaluate the potential for reproductive toxicity and effects on neonatal growth and survival. OECD guideline 416/GLP/QA.

Results P1 and P2 adults: Three deaths occurred prior to scheduled termination. Two P1 males (one control and one low dose), and one P1 high-dose female. Choke syndrome (i.e. oesophagus was distended with placental tissue) most likely caused the death in the female. Moreover, one P2 mid-dose male died 6 days prior to the start of the pre-breeding phase. In other studies have caecum showed to be the target organ in rats, and this study is no exception (Annex T1, Table B.6.53). Hence, increased caecal weight (full and empty) were identified in 250 mg/kg bw/day and above P1 and P2 rats (both sexes). P2 males at 50 mg/kg bw/day also revealed an increase in caecal weight. The only gross pathology observation documented were increased caecal size in a few males at 250 mg/kg bw/day and most high-dose animals. There were no histopathologic changes in any tissue, including the caecum.

Results F1 & F2 litter and pups: Increases in pup weigh on lactation days 7 and 14 were documented. Effects were not seen at day 21, nor a dose-response relationship. F1 female weanlings (250 mg/kg bw/day) exhibited a significant decrease in absolute uterine weight, but there was absence of a dose-response relationship and lack of effects in the F2 weanlings.

NOAEL (parental toxicity): 1000 mg/kg bw/day (the highest dose tested) based upon the absence of any significant adverse effects. Increased caecal weight and/or size in adult rats at 50 mg/kg bw/day and above is regarded as an adaptive effect and not taken into account.

NOAEL (offspring): 1000 mg/kg bw/day based upon no adverse effects observed at the highest dose level.

NOAEL (reproduction): 1000 mg/kg bw/day based upon the absence of adverse effects at the highest dose tested (Zablotny and Thomas, 2003).

5.1.8 Teratology

Rat

Study design: Time-mated female CD rats (25/dose level) were administrated aminopyralid by gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day on days 6-20 of gestation. OECD guideline 414/GLP/QA.

Results: One animal (300 mg/kg bw/day dose group) was found dead on gestation day (GD) 10. However, no gross lesions were observed in this pregnant female. All her foetuses seemed to be normal. Slight decrease in feed consumption was noted for the high-dose group on GD 6-9, whereas no differences were seen in body weight. It is worth mentioning that no gross lesions were noted in the caecum.

After administration of the test-substance some small alterations could be seen in foetuses, but these changes had neither a dose-response relationship nor occurred at high frequencies. In exposure order (0, 100, 300 and 1000 mg/kg bw/day) 6, 1, 0 and 2 malformed foetuses observed, respectively. One foetus exhibited haemorrhage in the pericardial fluid and another was found with a cervicalrib in the high-dose group, however these effects were not seen at lower doses. Top-dose level also had increased incidence of delayed thoracic rib ossification, but this is believed to be of no concern on the basis of delayed ossification of other bones seen in controls.

Aminopyralid do not have teratogenic properties.

NOAEL(maternal and developmental): 1000 mg/kg bw/day based on the absence of toxicologically relevant effects at any dose level (Carney and Tornesi, 2001, Anon, 2005).

Rabbit

Study design: New Zealand White female rabbits (26/dose level) were administrated aminopyralid by oral gavage at dose levels of 0, 25, 100 or 250 mg/kg bw/day (Phase I), or 0, 500, and 750 mg/kg bw/day (Phase II) on GD 7-27. OECD guideline 414/GLP/QA.

Results: There is some evidence of increased incidence of liver haemorrhage in all treated groups compared with concurrent controls, but in the absence of a dose response. However, the incidence at the selected dose levels exceeded the historical control values. Moreover, the liver has not been indentified as a target organ in previous studies with aminopyralid, including the reproductive toxicity study in rats. Although, a slight hypertrophic response was seen in high-dose animals in 1-year dog study.

Table 5.20: Clinical observations (days 7-28) in rabbit developmental toxicity study

Clinical observation	**Number of dams showing effect (N=26/dose group)**					
mg/kg bw/day	**0/0**	**25**	**100**	**250**	**500**	**750**
Gait						
Incoordinated	0	0	0	0	23 **First seen†** D7= 0 D8=1 D9=1 D10+=21	23 **First seen†** D7=1 D8=2 D9=4 D10+=16

† **First seen** = number of rabbits which **first showed** this clinical effect (any faecal alteration or incoordination) on gestation days 7, 8 or 9 or/after gestation day 10.
Note: Overall incidence (number rats/day) of observed incoordination at 500 mg/kg bw/day: 0-2 on days 7-9, 2-6 on days 10-21, 9-11 on days 22-23, 13-18 on days 24-27.

Table 5.21: Body weights, body weight gains and food consumption of dams in rabbit developmental toxicity study (means + SD)

BW or BW gain (g)	**Phase I**				**Phase II**		
	0	**25**	**100**	**250**	**0**	**500**	**750**
BW gd 10[a]	3387.2	3280.1	3328.2	3363.2	3265.0	3246.7	**3068.7***
Gain gd 7-10	38.5 (±35.8)	11.3 (±54.0)	24.8 (±32.0)	15.1 (±39.6)	25.0 (±37.7)	**-11.8$** (±54.3)	**-70.0$** (±111.3)
Gain gd 10-13	49.4 (±36.5)	42.3 (±52.4)	56.0 (±49.5)	36.0 (±46.0)	39.5 (±53.3)	51.9 (±68.8)	63.5 (±104.2)
Gain gd 13-16	74.4 (±56.2)	81.4 (±52.5)	66.1 (±65.7)	53.6 (±56.7)	97.3 (±41.6)	91.8 (±51.3)	33.2 (±104.6)
Gain gd 7-28	299.2 (±124.8)	271.3 (±150.2)	319.3 (±119.1)	250.8 (±138.1)	282.6 (±124.9	305.9 (±93.9)	no data
Gain gd 0-28	406.5 (±188.9)	415.7 (±167.4)	437.6 (±134.1)	364.8 (±162.6)	359.7 (±152.3	374.5 (±117.9	no data
Food consumption (g/animal/day)							
gd 7-8	186.0 (± 38.0)	169.4 (± 36.0)	186.7 (± 31.6)	175.0 (± 30.5)	175.4 (±33.1)	**143.8* (±43.1)**	**105.7* (± 52.5)**

[a] *gd = gestation day.*
**Statistically different from control mean by Dunnett's test, alpha = 0.05.*
$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

Phase I: No effects on feed consumption, body weight or body weight gain after exposure to aminopyralid were noted.

Unscheduled deaths arising during the study: One rabbit was removed prior to initiation of treatment because it did not eat. One rabbit was killed because it had aborted one foetus by resorption on GD 19 with no prior clinical observations. Gross finding revealed pale kidneys (bilateral). The cause of this abortion was not determined. Due to a gavage complication resulting in misdosing, one rabbit was found dead on GD 15. The three animals mentioned above had been assigned to the 250 mg/kg bw/day group. Finally, one 25 mg/kg bw/day rabbit was killed due to it aborting four foetuses on GD 26. It was reported that this female had decreased and mucoid faeces in her cage pan. This female had seven normally developed foetuses and one late resorption still within the uterus, which was documented after gross examination. Other observations included perineal soiling and dark ingesta/watery contents in the caecum. The cause of the abortion was not determined.

Evaluation of the litter data showed that mean percent pre-implantation loss was significantly increased at 25 mg/kg bw/day, but the effect lacked a dose-response relationship and the incidence was less than for the phase II controls.

Taking all measured parameters into account, phase I rabbits given ≤250 mg/kg bw/day did not exhibit any maternal or developmental toxicity (Annex T1, Table B.6.59 and B.6.60).

Phase II: Significant reductions in feed consumption, body weight (only at 750 mg/kg bw/day) and body weight gain in the two top-dose groups were noted after exposure to aminopyralid.

750 mg/kg bw/day: Maternal toxicity (incoordinated gait with reduced body weight gain (GD 7-10)) was so severe, that this group was removed from the study before the end of the dosing period and not used to investigate developmental toxicity. Gross examination revealed lesions of the glandular mucosa of the stomach (including multifocal erosion-ulcers, which are consistent with the substance being classified as a severe eye irritant) and kidneys (pale cortex) in several does. No histopathological examination was conducted, therefore it is not known if there could have been adverse histopathological effects, especially in the stomach, at doses below 500 mg/kg bw/day.

Clinical signs included increased incidences of decreased amounts of faeces and incoordinated movement shortly following dosing. Stiffening/dragging of the limbs was noted and also several rabbits tipped onto their sides when movement was attempted. These effects were transient and did not progressively worsen with time.

Unscheduled deaths: One animal died due to gavage complications. Two more animals were sacrificed on GD 17 (moribund) with clinical signs that included incoordinated gait, perineal urine soiling, decreased faeces, and decreased activity. Additionally, these two rabbits had significant decreases in body weight gains and feed consumption. Gross examination revealed that both were pregnant, and had pale kidneys (cortex), watery dark caecal contents, erosions/ulcers in the glandular mucosa of the stomach, and hairballs.

Organ weights were not evaluated for the high-dose group which was terminated early due to excessive toxicology. Gross pathology of one of twenty-five 500 mg/kg bw/day rabbits showed multifocal erosion-ulcers of the glandular mucosa of the stomach. Twelve rabbits in the high-dose group also had lesions of the glandular mucosa, and kidney alterations (pale cortex) were observed in eleven rabbits. Watery dark caecal contents and hair balls were also noted in two rabbits in this group.

500 mg/kg bw/day: One death was attributed to misdosing/gavage complication. Transient incoordinated gait was observed at similar incidence as for the 750 mg/kg bw/day group, however the degree of incoordination was much less pronounced than at the top-dose level.

There were no alteration in faeces, but a slight decrease in body weight gain. There was no evidence of embryonal/fetal effects at 500 mg/kg bw/day, except a single incidence of a fetus with a missing testis. No other occurrences of unilateral anorchia in fetal rabbits have previously been observed in this particular laboratory. This malformation can occur spontaneously, yet the isolated occurrence of this alteration may be a cause of concern.

NOAEL (maternal): 250 mg/kg bw/day based on severe clinical signs which included transient incoordinated gait (23/26 does), decreased body weight gain as well as erosion of the stomach mucosa. High-dose group was removed from the study due to excessive toxicology.

NOAEL(developmental): 500 mg/kg bw/day due to no adverse effects at the highest dose tested (Marty, Liberacki and Thomas 2002, Anon 2005).

Summary (reproductive toxicity and teratology):

No adverse effects were observed in the reproduction toxicity study in rats. Aminopyralid has been shown not to be a selective reproductive toxin. Increased caecal weight and/or caecal size was seen in adult rats at 50 mg/kg bw/day and above. **NOAEL(maternal, foetuses and reproduction)**: 1000 mg/kg bw/day (the limit dose) based upon no adverse effects observed at the highest dose level tested.

No adverse effects were seen in the developmental toxicity study in rats. However, at the highest dose tested in rabbits, incoordinated gait accompanied by decreased amounts of faeces, significant reductions in body weights, body weight losses, decreased body weight gains, and decreased feed consumption were observed. The animals that died had pale kidneys, watery, dark cecal contents, erosions/ulcers of the stomach (glandular mucosa) and hairballs. 750 mg/kg bw/day exceeded the maximum tolerated dose, and all rabbits in the dose group were sacrificed. Under the conditions of this study, dietary administration of the test compound did not induce teratogenic effects in rabbits.
NOAEL (maternal toxicity) 250 mg/kg bw/day, based upon transient incoordinated gait, decreased body weight gain as well as multifocal erosion-ulcer of the glandular mucosa of the stomach in rabbits.
NOAEL (fetal toxicity) 500 mg/kg bw/day, based upon no adverse effects observed at any dose level.

5.1.9 Neurotoxicity

No test of delayed neurotoxicity is required and none was conducted, on the basis that delayed neurotoxicity is relevant only to anticholinesterases. Aminopyralid is not an anticholinesterase, hence it has no potential to cause delayed neurotoxicity.

Rat, acute oral

Study design: Fisher 344 rats (10/sex/dose level) were administrated aminopyralid by oral gavage, as a suspension in 0.5 % aqueous Methocel at 0, 500, 1000, or 2000 mg/kg bw/day. OECD guideline 424/GLP/QA.

Results: Clinical observations were restricted to increased incidence of faecal soling high-dose males and urine soiling in females at the top-dose level. However, all incidences were transient (resolved within the study period) and occurred in the absence of any gross or neuropathologic changes. Whether this soiling was indicative of a neurotoxic potential at the highest dose is not clear. There were no other signs of neurotoxicity or systemic toxicity.

NOAEL: 1000 mg/kg bw/day based on higher incidence of faecal soiling in males and urine soling in females (Marable, Andus, and Stebbins, 2002).

Table 5.22: Number of rats showing perineal soiling during clinical examinations on days 2-4 (n=10 rats/sex/dose):

	0 mg/kg bw	500 mg/kg bw	1000 mg/kg bw	2000 mg/kg bw
males				
Perineal, urine	0	0	0	0
Perineal, faeces	2	1	3	**8**
females				
Perineal, urine	0	0	0	**4**
Perineal, faeces	0	0	0	0

Rat, 12 months chronic

This study (chronic neurotoxicity) represent one component of a three-part, combined chronic neurotoxicity/chronic toxicity/oncogenicity study (other parts of the study are reported earlier).

Study design: Fisher 344 rats (10/sex/dose level) were fed diets containing aminopyralid at dose levels of 0, 5, 50, 500, or 1000 mg/kg bw/day for up to 24 months. 5 rats/sex/group were pre-selected for neurotoxicity evaluation and 5 rats/sex/group were used to evaluate both toxicity and neurotoxicity after 12 months. At termination, 5 rats/sex/group (and all rats in the control group and the high-dose group) were subjected to neuropathological evaluation. OECD guideline 424/GLP/QA.

Results: There was no observed effect on gait or motor activity. Within the neurotoxicity sub-group, no significant effects were seen on body weight, though significant body weight reduction were observed in males at the two top-dose levels when all study animals (n=65/sex/group) were considered.

In high-dose males an increase in defaecation level was observed. From 6 months onwards, there was some evidence of increase in degree of defaecation in males exposed to 500 and 50 mg/kg bw/day. A slight increase in the severity of urination was seen in males at 12 months at the two top-dose levels, although this is an uncertain evidence of substance-related effect.

Gross pathology revealed the same result as seen in the chronic toxicity/carcongenicity parts of this study, i.e. caecum enlargement in all rats given either 500 or 1000 mg/kg bw/day. Histopathological investigation showed spontaneous degeneration of individual nerve fibres in addition to swollen axons (in males) in high-dose animals. These lesions are considered typical of Fischer 344 rats of this age. Further findings in the top-dose group considered to be typical spontaneous lesions found in Fischer 344 rats of this age and husbandry conditions are; slight increase in degeneration of the retina in males, arterial mineralization of the eye of females and sinusoidal dilation in the pars distalis region of the pituitary of males and females. This assumption is based on the lack of clear effects in the chronic toxicity/carcinogenicity part of this study where lager groups of animals were investigated.

NOAEL: 1000 mg/kg bw/day based on no adverse effects observed after exposure to aminopyralid. The caecal changes and the increase of defaecation are not regarded as adverse effects (Maurissen, Andrus, Johnson, and Dryzga, 2003).

Summary (neurotoxicity):

Aminopyralid did not result in any neurotoxic effects. **NOAEL (neurotoxicity):** 1000 mg/kg bw/day (highest dose tested), based on no effects observed.

5.1.10 Special studies

Investigation of incoordination in rabbits exposed to aminopyralid

These supplementary studies were conducted as a follow-up investigation to the developmental toxicity probe study of pregnant New Zealand rabbits dosed with aminopyralid (Marty, Liberacki and Thomas 2002, Anon 2005).

Studies with aminopyralid TIPA were conducted using the formulation GF-871. GF-871 is a soluble concentrate that contains predominantly aminopyralid TIPA and water, plus a very small percentage of TIPA. The formulation GF-871 was used for these toxicity studies, rather than aminopyralid TIPA itself, because the TIPA salt of aminopyralid is a waxy solid and therefore presents some practical difficulties for preparing homogeneous solutions and dietary mixes; these are much easier to prepare using GF-871. The TIPA salt of aminopyralid rapidly dissociates in water which makes the findings relevant for the risk assessment of aminopyralid.

Study 1: Oral gavage pilot study in non-pregnant NZW rabbits exposed to aminopyralid

The purpose of this pilot study was to characterise the behavioural effects of aminopyralid in non-pregnant New Zealand white rabbits.

Study design: Three sexually mature (non-pregnant) female rabbits were dosed with a suspension of aminopyralid in 0.5% aqueous Methocel. There is no appropriate guideline for such a study and no GLP. The study is not acceptable even as a supplementary study because of the limitations to the experimental design (in particular the small sample size and lack of a control group) and the apparently inaccurate clinical description of the observed incoordination.

Results: This pilot study shows that gavage exposure to 1000 mg aminopyralid/kg bw/day can cause incoordination in non-pregnant rabbits (which was first seen after 1 or 2 doses). The study suggests that non-pregnant rabbits may be less sensitive than pregnant rabbits to incoordination caused by exposure to aminopyralid because incoordination was not seen at 500 mg aminopyralid/kg bw/day, a dose level at which incoordination has been seen in pregnant rabbits. However, the present study is too limited to draw any firm conclusions about the relative sensitivity of pregnant and non-pregnant rabbits to aminopyralid-induced incoordination (Marable and Day, 2004).

Study 2: Oral gavage developmental toxicity study in NZW rabbits exposed to GF-871 (an aqueous formulation consisting of 41.3% triisopropanolammonium (TIPA) salt of aminopyralid)

Study design: NZW time-mated female rabbits (26/group) were administrated by gavage GF-871 (OECD 414/GLP).

Table 5.23: The following dose levels were administered:

	Dose levels (mg/kg bw/day)			
GF-871	0	484	1211	2421
Aminopyralid TIPA (target doses)	**0**	**200**	**500**	**1000**
Aminopyralid acid equivalents	0	102	256	512

Results: Three high-dose and one 500 mg/kg bw/day females were killed because of severe inanition and body weight loss. Furthermore, one high-dose and one control female aborted.
The overall incidence of observed incoordination in the high-dose animals increased during the study. The only maternal effect observed at 200 mg aminopyralid TIPA/kg bw/day were a single transient incidence of incoordination in one animal. The same day decreased activity was seen in the same animal.

Repetitive transient chewing behaviour and absent/reduced faeces were seen in some rabbits. The latter may be temporally correlated with reduced food consumption.

Table 5.24: Notable clinical signs in pregnant rabbits dosed by gavage with aminopyralid TIPA (number of individuals showing effect):

Clinical sign	**Dose of aminopyralid TIPA** (and as aminopyralid acid equivalents) : mg/kg bw/day			
	0	**200** (102)	**500** (256)	**1000** (512)
Faeces absent	1/26 =4%	0/26	2/26 = 8%	5/26 = 19%
Faeces reduced	3/26 = 12%	5/26 =19%	11/26 =42%	18/26 = 69%
Incoordinated gait	**0/26**	**1/26 = 4%**	**2/26= 8%**	**19/26 = 73%**
Repetitive chewing behaviour	0/26	0/26	0/26	6/26 = 23%

Significant reductions in body weight gain and food consumption were seen in high-dose females.

Additionally, no lesions in the stomach or the caecum were noted. At 1000 mg aminopyralid TIPA/kg bw, mean fetal weight was reduced in females (by 10%) and in both sexes combined (by 10%). These mean litter weights were outside recent historical control ranges. Malformed foetuses (1-3) at each dose level (including controls) occurred in the absence of any consistent pattern of effect and/or lack of dose response.
There was a slight increased foetal and litter incidence of delayed ossification of sternebrae and pubis after high-dose exposure which is consistent with the smaller foetal size at this dose level.

Table 5.25: Some fetal skeletal alterations in the rabbit developmental toxicity study with aminopyralid TIPA

		Dose of aminopyralid TIPA (and as aminopyralid acid equivalents) : mg/kg bw/day			
		0	**200** (102)	**500** (256)	**1000** (512)
Sternebrae; delayed ossification	Fetal incidence	35/179 (20%)	238/207 (18%)	43/196 (22%)	45/155 (29%)
	Litter incidence	15/24 (63%)	16/25 (64%)	14/24 (58%)	16/18 (89%)
Pubis; delayed ossification	Fetal incidence	2/179 (1%)	4/207 (2%)	3/196 (2%)	6/1552.2 (4%)
	Litter incidence	1/24 (4%)	2/25 (8%)	2/24 (8%)	5/18 (28%)

NOAEL (maternal) <200 mg aminopyralid TIPA/kg bw/day (<102 mg aminopyralid/kg bw/day) based on inanition, severe body weight loss and incoordination at the two top-dose levels.

NOAEL (developmental) 500 mg aminopyralid TIPA/kg bw/day (256 mg aminopyralid /kg bw/day) based on reduced foetal weight at 1000 mg aminopyralid TIPA /kg bw/day (supported by the increase in delayed ossification in foetuses at 1000 mg aminopyralid TIPA /kg bw/day) (Carney and Tornesi 2004).

Study 3: Supplemental report: Oral gavage developmental toxicity study in NZW rabbits exposed to GF-871

Establishment of NOEL for clinical effects (incoordination) in pregnant rabbits exposed to a formulation (GF-871) containing aminopyralid TIPA.

Study design: NZW time-mated female rabbits (26/group) were administrated by gavage GF-871(OECD 414/GLP).

Results: Transient incoordination at 150 mg aminopyralid TIPA/kg bw/day was seen on one day only in three rabbits. It presented as a slight stumbling behaviour when the rabbits tried to walk or turn. The rabbits appeared normal when sitting upright or lying recumbent. The incoordination was similar to that reported previously for aminopyralid TIPA (Carney and Tornesi 2004a) and aminopyralid (Marty et al 2002).

Incoordination (involving a weakness of the right forelimb) and death (probably a consequence of misdosing) in one rabbit at 50 mg aminopyralid TIPA/kg bw/day was seen. This animal was found to have a lacerated oesophagus (with necrotising muscular inflammation of neck, muscles of the ventral spinal cord and particularly the right axillae and thoracic areas). This rabbit also showed decreased activity, bright yellow urine, absent faeces and pale mucous membranes. Raised body temperature was noted. Additionally, repetitive chewing at 150 mg aminopyralid TIPA/kg bw/day was seen in two animals at the same time that incoordination was observed. No lesions in stomach or caecum were seen.

Table 5.26: The following dose levels were administered:

	Dose levels (mg/kg bw/day)		
GF-871	0	121	363
Aminopyralid TIPA (target doses)	**0**	**50**	**150**
Aminopyralid acid equivalents	0	26	77

Table 5.27: Notable clinical observations in pregnant rabbits dosed by gavage with aminopyralid TIPA (number of individuals showing effect)

Clinical sign	**Dose of aminopyralid TIPA** (and as aminopyralid acid equivalents) mg/kg bw/day		
	0	**50** (26)	**150** (77)
Incoordination	**0/26**	**1/26 = 4%***	**3/26 = 12%**
Repetitive chewing behaviour	0/26	0/26	2/26 = 8%
Unscheduled death	0/26	1/26	0/26

NOAEL (maternal) 50 mg aminopyralid TIPA/kg bw/day (26 mg aminopyralid/kg bw/day) based on transient incoordination and repetitive chewing in a few 150 mg aminopyralid TIPA/kg bw/day (77 mg aminopyralid/kg bw/day) pregnant rabbits (Carney & Tornesi, 2004).

Study 4: Behavioural study in pregnant NZW rabbits exposed to GF-871.

The purpose of the study was to make a detailed evaluation of clinical signs (incoordination) in pregnant New Zealand White rabbits exposed to GF-871, which is an aqueous formulation consisting of 41.3 % aminopyralid TIPA. These signs of incoordination had been observed in previous studies with GF-871 (Carney and Tornesi 2004).

Study design: A test group of pregnant rabbits (5 does) and a control group (2 does) were used to evaluate NZW rabbit's behaviour. There is no appropriate guideline for such a study or GLP. The study is small and poorly reported. Unfortunately it is not clearly stated if the dose levels quoted (500 and 1000 mg/kg bw/day) in the study report refer to GF-871 or to aminopyralid TIPA.

Five time-mated NZW rabbits received GF-871 via gavage according to the following dosing regimen:
500 mg/kg bw/day from GD 7-15, followed by
1000 mg/kg bw/day from GD 16-23, with
two doses of 1000 mg/kg bw/day given 1 hr apart on GD 23.

Results: No effects were seen in the control group. No clinical signs were noted in the exposed animals during the first phase of dosing with 500 mg/kg bw/day. However, after increasing the dose to 1000 mg/kg bw/day on GD 16, incoordination was noted at least once in all five rabbits, with these signs first appearing as early as GD 18 and as late as GD 23. At the expanded clinical observations on GD 23, all dosed rabbits exhibited abnormal behaviour characterized by incoordination during voluntary movement. Both hind limbs and forelimbs were affected. The following were also noted on GD 23:

- No consistent changes in muscle strength as evaluated by observing external ear position, head position and extensor thrust reflexes.
- No autonomic or somatic signs of convulsive activity, such as urination, defaecation, or paroxysmal movements (recurring intensification of movements).
- No signs of myoclonus (shock-like contractions of muscle) or tremors. No apparent changes in level of consciousness as evaluated by observations of the rabbit's attention to external stimuli in the environment.

- No apparent change in vestibular function (control of balance) as evaluated by observing head position and spontaneous nystagmus (ie there was no involuntary, rapid, rhythmic movement of eye ball).
- Optokinetic nystagmus (eye movement in response to object moving across field of vision) was normal.
- Additionally, the most severely affected rabbits had slowed righting reaction.

In conclusion, the primary neurological change was incoordination of all four limbs. This study can not be used to determine a reliable NOAEL for effects in maternal behaviour due to the limitations in reporting and design of the study. Although, incoordination was noted at 1000 mg aminopyralid TIPA/kg bw/day (512 mg aminopyralid/kg bw/day), with no effects noted at 500 mg aminopyralid TIPA/kg bw/day (256 mg aminopyralid/ kg bw/day).

Study 5: Oral gavage neuropathology study in time-mated NZW rabbits exposed to GF-871

This study is based only on the Draft assessment report (August 2006) relating to lack of submitted dossiers from the applicant.

Study design: Groups of 10 time-mated pregnant female New Zealand White rabbits (aged 5-6 months) were administered GF-871 by gavage at targeted dose levels of 0 or 800 mg aminopyralid TIPA (410 mg aminopyralid) /kg bw/day on gestation day (GD) 7 through 27 to evaluate the potential for neuropathologic changes. The test material was dosed as a solution. The concentration of test dose was 200 mg aminopyralid TIPA/ml, which is equivalent to c. 104 mg aminopyralid/ml. For more detailed information of the study design see Annex T1 page 219-222 (OECD 414 and 424/GLP).

Results: Infrequent and transient incoordination was seen in 3 of 10 rabbits exposed to aminopyralid TIPA. Consistent with prior reports, the incoordination presented itself as a slight stumbling behaviour when the rabbits were trying to walk or turn, and affected both fore-and hind limbs. For clinical examination, incoordination during voluntary movement was graded as followed in Annex T1 page 220.

Slight transient incoordination was noted in one rabbit on the first day of dosing (GD7) and in another rabbit on GDs 9 and 27; in these animals both the onset and resolution of incoordination occurred within 1-2 h after dosing.
The third rabbit to showed moderate incoordination on GD14 that progressed to severe incoordination. This rabbit died a few hours later following an inadvertent fall from its cage during the clinical examination.

Absent or decreased faeces were occasionally noted in three rabbits (first observed on GD9), which could be correlated with reduced food consumption and body weight.
Body weight loss occurred from GD 7-10. Body weight gain was then slightly reduced up to day 16. From day 16 to termination, the test group gained slightly more weight than controls.
Feed consumption was decreased over the first 2 weeks of the exposure period (including a 28% decrease on days 7-8). The last week food consumption was comparable to controls.

There were no histopathologic observations in the central or peripheral nervous systems of rabbits given aminopyralid TIPA. Lesions of the nervous system were low in incidence and severity and comparable to controls. There was no particular association between neuropathological findings and the three animals that showed incoordination.
The cause of death for the rabbit that fell from its cage was not determined, but the study authors considered that it was likely secondary to the gross pathologic observations of hydrothorax and pulmonary congestion (there was blood-tinged fluid in the thoracic cavity). Histopathological examination of this rabbit showed multiple skeletal muscles (including anterior tibial and gastrocnemius muscles) with acute degeneration of muscle fibres. Similar findings were not observed in any of the other rabbits given aminopyralid TIPA or control rabbits. The study authors attributed this finding most likely either to ischemia or to aminopyralid. The study authors also report that skeletal muscle lesions were not observed in previous studies (species not defined) with aminopyralid or aminopyralid TIPA.

In conclusion, repeated oral gavage administration of 800 mg aminopyralid TIPA/kg bw/day (equivalent to 410 mg aminopyralid/kg bw/day) to pregnant rabbits from GD 7-27 caused incoordination in a few rabbits, as well as other evidence of toxicity, i.e. reduced food consumption, initial loss of body weight and reduced faeces. There was no evidence of neuropathological lesions. However, the rabbit with severe incoordination also showed degeneration of skeletal muscle fibres (Yano and Zablotny, 2005).

Study 6: Metabolism and pharmacokinetics of 14C-DE-750 in non-pregnant and pregnant NZW rabbits following single and repeated oral administration (Hansen, Mendrala, Markham and Saghir, 2005)

This study is described under the toxicokinetics section earlier in this assessment (5.1.1.)

Summary (special studies):

Transient incoordination is a consistent finding in rabbit studies with aminopyralid and aminopyralid TIPA. This finding has mostly been investigated in pregnant rabbits.
However, it is notable that in the acute oral study of rats given a high dose of aminopyralid (5000 mg/kg bw administered as two fractional doses approx 1h apart) incoordinated gait was seen only in the rat that died (Annex T1 section B.6.2.1). Decreased muscle tone was also seen in this rat, and in two other rats. This suggests that high doses of aminopyralid might have effects on coordination in rats similar to that seen in rabbits.

There was no evidence for incoordination occurring after a single dose of up to about 500 mg aminopyralid/kg bw/day, the highest dose relevant to setting an acute reference dose (ARfD), i.e. there was no strong evidence supporting the setting of an ARfD. At doses up to 500 mg aminopyralid/kg bw/day, the earliest incoordination was seen was on the 6th day of dosing (GD 12). However, as incoordination is a transient effect and it would appear that observation of the animals was periodic (not continuous) it is possible that some instances of incoordination may have been missed. There is evidence for incoordination in a non-pregnant rabbit after 1 or 2 doses of 1000 mg aminopyralid/kg bw.

Clinical observation of the behaviour of pregnant rabbits treated with high doses of aminopyralid TIPA revealed the primary abnormal finding to be incoordination of all 4 limbs. There were no other significant neuro-muscular findings (including evidence of muscular weakness or CNS depression).

The findings of the neuropathy study in pregnant rabbits indicate that the clinical observations of incoordinated gait in pregnant rabbits caused by exposure to aminopyralid are not the result of a neuropathological lesion (CNS and PNS were examined, including hind limb nerves). However, degeneration of skeletal muscle fibres was seen in one pregnant rabbit with severe incoordination.

The ADME study showed that there were differences in toxicokinetics between late stage pregnant rabbits exposed repeatedly to aminopyralid from GD 7 and non-pregnant and early-stage pregnant rabbits following a single acute exposure to aminopyralid. The evidence suggests that the bioavailability of aminopyralid would be greater in late-stage pregnant rabbits than non-pregnant and early-stage pregnant rabbits.

Less binding of aminopyralid to plasma proteins in pregnant rabbits (particularly those at a late stage in pregnancy) than in non-pregnant rabbits or non-pregnant rats, would have resulted in higher levels of free aminopyralid in plasma of pregnant rabbits and hence would have made them more susceptible to the systemic effects of aminopyralid.

Incoordination following exposure to aminopyralid were only been observed in rabbits (following gavage dosing). It has not been seen in rats, mice or dogs following dietary exposure to up to 1,000 mg aminopyralid/kg bw/day, or in rats following gavage dosing (apart from one rat exposed to 5,000 mg/kg bw by gavage in an acute toxicity study; this animal subsequently died).

The cause of the incoordination in rabbits is not known. Speculations and explanations are discussed by the RMS in the Annex T1 (see page 229-232). Though, in the absence of clear evidence as to the mode of action for the occurrence of incoordination in rabbits it is not possible to comment on the

relevance of this hazard for humans. The precautionary approach of assuming that this effect is relevant for human hazard assessment is therefore appropriate.

The incoordination in pregnant rabbits is a critical effect, and should be considered as a severe functional disturbance (the criteria for classification as **Xn; R48/22 danger of serious damage to health by prolonged exposure if swallowed** are fulfilled).

5.1.11 Human data

No human data was available.

5.1.12 Classification and labelling

Aminopyralid is classified as **Xi; irritant, R41 risk of serious damage to eyes and Xn; harmful to health, R48/22 danger of serious damage to health by prolonged exposure if swallowed.** Aminopyralid is not included in Annex I.

There is an ongoing evaluation of aminopyralid in the EU.

5.1.13 Reference values

ADI

An ADI of 0.26 mg/kg bw/day is proposed for aminopyralid based on applying a 100-fold assessment factor to NOAEL of 26 mg aminopyralid/kg bw/day determined in the clinical-effects study with pregnant rabbits (Carney and Tornesi 2004b). The uncertainty factor accounts for interspecies extrapolation (10X) and intraspecies variability (10X).

The RMS: 0.26 mg/kg bw/day based on the statement above.
There is an ongoing evaluation of aminopyralid in the EU, and no reference values have been proposed at the current time.
The Applicant: 0.932 mg/kg bw/day based on a NOAEL of 93 mg/kg bw/day the 1-year dog study (even though the Applicant considered that 50 mg/kg bw/day was a NOAEL in the rat carcinogenicity study).
The JMPR: 0-0.9 mg/kg bw/day based on a NOAEL of 93.2 mg/kg bw/day identified on the basis of histological changes in the gastric mucosa at higher doses in a 1-year study in dogs, and a safety factor of 100.
The USEPA: 0.5 mg/kg bw/day, based on the NOAEL of 50 mg/kg bw/day in the rat combined toxicity/carcinogenicity study with a 100-fold uncertainty factor.

AOEL

An AOEL of 0.26 mg/kg bw/day is proposed for aminopyralid based on applying a 100-fold assessment factor to the NOAEL (for incoordination in pregnant rabbits) of 26 mg aminopyralid acid equivalents/kg bw determined in the study of Carney and Tornesi (2004b).

The RMS: 0.26 mg/kg bw/day based on the statement above.
The Applicant: 1.16 mg/kg bw/day based on applying a 100-fold assessment factor (together with an additional correction factor of 0.5 for oral absorption based on the rat ADME study) to the NOAEL of 232 mg/kg bw/day determined in the 90-day dog study.

ARfD

An ARfD of 0.26 mg/kg bw is proposed based on applying a 100-fold assessment factor to the NOAEL of 26 mg aminopyralid acid equivalents/kg bw determined in the rabbit study of Carney and Tornesi (2004b), i.e. the same study used for setting the ADI.

The Applicant states that an ARfD is not applicable for aminopyralid.
The RMS: of 0.26 mg/kg bw/day, based on applying a 100-fold assessment factor to the NOAEL of 26 mg aminopyralid acid equivalents/kg bw determined in the rabbit study of Carney and Tornesi (2004b).
The USEPA: an acute Reference Dose (RD) for the general population is not required, based on aminopyralid's low toxicity.
The JMPR concluded that it was not necessary to establish an ARfD for aminopyralid (see Annex T2).

5.2 Metabolites

No toxicity studies of metabolites have been conducted and none is considered necessary because the available data indicate that aminopyralid is not metabolised in rats (or rabbits).

5.3 Co-formulants

The product does contain a co-formulant above the limit; 30% aromatic hydrocarbon solvent, and hence meet the criteria for R66 Repeated exposure may cause skin dryness or cracking (classification not needed, covered by R38) and **R67 vapours may cause drowsiness and dizziness.**

The VKM Working group 2 will receive the entire composition and the evaluation of the properties of each co-formulant.

5.4 Product - Simplex

5.4.1 Acute toxicity

Oral, rat

Acute oral LD50 in female Fischer 344 rats was found to be >5000 mg/kg bw in an up/down study design by gavage (GLP/QA/OECD 425) (Smedley, 2003).

Dermal, rat

Acute dermal LD50 in Fischer 344 rats was found to be >5000 mg/kg bw (the top-dose) for males and females (GLP/QA/OECD 402). Dermal irritation consisting of erythema, edema and desquamation was noted at the test site; signs of irritation persisted up to day 12 (Smedley, 2003).

Inhalation

No inhalation studies have been conducted and no study is needed on Simplex (Annex T1, B.6.11.3).

Summary (acute toxicity):

Simplex was not harmful by swallowing, skin contact or by inhalation. Hence, no classification for acute oral, dermal and inhalation toxicity is required.

5.4.2 Irritation and sensitisation

Dermal, rabbit

Three New Zealand White rabbits were used in the study. Mean dermal irritation index (24, 48 and 72 hours) was 1.33 for erythema and 0.67 for oedema (Table 5.28).
Slight to well-defined erythema on 3/3 test sites and very slight to slight oedema on 2/3 test sites was observed at the 1-hour scoring interval. Additional dermal findings included desquamation (3/3 test sites) and superficial lightening (1/3 test sites).
Erythema and oedema had resolved completely on 1/3 test sites by the 72-hour scoring interval (when observation of this animal terminated) on 2/3 test sites by day 7 (when observation of these two animals terminated). However, at termination of the observations desquamation was still present in all animals. The criteria for classification as **Xi; R38 Irritating to skin** is fulfilled (GLP/QA/OECD 404) (Smedley, 2003).

Table 5.28: Mean of scores for skin irritation at 24, 48 and 72 h:

Animal	Erythema	Oedema
1	2	2
2	1.33	0
3	0.67	0
EC trigger values	*≥ 2 (in 2 or more animals)*	*≥ 2 (in 2 or more animals)*

Eye, rabbit

Three New Zealand White rabbits were used in the study. Ocular irritation was evaluated by the method of Draize *et al.* (1944). One hour after test substance instillation, all three treated eyes exhibited conjunctivitis. By 72 hours, all animals also developed corneal opacity and iritis. Mean 24-72h irritation scores are summarised in Table 5.29. Iritis persisted only up to days 4-10. However, corneal opacity (grade1) and conjunctivitis (grade 1 redness) was still present at day 21 (study termination) in 2 rabbits. These two rabbits also showed pannus of the cornea from days 10 or 14 to day 21. Simplex is classified as severely irritating to the eye (**Xi; R41**) based on persistence of effects through to termination on day 21 (GLP/QA/OECD 405) (Moore, 2004).

Table 5.29: Mean values for ocular lesions 24, 48 and 72 h after instillation

Animals	Corneal opacity	Iridial lesions	Conjunctival	
			Redness	Chemosis
1	1.0	0.33	2.0	1.33
2	1.0	0.33	2.0	1.0
3	1.0	**1.0**	2.0	1.33
EC trigger values*: (R36)	≥2.0, <3	≥1.0, <2.0	≥2.5	≥2.0
EC trigger values*: (R41)	≥3	= 2	na	na

**Classification triggered if any EC value is attained by two or more animals.*
R41 also triggered by ocular lesions present at the end of the test in any animal
na not applicable

Skin sensitisation, guinea pig

Simplex has not been found to induce a dermal sensitisation response using the Maximization Design in Hartely-derived albino guinea pigs. Guinea pigs (10/sex) were treated with 25% ww Simplex in deionized water. Simplex caused a positive skin response (grade1 erythema) in a maximum of 5/20 test animals (25%). The compound is not classified as a contact sensitiser in guinea pigs because it does not fulfil the EU criteria which require a positive response of at least 30% (GLP/QA/OECD 406) (Smedley, 2003).

Summary (irritation and allergy):

Simplex is characterized as extremely irritating to the eye based on the irritation persisting in the eyes of two of the three rabbits (The criteria for classification as **Xi; R41 Risk of serious damage to eyes** are fulfilled). Simplex is also found irritating to the skin (the product should be classified with **Xi; R38**) based on the persistence of desquamation in all 3 animals, whereas it was not found to be a dermal sensitiser.

Study findings are summarised in the table below.

Table 5.30: Summary of acute toxicity, irritation and skin sensitisation studies with Simplex:

Test	Species	Result	Classification (99/45/EC)	Reference
Acute oral	Rat	LD50> 5000 mg/kg bw	None	Smedley 2003a
Acute dermal	Rat	LD50> 5000 mg/kg bw	None	Smedley 2003b
Acute inhalation	Rat	No study conducted, no study needed	-	-
Skin irritation	Rabbit	Persistent desquamation to end of study (day 3 or 7) in all 3 rabbits	**R38**	Smedley 2003c
Eye irritation	Rabbit	Persistent corneal opacity and conjunctivitis to end of study (day 21) in 2 out of 3 rabbits	**R41**	Moore 2004
Skin sensitisation	Guinea	In M and K assay, skin responses in test animals not sufficient to classify product as a skin sensitiser	None	Smedley 2003d

5.4.3 Classification and labelling

The following classification and labelling for human health effects has been proposed for Simplex; **Xi; irritant, R41 risk of serious damage to eyes, R38 irritating to skin, and R67 vapours may cause drowsiness and dizziness.** These proposals take into account the classification of the product, the non-active substances present in the product and the two active substances which include aminopyralid and fluroxypyr.

5.4.4 Dermal absorption

No studies on dermal absorption have been conducted for aminopyralid or fluroxypyr. As default value of 100% would normally be applied in the absence of dermal absorption data, until a dermal absorption study is provided to support a lesser value. However, the dermal absorption is not expected to be higher than the oral absorption. The higher oral absorption in the rabbit (~80%) compared to the rat (50-60%) should not be disregarded. As a surrogate for a real dermal absorption value the dermal absorption is set to 80% for both the concentration and the diluted solution.

Summary (dermal absorption):

In the exposure calculations and the risk assessment the dermal absorption was set to 80% (both the concentration and the diluted solution) as a surrogate for a measured dermal absorption value.

5.5 Exposure data

Simplex (the product GF-839) is a dilutable concentrate formulation of water in oil emulsion (a modified EO) containing 100 g /l fluroxypyr (present as 148 g/l fluroxypyr-methyl heptyl ester) and 30 g/l aminopyralid (present as 37 g/l present as the potassium salt) as the active substances. The product is to be packaged in 5 litre PET bottles, which have a 63 mm closure. Applications of Simplex will be achieved via tractor-mounted (boom) sprayers and knapsack sprayers. Water will be the diluent/carrier. The toxicological data supplied proposed that Simplex should be classified as Xi, Irritant with the associated risk phrase's irritating to skin (**R38**) and a risk of serious damage to eyes (**R41**). With respect to the hazard classification shown above, a faceshield and gloves are necessary PPE to be worn during mixing and loading operations.

Operator exposure

Exposure to aminopyralid applied to grassland using tractor-mounted/trailed boom sprayers (hydraulic nozzles) and hand-held sprayers has been considered by this evaluation.

NAD is 200 ml/daa for Simplex.

Growers using Simplex as proposed are likely to apply the product on no more than a few days each year, whereas contract operators might use the product for a few consecutive weeks. In all situations, operators will only experience short-term exposure (i.e. up to 90 days/year) to aminopyralid/fluroxypyr. Therefore, considering the proposed use pattern of the product Simplex, it is appropriate to compare predicted levels of operator exposure to aminopyralid with an AOEL derived from toxicity studies for the effects of exposure up to 90 days. An AOEL of 0.26 mg/kg bw/day is proposed based on applying a 100-fold assessment factor to the NOAEL (for incoordination in pregnant rabbits) of 26 mg aminopyralid acid equivalents/kg bw determined in the study of Carney and Tornesi (2004b).
No correction for oral absorption is necessary when calculating this AOEL. This is because aminopyralid is extensively absorbed following gavage dosing of pregnant rabbits (Annex 1, Section B.6.8.2.b). When pregnant rabbits were dosed singly with 362 mg/kg bw or repeatedly with 279 mg/kg bw/day (ie even at dose levels higher than the NOAEL of 26 mg/kg bw/day), absorption was 83-86% based on the extent of urinary excretion (Annex 1, Section B.6.10).

A dermal absorption value for aminopyralid of 80% for the concentrate and in-use dilution is considered appropriate based on a default value for dermal absorption adjusted to take into account the extent of oral absorption (Annex 1, Section B.6.12.3).

Table 5.31: UK POEM Model exposure estimates for Simplex

PPE	* Total exposure (mg/kg bw/day)	Exposure as a % of AOEL
Grassland - Tractor-mounted / trailed boom sprayer (hydraulic nozzles)		
None	1.13	434.6
Gloves	0.12	46.15
Grassland – Knapsack sprayer; Hand-held sprayer (15 l tank): hydraulic nozzles		
None	0.57	219.23
Gloves	0.21	80.77

PPE: Personal protective equipment.
Please note, the UK POEM estimate have used the organic solvent-based data for UK POEM as this formulation type (a modified EO) is not common. The estimation is based on an AOEL of 0.26 mg/kg bw/day for aminopyralid.
** Assumes a 60 kg operator, 80% absorption via the dermal route for the concentrate and in-use concentration for aminopyralid. Duration of spraying is set to 6 hours, with an application volume of 200 l/ha. Work rate/day (tractor-mounted/trailed boom sprayer): 30 ha. Work rate/day (knapsack-sprayer): 0.1 ha.*

Estimation preformed with UK Poem do not exceed the suggested AOEL when gloves are been used. The exposure estimations revealed that is was minimal exposure danger regarding worker, thereby

minimal exposure danger for bystanders, although a precautionary establishment of the dermal absorption.

Bystanders and Workers exposure

For bystanders or for workers re-entering treated crops, predicted exposure to aminopyralid/aminopyralid potassium is less than 10% of the active substance's AOEL. Therefore, the risk to bystanders and workers from exposure to aminopyralid/aminopyralid potassium is acceptable.

Summary of exposure:

UK Poem model estimate of exposure suggest levels of exposure will be within acceptable levels for operators without PPE for application using a boom sprayer. For application with knapsack sprayers the UK POEM estimate of exposure require gloves to be worn when handling the undiluted product during mixing and loading and during application of the diluted spray solution. However, as a result of the hazard classification, a face shield and gloves are necessary PPE to be worn during mixing and loading operations, due to the risk of causing serious damage to eyes (**R41**) and since the product is irritating to skin (**R38**).

6. Residues in food or feed

Residues in food and feed are not discussed in this report.

7. Fate and Behaviour in the Environment

The assessment is based on the EU Draft Assessment Report (DAR, E1) on aminopyralid from August 2006 (RMS UK). The DAR has not yet been subject to peer review at EFSA. This assessment only addresses the substance aminopyralid. The substance fluroxypyr was reassessed by the Norwegian Food Safety Authority in 2009 but last assessed by The Scientific Committee in Norway in 2000.

Application rate: 6 g a.s./decare (60 g a.s./ha). Crop: Grass, pasture. Number of applications pr season: 1. Time of application: May/June or July/August.

7.1 Aminopyralid

7.1.1 Degradation in soil

Degradation pathway
The route and rate of aerobic degradation of aminopyralid has been determined in four soils under laboratory conditions. Aminopyralid was steadily degraded in soil under aerobic conditions. The only metabolite observed was CO_2 indicating that the phenyl ring of aminopyralid is mineralised. No other degradation products were detected. Little or no mineralisation was observed under sterile conditions, demonstrating that the degradation was microbial. After 92 days at 20°C, the CO_2 accounted for 24-69 % AR, whilst the unextracted radioactivity accounted for 10-22 % AR. Aminopyralid was essentially stable under anaerobic conditions.

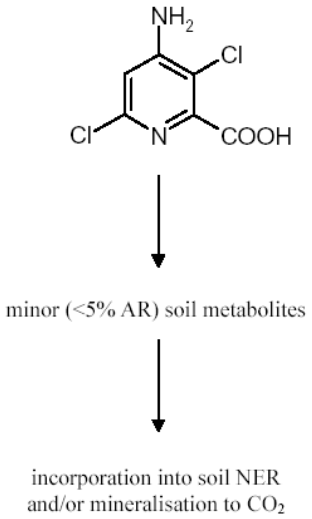


Fig. 7.1: Proposed degradation pathway of aminopyralid in soil under aerobic conditions.

Aerobic degradation
The aerobic degradation rate is medium, DT50: 26-147 days (geometric mean 56 days). DT90: 88-488 days. The degradation rate depends on temperature. Bound residue and mineralization accounted for 10-23 and 24-69 % of AR (Applied Radioactivity) respectively. No other degradation products than CO_2 were observed. The choice of degradation kinetics seems to be describing the degradation satisfactory. A couple of the DT90 values are extrapolated beyond the duration of the study and must be regarded as rather uncertain. Details of the degradation studies are summarized in Table 7.1. Normalised DT50 values did not differ very much from the originally calculated values.

At 10 °C the degradation rate is slow. DT50: 402 days. DT90: 1335 days. Bound residue and mineralization accounted for 6 and 9 % of AR, respectively. No other degradation products than CO_2 were observed. The choice of degradation kinetics seems to be describing the degradation satisfactory even though DT50 and DT90 values are extrapolated well beyond the duration of the study. These values must be regarded as rather uncertain, even though they give an indication that aminopyralid might be rather persistent at lower temperatures.

Anaerobic degradation

Aminopyralid was very stable under anaerobic conditions and no degradation rate was estimated. Metabolites were observed, but they were minor (< 5 %) and not identified.

Table 7.1: Detailed overview of the anaerobic/aerobic/sterile degradation studies on aminopyralid.

	Cuckney, sand	North Dakota, sandy loam sediment	Thessaloniki, clay loam	Cuckney, sand	Charentilly, clay loam	Parabraum Erde, sandy silt loam		
Substance	Aminopyralid							
Aerobic/ anaerobic	Anaerobic		Aerobic					Sterile
Temperature (°C)	20	25	20				10	20
Study Duration, days	120	636	123					
Sand (%)	89	57	41	91	42	26		
Silt (%)	8	36	36	6	32	60		
Clay (%)	3	7	23	3	26	14		
pH	6.0	8.1	7.7	5.6	5.8	7.7		
Organic Carbon (%)	1.3	4.9	1.5	1.5	1.0	1.0		
MWHC (%)	-	-	86.9	43.7	68.4	56.0		
% MWHC	-	-	40	40	40	40		
Microbial biomass (µg C/g soil)	Start: 111 End: 40	Start: 43 End:54	Start: 216 End: 94	Start: 111 End: 40	Start: 55 End: 51	Start: 41 End: 72		
DT50, days	Stable	Stable	26*	147*	29*	86*	402*	Stable
DT90, days	-	-	88*	488*	96*	286*	1335*	-
CO2 (%). Maximum within 100 days	0.3 (59 d.) 0.4 (120 d.)	0.6 (90 d.)	68 (92 d.)	24 (92 d.)	69 (92 d.)	35 (92 d.)	9 (92 d.)	2 (92 d.)
Bound residue (%).Maximum within 100 days	0.6 (59 d.) 0.7 (120 d.)	1.9 (90 d.)	23 (61 d.)	10 (92 d.)	21 (60 d.)	14 (92 d.)	6 (92 d.)	2 (92 d.)
Metabolites > 5 %. Maximum within 100 days	None							
References	Rutherford and Meitl, 2004		Yoder and Smith, 2003a					

* Single First Order (SFO)

Photolysis in soil

Photolysis can be regarded as an important route of degradation for aminopyralid as the substance degrades much more rapid when irradiated (12 hour light/12 hour dark) than under dark conditions. It is uncertain though of the contribution of photolysis to the overall degradation of aminopyralid in soil.Photolytic DT50 and DT90 of aminopyralid were calculated to be 61 days in the test system (40 days with summer sunlight at 40 °N) and 203 days (132 days with summer sunlight at 40 °N) respectively. After 44 days 69 % of AR still remained as aminopyralid in the irradiated samples. An unknown component reached 4.6 % AR after 44 days but this was not identified as it was < 5 % AR. CO_2 accounted for approximately 3 % AR after 44 days in the irradiated samples (E1).

Field Dissipation
The dissipation of aminopyralid was found to be medium to high with DT50: 4-22 days, geometric mean 12 days, and DT90: 13-72 days. Information on the microbial activity in the soils was not mentioned in the study report. Climate data are missing and the relevance to Norway is difficult to assess.

Table 7.2: Summary of field dissipation studies performed with the active substance aminopyralid.

Field site	Melbourne, Derbyshire, UK	Dollern, Germany	Chalons le Verger, Northern France	Sorgues, Southern France
Substance and application rate (kg as/ha)	Aminopyralid, 0.060	Aminopyralid, 0.0567	Aminopyralid, 0.0612	Aminopyralid, 0.0618
Application date	8th May	26th April	28th May	26th April
Duration (days)	119	158	127	125
soil type	clay loam	sandy loam	sandy loam	clay
Sand (%)	44	71	76	18
Silt (%)	32	19	15	35
Clay (%)	24	10	9	47
pH	6.6	6.2	7.5	8.0
Org. C (%)	1.5	3.6	0.9	3.0
Average soil temp. (°C) during study period	19.1	13.2	16.0	19.9
DT50, days	35/22*	32/19*	26/14*	8/4*
DT90, days	116/72*	105/64*	87/46*	26/13*
References	Unsworth et al., 2003, Havens, 2004, Anon, 2004 a and b			

* Normalised values, SFO

Table 7.3: Soil temperature from Ås and Værnes. Based on data from 1995 at 10 cm depth.

		April	Mai	Juni	Juli	August	September	Oktober
Ås	Temp. (°C)	3.1	8.7	15.0	16.9	17.5	12.4	9.7
Værnes	Temp. (°C)	1.6	7.2	12.5	13.9	14.4	11.5	7.8

Soil accumulation
Not relevant as $DT90_{field}$ < 1 year.

7.1.2 Sorption and mobility in soil

Sorption
The sorption of aminopyralid to soil can be classified as low with Kf: 0.01-0.73 (arithmetic mean 0.17) and Koc: 0.31-21.7 (arithmetic mean 8.3). The Freundlich exponent (1/n) ranged from 0.32 to 1.52 (arithmetic mean 0.85) indicating some non-proportional dependence of adsorption on the concentration. There is evidence that the sorption may be stronger at low pH (acidic soils). The $Koc_{desorption}$ was generally higher than the $Koc_{adsorption}$ indicating that the sorption was not completely reversible.

Table 7.4: Sorption of aminopyralid in soil.

	Dowling Clay, Mississippi	Norfolk Loamy Sand, North Carolina	Barnes Clay loam, North Dakota	Ryerson, Canada	Thessaloniki, Hellas	Cuckney, UK	Charentilly, France	Farringdon, UK
Sand (%)	8	86	34	17	37	90	27	25
Silt (%)	24	10	34	46	46	6	46	29
Clay (%)	68	4	32	37	17	4	27	46
pH	6.9	4.5	4.8	7.8	7.8	6.6	6.1	7.5
Org. C. (%)	1.5	0.6	3.6	3.9	1.0	1.6	1.0	3.2
Kf (ads)	0.05	0.13	0.73	0.26	0.04	0.05	0.07	0.01
Koc (ads)	3.3	21.7	20.3	6.7	4.0	3.13	7.0	0.31
1/n (ads)	1.52	0.85	0.9	0.87	0.81	0.74	0.81	0.32
Kf (des)	-	2.12	2.88	3.09	1.97	1.72	1.24	-
Koc (des)	-	353	80	79	197	107	124	-
References	Rutherford, 2002							

7.1.3 Degradation in water

Hydrolysis

The hydrolysis of aminopyralid has been studied at a concentration of 0.4 mg/l in the dark at both 20°C and 50°C in sterile aqueous buffered solutions at pH5, pH7, and pH9. This showed that aminopyralid was completely stable to hydrolysis under all these conditions (E1).

Photolysis in water

Photolysis may be an important degradation pathway for aminopyralid if one compares half lives from the irradiated samples with half lives from the dark controls. After 8 hours in irradiated samples at pH 5 and 20 °C, the recovery was 43 %, while the recovery was 95 % after 2 days in the dark control indicating that irradiation increased the degradation rate. The samples were irradiated continuously using a xenon lamp. Samples were also prepared to identify the photoproducts. Two major photoproducts were seen in the irradiated samples, oxamic acid and malonamic acid. These were contained in a fraction which reached up to 68.8 % AR in total, but this fraction also contained other (up to 6) multiple short-chain degradates. At 15 days, only oxamic acid and malonamic acid were >10% AR.

The predicted DT50 and DT90 values at various latitudes and seasons were then derived using the quantum yield. Predicted DT50 and DT90 values are summarised in Table 7.5 and Figure 7.2 shows the proposed photolytic degradation pathway (E1).

Table 7.5: Predicted DT50 and DT90 values for the photodegradation of aminopyralid at various latitudes and seasons.

Latitude and season	DT_{50} (days)	DT_{90} (days)
0°N Summer	0.7	2.4
20°N Summer	0.6	1.9
20°N Winter	1.0	3.2
40°N Summer	0.6	2.0
40°N Winter	2.0	6.6
60°N Summer	0.7	2.3
60°N Winter	1.4	4.7

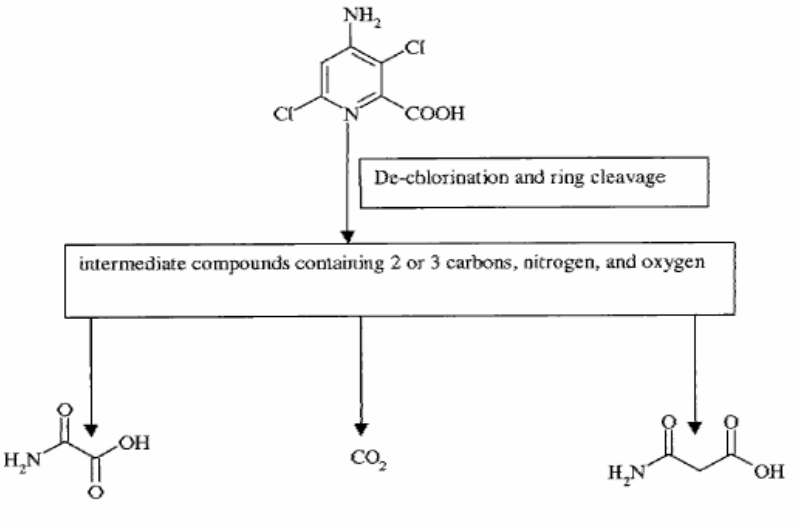


Fig. 7.2: Proposed aqueous photolysis pathway for aminopyralid

Ready biodegradation
Aminopyralid is classified as 'not readily biodegradeable' under the conditions of the test (E1).

Water/sediment
The degradation for the whole system was recalculated by RMS using the biphasic Hockey Stick approach and can be classified as moderate with DT50: 83-104 days when assessing the first phase. (geometric mean 93 days). When assessing the second phase DT50 was 829-1495 days (geometric mean 1113 days). Data from the French system were excluded due to very poor fit. DT90 values were much higher than the duration of the study and were regarded as too uncertain. The Hockey Stick approach was by RMS regarded more appropriate to describe the degradation in this case, thus only taking into account half lives of the two phases and not assessing DT90.

Bound residue constitutes between 3 and 15 % of AR after 101 days. The mineralization was low, reaching between 1 and 3 % of AR after 101 days in the different systems. Only minor unknown metabolites < 5 % observed at any time point. Aminopyralid dissipates only in small amounts to the sediments.

Table 7.6: Degradation/dissipation of aminopyralid in water/sediment. Using first order biphasic hockey stick approach due to very high DT90 values (much higher than study duration). Calculations made by RMS.

	Haut Languedoc, France	**Alto Garda, Italy**	**North Dakota, US**
Aerobic/anaerobic	Aerobic		
Temperature (°C)	20		
Sand (%)	91	39	53
Silt (%)	8	58	40
Clay (%)	1	3	7
pH	6.1	7.9	8.1
Organic C. (%)	0.8	12.0	6.2
DT50 (water)	23/188*	31/491*	36/919*
DT50 (whole system)	1890/802* 810**	83/829*	104/1495*
DT90 (whole system)	2689**	-	-
CO_2 (%) Maximum within 100 days	2.7 (101 d.)	1.7 (62 d.)	1.2 (101 d.)
Bound residue Maximum within 100 days	3.2 (101 d.)	14.8 (101 d.)	12.1 (62 d.)
Metabolites > 5 % Maximum within 100 days	-		
References	Yoder and Smith, 2003b		

* 1st phase DT50/2nd phase DT50. In French system Hockey Stick approach not appropriate due to very poor dataset (r^2 0.451).
** 1st order non linear regression (r^2 0.605)

7.1.4 Fate and behaviour in air

Volatility
The volatility of aminopyralid from the soil surface and dwarf runner bean has been studied. The volatilization rate of formulated 14C-phenyl-aminopyralid and its atmospheric half-life due to photochemical oxidation were calculated. The Atmospheric Oxidation Program for Microsoft Windows (AOPWIN, version 1.90; US EPA) was used to carry out the calculation according to the methods of Atkinson. No significant volatilisation (0.2 % AR) of 14C-phenyl-aminopyralid was observed from plant surfaces, although a small loss (2.6 % AR) was seen from soil after an exposure time of 24 hours in the wind tunnel. The estimated atmospheric half-life was 6.4 days, equivalent to 77 hours, assuming an OH-radical concentration of 1.5 x 106 molecules/cm^3, an overall OH rate constant of 1.7 x 10^{-12} cm^3/molecule-sec, and a 12-hour day.

7.2 Exposure assessment

7.2.1 Soil

The initial aminopyralid PECs following application was calculated from the proposed use of Simplex in pasture grass according to the GAP, i.e. one annual application at 60 g a.s./ha. It was assumed that aminopyralid was evenly distributed in the top 5 cm soil horizon with a soil bulk density of 1.5 g/ml. A worst-case 50 % crop interception was used to represent foliar application, together with a refinement using 90 % crop intercept to represent a more realistic interception provided by established grassland as outlined in the FOCUS groundwater guidance. Aminopyralid concentrations were calculated assuming a worst case DT50 (lab, 20 °C) of 147 days.

PIEC (predicted initial environmental concentration) in soil after the application of 60 g a.s./ha is 0.04 mg a.s./kg with 50 % interception and 0.008 mg a.s./kg with 90 % interception.

7.2.2 Groundwater

The leaching behaviour of aminopyralid was estimated using all the nine FOCUS groundwater scenarios and the two recommended models, i.e. FOCUS-PELMO (version 3.3.2) and FOCUSPEARL (version 2.2.2). The simulations were performed for every month and maximum predicted values are presented in Table 7.8 along with the application dates simulated by the Rapporteur. Where concentrations were predicted to exceed 0.1 µg/l, results are also presented for the months preceding and following the peak month. The use considered was Simplex in pasture grass according to the representative GAP, i.e. a single application of 60 g as/ha. The cropping scenario chosen for these simulations was "grass/alfalfa", which is a cropping pattern available for all of the FOCUS gw scenarios. Each use was investigated as consecutive annual applications for a period of 20 years, with irrigation included in all cases. For the grass/alfalfa scenario with established turf, the recommended interception is 90 %, thus the application rate used for all scenarios was 60 g as/ha x 10 %, or 6 g as/ha.The input values are outlined in Table 7.7. These modelling assessments indicate that contamination of groundwater > 0.1 µg/l is likely to occur. This was seen in 6 of the 9 scenarios, even though worst case input values and assumptions were not used. Both the use of geo mean DT50 from field studies (12.1 days) and the use of 90 % interception can be questioned.

The leaching behaviour of aminopyralid was also estimated using the Norwegian (Heia) and Swedish (Önnestad and Krusenberg) groundwater scenarios with the FOCUS crop grass/alfalfa, using the model MACRO (4.4.2). The same input values for the active substance were used, except that more realistic application dates were used and the simulations were run with geometric mean half lives from laboratory (56 days) studies. In addition the simulations were run with both 90 and 50 % interception with full dose and with 90 % interception and half dose. Only 50 % plant cover at application date may be realistic under Norwegian conditions in June. The results of these simulations are summarised in Table 7.9. All simulations show that aminopyralid will enter groundwater at amounts well above the threshold value of 0.1 µg/l, included when only half the dose is applied and an interception of 90 % is used.

Table 7.7: Input-parameters for FOCUS groundwater modelling (PECgw).

Input parameter	Unit	aminopyralid
Physical-chemical parameters		
Molecular mass	$g.mol^{-1}$	207
Vapour pressure	Pa	$2.6x10^{-8}$
Water solubility	$mg.l^{-1}$	2480
Plant uptake factor		0.5
Degradation parameters		
Half-life soil, field values	days	12.1 (geometric mean)
Normalised for temperature	°C	20
Q_{10} factor		2.2
Exponent for moisture content relative to field capacity	%	100
Sorption parameters		
Kfoc	$ml.g^{-1}$	4.07
Kfoc (Porto scenario)	$ml.g^{-1}$	20.3
Freundlich exponent		0.85
Freundlich exponent (Porto scenario)		0.9

Table 7.8: 80th Percentile annual average leachate concentrations at 1 m depth (µg/l) derived

from FOCUS-PELMO and FOCUS-PEARL.

Scenario	Application date	PEC_{GW}	
		FOCUS-PELMO	FOCUS-PEARL
Châteaudun	15-Oct	0.019	0.044
Hamburg	15-July	-	0.057
Hamburg	15-Aug	0.045	0.106
Hamburg	15-Sept	0.108	0.172
Hamburg	15-Oct	0.151	0.246
Hamburg	15-Nov	0.235	0.227
Hamburg	15-Dec	0.142	0.123
Hamburg	15-Jan	0.022	0.030
Jokioinen	15 July	0.043	0.040
Jokioinen	15-Aug	0.125	0.117
Jokioinen	15-Sept	0.200	0.142
Jokioinen	15-Oct	0.311	0.242
Jokioinen	15-Nov	0.241	0.209
Jokioinen	15-Dec	0.096	0.098
Kremsmünster	15-Oct	0.072	0.074
Okehampton	15-Aug	0.039	0.044
Okehampton	15-Sept	0.107	0.109
Okehampton	15-Oct	0.149	0.147
Okehampton	15-Nov	0.167	0.165
Okehampton	15-Dec	0.095	0.095
Piacenza	15-Aug	0.048	0.049
Piacenza	15-Sept	0.128	0.101
Piacenza	15-Oct	0.194	0.132
Piacenza	15-Nov	0.197	0.153
Piacenza	15-Dec	0.171	0.131
Piacenza	15-Jan	0.069	0.070
Porto	15-Nov	<0.001	<0.001
Sevilla	15-Nov	0.028	0.049
Thiva	15-Sept	-	0.027
Thiva	15-Oct	0.095	0.106
Thiva	15-Nov	-	0.059

Table 7.9: 80th Percentile annual average leachate concentrations at 1 m depth (µg/l) derived from MACRO (4.4.2) with Norwegian and Swedish groundwater scenarios and with different half lives and interception values and application dates.

Substance	**Crop**	**Scenario**	**80th percentile (µg/l) 10th August**	**Simulation nr.**
Geo mean half life from laboratory studies, 56 days, 90 % interception				
Aminopyralid	Grass/alfalfa	Önnestad (SE)	0,71	108
		Krusenberg (SE)	0,72	109
		Heia (NO)	0,7	110
Substance	**Crop**	**Scenario**	**80th percentile (µg/l) 31st May/ 10th June**	**Simulation nr.**
Geo mean half life from laboratory studies, 56 days, full dose (60 g a.s./ha), 90 % interception				
Aminopyralid	Grass/alfalfa	Önnestad (SE)	0.41/0.43	76/82
		Krusenberg (SE)	0.27/0.33	77/83
		Heia (NO)	0.29/0.32	78/84
Geo mean half life from laboratory studies, 56 days, full dose (60 g a.s./ha), 50 % interception				
Aminopyralid	Grass/alfalfa	Önnestad (SE)	2.0/2.1	79/85
		Krusenberg (SE)	1.4/1.7	80/86

		Heia (NO)	1.4/1.6	81/87
Geo mean half life from laboratory studies, 56 days, half dose (30 g a.s./ha), 90 % interception				
Aminopyralid	Grass/alfalfa	Önnestad (SE)	0,19/0.2	136/140
		Krusenberg (SE)	0.69/0.71	135/141
		Heia (NO)	0,14/0.16	137/142

7.2.3 Surface water

Modelling/PECsw, PECsed.
FOCUS SWASH was used to estimate the exposure of surface water and sediment. Input parameters for the different substances are summarized in Table 7.10 and the most relevant results are summarized in Table 7.11. Concentrations used in risk assessment are in bold.

Table 7.10: Input parameters for the FOCUS surface water modelling.

Aminopyralid	
FOCUS surface water step	3
Application rate	1x 60 g a.s./ha
Crop	grass/alfalfa
Time of applications	March-May June-September October-February
Plant uptake	0.5 “average”
“Wash off” factor	0.5/cm (MACRO) 0.05/mm (PRZM)
Molecular weight [g mol-1]	207
Water solubility [mg L-1]; 20 °C	2480
Vapour pressure [Pa]	$2.6x10^{-8}$
K_{FOC} [L kg-1]	4.07
1/n	0.85
DT50 on crop [days]	10 (default)
DT50 soil [days]	9.2 (geo. mean)*
DT50 whole system [days]	1000
DT50 water [days]	1000
DT50 sediment [days]	1000
Plant uptake	0.5

* The half life of 9.2 days was wrong due to an error in the applicant’s calculations. A half lie of 12.1 days should have been used, but this error does not affect the further risk assessment and hence the modelling was not changed.

Table 7.11: Summary of Global maximum PEC-values for aminopyralid, step 3.

Scenario	**Surface water compartment**	**Default buffer zone (m)**	**PEC_{SW} (µg/L)**	**PEC_{SED} (µg/kg dw)**
			Global Max	**Global Max**
D1, Lanna	Ditch	1	5.7	1.8
D1, Lanna	Stream	1.5	4.1	1.1
D2, Brimstone	Ditch	1	**22.6**	**3.3**
D2, Brimstone	Stream	1.5	14.5	1.7
D3, Vredepeel	Ditch	1	0.4	0.1
D4, Skousbo	Pond	3.5	0.3	0.3
D4, Skousbo	Stream	1.5	0.5	0.2
D5, La Jailliere	Pond	3.5	0.4	0.3
D5, La	Stream	1.5	0.4	0.1

			PEC_{SW} (µg/L)	PEC_{SED} (µg/kg dw)
Jailliere				
R2, Porto (Terraced)	Stream	1.5	0.3	0.1
R3, Bologna	Stream	1.5	2.1	0.2

8. Ecotoxicology

This assessment is based on documentation submitted by the applicant (Dow AgroSciences, 2006), and Aminopyralid - Draft Report and Proposed Decision, August 2006, Volume 3, Annex B.9. (RMS: UK) (Appendix E1).

There is an ongoing evaluation of aminopyralid in EFSA. Fluroxypyr was evaluated by the Norwegian Food Safety Authority in 2006, and is not considered further in this report.

Application rates result in a release of 200 ml Simplex/decare (6 g aminopyralid/decare and 20 g fluroxypyr/decare) to the environment.

8.1. Aminopyralid

8.1.1. Terrestrial organisms

Mammals
Data on mammalian toxicity of aminopyralid have been assessed in chapter 5 of this report. For ecotoxicological risk assessment purposes the following endpoints will be used: Low acute toxicity, LD50: >5000 mg a.s./kg bw, reproductive NOEL: 1000 mg a.s./kg bw/d.

Birds
Low acute oral toxicity (LD50: >292->2250 mg/kg bw/d, low dietary toxicity (LC50: >5620 mg/kg diet, and low reproductive toxicity (NOEC: 2700 mg/kg diet).

Test substance	Species	Type of study	LD50/LC50	NOEL/ NOEC	Endpoint expressed as mg/kg bw/d	Reference
Aminopyralid	Bobwhite quail *Colinus virginianus*	Acute oral	>2250 mg a.s./kg bw	<63 mg a.s./kg bw	>2250	Gallagher et al 2001, 8.1.1/01
Aminopyralid	Bobwhite quail *Colinus virginianus*	Acute oral	>292 mg a.s./kg bw	14 mg a.s./kg bw	>292	Gallagher et al 2003a, 8.1.1/02
Aminopyralid	Bobwhite quail *Colinus virginianus*	Dietary	>5620 mg a.s./kg feed	5620 mg a.s./kg feed	>1457	Gallagher et al 2001a, 8.1.2/01
Aminopyralid	Mallard duck *Anas platyrhynchos*	Dietary	>5620 mg a.s./kg feed	5620 mg a.s./kg feed	>2409	Gallagher et al 2001b, 8.1.2/02
Aminopyralid	Bobwhite quail *Colinus virginianus*	Subchronic/ reproductive	-	2700 mg a.s./kg feed	185.6	Mach 2003a, 8.1.3/01
Aminopyralid	Mallard duck *Anas platyrhynchos*	Subchronic/ reproductive	-	2700 mg a.s./kg feed	237.8	Mach 2003b, 8.1.3/02

Bees
Low contact (LD50: >100 µg/bee) and oral (LD50: >120 µg/bee) toxicity to bees.

Test substance	72-hr Contact LD50	48-hr Oral LD50	Reference
Aminopyralid	-	>120	Aufderheide 2001b 8.3.1.1/02
Aminopyralid	>100	-	Aufderheide 2001a 8.3.1.1/01

Non-target arthropods
To test the toxicity of aminopyralid without the presence of fluroxypyr, a SL-formulation with 24 % aminopyralid was tested. In Tier 1 laboratory acute contact toxicity studies, GF-871 showed low effects on parasitoids and predatory mites.

Test substance	Type of study	Species/ Life stage	Application rate (g a.s./decare)	Effect (%)	LR50 (g a.s./ decare)	Reference
GF-871 (24 % aminopyralid)	Laboratory glass plates, exposure of adults for 48 hours followed by 12 d fecundity assessment	*Aphidius rhopalosiphi* (parasitoid wasp)	3.0 6.0 3.0 6.0	M: 21.5 M: 38.9 F: 6.2 F: 9.7	-	Smith 2003a 8.3.2/01
GF-871 (24 % aminopyralid)	Laboratory glass plates, exposure of adults for 7 days followed by 7 days fecundity assessment	*Typhlodromus pyri* (predatory mite)	0.83 3.0 6.0 0.83 3.0 6.0	M: 12.6 M: -5.8 M: -4.6 F: 18.1 F: 16.0 F: 16.5	-	Smith 2003b 8.3.2/02

M = Mortality (corrected for control mortality)
F = Fecundity as % of control fecundity
Negative values indicate a lower level of mortality, a higher level of fecundity and a higher level of egg viability compared with the control.

Earthworms
Low acute toxicity of aminopyralid (LC50: >1000 mg/kg d.w. soil).

Test substance	Species	Exposure	LC50 (mg/kg)	NOEC (mg/kg)	Reference
Aminopyralid	*Eisenia foetida*	14 days	>1000	1000	Ward and Boeri 2001 8.4.1/01

Other soil macroorganisms
No studies were submitted.

Microorganisms
The effects of aminopyralid on microbial mediated carbon mineralization (respiration) and nitrogen mineralization in soil were investigated in laboratory tests with application rates of 1.68 mg a.s./kg dry soil (120 g a.s./decare, 20 x the max. dose) and 8.4 mg a.s./kg dry soil (600 g a.s./decare, 100 x the max. dose). No significant effects above the 25 % trigger were seen. (McMurray 2002, 8.5/01).

Terrestrial plants
No studies were submitted.

8.1.2 Aquatic organisms

Fish
Low acute toxicity (96h LC50: >100 mg a.s./l) and low chronic toxicity (32d NOEC: 1.36 mg a.s./l).

Test substance	Species	Exposure	LC50 (mg/l)	NOEC (mg/l)	Reference
Aminopyralid	Rainbow trout *Oncorhynchus mykiss*	96 hr	>100	100	Marino et al 2001 8.2.1/01

Test substance	Species	Exposure	LC50 (mg/l)	NOEC (mg/l)	Reference
Aminopyralid	Bluegill sunfish *Lepomis macrochirus*	96 hr	>100	100	Machado 2002a 8.2.1/02
Aminopyralid	Sheepshead minnow *Cyprinodon variegatus*	96 hr	>100	100	Machado 2002b 8.2.1/03
Aminopyralid	Fathead minnow *Pimephales promelas*	Early life stage test 32 days	-	1.36	Marino et al 2002 8.2.2.2/01

Bioconcentration
No studies were submitted.

Invertebrates
Low acute toxicity to daphnids, mysids and eastern oyster (EC50: >89 mg a.s./l).
Low chronic toxicity to Daphnia magna (21d EC50: >100 mg a.s./l, NOEC: 100 mg a.s./l).

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
Aminopyralid	*Daphnia magna*	48 hr	>100	100	Marino et al 2001, 8.2.4/01
Aminopyralid	*Mysidopsis bahia*	96 hr	>100	100	Machado 2002c, 8.2.4/02
Aminopyralid	*Crassostrea virginica* (Eastern oyster)	96 hr	>89	89	Cafarella 2002 8.2.4/03
Aminopyralid	*Daphnia magna*	21 days	>100	100	Henry et al 2003, 8.2.5/01

Sediment-dwelling organisms
Low chronic toxicity to *C. riparius* larvae (28d NOEC: 130 mg a.s./l, NOEC: 46.7 mg a.s./kg sediment).

Test substance	Species	Exposure	EC50	NOEC	Reference
Aminopyralid	*Chironomus riparius*	28 days	680 mg/l (emergence) 313 mg/kg sediment (emergence)	130 mg/l (emergence) 46.7 mg/kg sediment (emergence)	Putt 2002 8.2.7/01

Aquatic plants
Low toxicity to duckweed (14d EC50: >88 mg a.s./l).

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
Aminopyralid	Duckweed *Lemna gibba*	14 days	>88	88	Hoberg 2002e 8.2.8/01

Algae
Low to moderate toxicity to algae (72h EC50: 18->100 mg a.s./l).

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
Aminopyralid	*Pseudokirchneriella subcapitata* (green algae)	72 hr	32 (biomass) 30 (growth rate)	23 (biomass) 23 (growth rate)	Hoberg 2002a 8.2.6/01
Aminopyralid	*Navicula pelliculosa* (freshwater diatom)	72 hr	18 (biomass) 22 (growth rate)	6.0 (biomass) (growth rate)	Hoberg 2002b 8.2.6/02
Aminopyralid	*Anabaena flos-*	120 hr	28 (cell density)	16 (cell density)	Hoberg 2002c

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
	aquae (bluegreen)				8.2.6/03
Aminopyralid	*Skeletonema costatum* (marine diatom)	72 hr	77 (biomass) >100 (growth rate)	13 (biomass) 13 (growth rate)	Hoberg 2002d 8.2.6/04

Microorganisms
The influence of aminopyralid on the respiration activated sewage sludge microorganisms was determined. Inhibition of respiration was ≤21 % at concentrations up to 1000 mg a.s./l. The EC50 was estimated to be >1000 mg a.s./l. (Heim and Heim 2002, 8.7/01)

Micro-/mesocosm studies
No studies were submitted.

8.2 Fluroxypyr

Fluroxypyr is classified as R52/53 in the EU, i.e. “Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment”. Fluroxypyr was evaluated by the Norwegian Food Safety Authority in 2006, and is not considered further in this report.

8.3 Co-formulants

One of the co-formulants is classified as N; R51/53, i.e. “Dangerous for the environment.” and “Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment”.

8.4 Plant Protection Product

8.4.1 Terrestrial organisms

Birds
Low acute oral toxicity (LD50: >2250 mg/kg bw). The study is performed with a formulation with higher concentrations of both aminopyralid and fluroxypyr than in Simplex.

Test substance	Species	Type of study	LD50	NOEL/ NOEC	Endpoint expressed as mg/kg bw/d	Reference
GF-839 (3.7 % aminopyralid + 14.8 % fluroxypyr)	Bobwhite quail *Colinus virginianus*	Acute oral	>2250 mg/kg bw	486	>2250	Gallagher et al 2003b 10.1.1/01

Bees
Low contact (LD50: >100 µg/bee) and oral (LD50: >200 µg/bee) toxicity to bees. The studies are performed with a formulation with higher concentrations of both aminopyralid and fluroxypyr than in Simplex.

Test substance	72-hr Contact LD50	48-hr Oral LD50	Reference
GF-839 (3.7 % aminopyralid + 14.8 % fluroxypyr)	>100	-	Aufderheide 2003b 10.4.1/02
GF-839 (3.7 % aminopyralid + 14.8 % fluroxypyr)	-	>200	Aufderheide 2003a 10.4.1/01

Non-target arthropods
In Tier 1 laboratory acute contact toxicity studies, Simplex showed negligible effects on lacewing. Extended lab studies did not show effects above the trigger effect level of 50 % on parasitoids and predatory mites.

Test substance	Type of study	Species/ Life stage	Application rate (ml/decare)	Effect (%)	LR50 ml/decare	Reference
Simplex	Extended laboratory exposure of adults on barley plants for 48h followed by 12h oviposition assessment.	*Aphidius rhopalosiphi* (parasitoid wasp)	13.85 100 200 13.85 100 200	M[1]: 10.7 M: -3.6 M: 14.3 F[2]: -47.8 F: -35.1 F: -23.5	>200	Smith 2003c 10.5.1/01
Simplex	Extended laboratory exposure of adults on bean leaves for 7d followed by 7d fecundity assessment.	*Typhlodromus pyri* (predatory mite)	13.85 100 200 13.85 100 200	M: 0 M: 3.3 M: 1.1 F: 19.2 F: 25.7 F: 32.8	>200	Philips 2003 10.5.1/02
Simplex	Laboratory glass plate, exposure of larvae until pupation followed by assessment of mortality, fecundity and egg viability.	*Chrysoperla carnea* (lacewing)	27.7 200 27.7 200 27.7 200	M: 11.8 M: -5.8 F: -28.6 F: -11.3 E[3]: 0.03 E: -2.51	>200	Smith 2003d 10.5.1/03

M[1] = Mortality (corrected for control mortality)
F[2] = Fecundity as % of control fecundity
E[3] = % reduction in egg viability
Negative values indicate a lower level of mortality, a higher level of fecundity and a higher level of egg viability compared with the control.

Earthworms
Moderate acute toxicity (LC50: 710 mg/kg d.w. soil). The study is performed with a formulation with higher concentrations of both aminopyralid and fluroxypyr than in Simplex.

Test substance	Species	Exposure	LC50 (mg/kg)	NOEC (mg/kg)	Reference
GF-839 (3.6 % aminopyralid + 14.8 % fluroxypyr)	*Eisenia foetida*	14 days	710	500	Boeri and Ward 2003 10.6.1.1/01

Microorganisms
The effects of GF-839 (3 % aminopyralid acid + 14.5 % fluroxypyr-meptyl) on microbial mediated carbon mineralization (respiration) and nitrogen mineralization in soil were investigated in laboratory tests with application rates equivalent to 5 and 25 g aminopyralid/decare. No significant effects above the 25% trigger were seen. (Hayward 2003, 10.7.1/01).

Litter bag
No studies were submitted.

Terrestrial plants
A 21 days vegetative growth test was performed with 2 monocots and 4 dicots, using Simplex. Applications were made post emergence at application rates of 1.6 to 200 ml/decare. Plants were assessed for visual injury, plant mortality and foliar fresh weight. EC50 values are summarised below. The application rate in Norway is 200 ml/decare.

	Oats	Ryegrass	Oilseed rape	Soybean	Cucumber	Sugar beet
EC50 (ml/decare)	>200	>200	171	34	46	52

8.4.2 Aquatic organisms

Fish
Acute toxic to rainbow trout (96h LC50: 7.6 mg/l).

Test substance	Species	Exposure	LC50 (mg/l)	NOEC (mg/l)	Reference
GF 839 (3.0 % aminopyralid + 14.5 % fluroxypyr)	*Oncorhynchus mykiss* (rainbow trout)	96 hr	7.6	<5.0	Hicks 2003a 10.2.1/01

Invertebrates
Moderate acute toxicity to *Daphnia magna* (48h EC50: 35 mg/l).

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
GF 839 (3.0 % aminopyralid + 14.5 % fluroxypyr)	*Daphnia magna*	48 hr	35	5.0	Hicks 2003b 10.2.1/02

Aquatic plants
No studies were submitted.

Algae
Toxic to algae (72h EC50: 1.42 mg/l).

Test substance	Species	Exposure	EC50 (mg/l)	NOEC (mg/l)	Reference
GF 839 (3.0 % aminopyralid + 14.5 % fluroxypyr)	*Navicula pelliculosa* (freshwater diatom)	72 hr	1.52 (cell density) 1.42 (biomass) 2.01 (growth rate)	1.04 (cell density) 1.04 (biomass) 1.04 (growth rate)	Hicks 2003c 10.2.1/03

Micro-/mesocosm studies
No studies were submitted.

8.5 Toxicity/Exposure estimates

8.5.1 Terrestrial organisms

Mammals
Aminopyralid showed low acute (LD50: >5000 mg a.s./kg bw) and reproductive (NOEL: 1000 mg a.s./kg bw/d) toxicity. The following table summarises the acute and long-term toxicity endpoints for the active substance, the respective ETEs for small herbivorous mammals and the TERs generated. Both TER values are above the relevant triggers (Acute: <10, Chronic: <5).

An acute oral LD50 value of >5000 mg GF-839/kg bw can be derived from Smedley (2003b). Similar calculations as for the active substance resulted in acute TER values above the trigger (<10).

Similar calculations based on low acute toxicity (LD50 >5000 mg/kg bw) of the formulated product GF-839 resulted in an acute TER value above the trigger (<10).

For the short-term and long-term scenarios it is assumed that the two active substances are unlikely to co-exist in the same proportions as in the original formulation. Hence it can be argued that since fluroxypyr has Annex I listing for use at rates up to 40 g/decare, that the lower rate of use in the product GF-839 (20 g/decare) should not pose unacceptable short or long term risks to mammals.

Test substance	Time scale	Species	Toxicity endpoint (mg/kg bw/d)	ETE	TER	Annex VI trigger
Aminopyralid	Acute	Small herbivorous	>5000	11.84	>600	10
Aminopyralid	Long-term reproductive	Small herbivorous	1000	3.36	298	5
GF-839	Acute	Small herbivorous	>5000	395	>13	10

Birds

Aminopyralid showed low acute, dietary and reproductive toxicity. The following table summarises the acute, short-term dietary and long-term toxicity endpoints for the active substance, the respective ETEs for herbivorous/insectivorous birds and the TERs generated. All TER values are above the relevant triggers (Acute: <10, Chronic: <5).

Similar calculations based on low acute toxicity of the formulated product GF-839 resulted in acute TER values above the trigger (<10).

For the short-term and long-term scenarios it is assumed that the two active substances are unlikely to co-exist in the same proportions as in the original formulation. Hence it can be argued that since fluroxypyr has Annex I listing for use at rates up to 40 g/decare, that the lower rate of use in the product GF-839 (20 g/decare) should not pose unacceptable short or long term risks to birds.

Test substance	Time scale	Species	Toxicity endpoint (mg/kg bw/d)	ETE	TER	Annex VI trigger
Aminopyralid	Acute	Large herbivorous	>2250	3.75	>600	10
Aminopyralid	Acute	Insectivorous	>2250	3.24	>693	10
Aminopyralid	Short-term dietary	Large herbivorous	>1457	2.01	>726	10
Aminopyralid	Short-term dietary	Insectivorous	>1457	1.81	>805	10
Aminopyralid	Long-term reproductive	Large herbivorous	185.6	1.81	103	5
Aminopyralid	Long-term reproductive	Insectivorous	185.6	3.36	175	5
GF-839	Acute	Large herbivorous	>2250	125	>18	10
GF-839	Acute	Insectivorous	>2250	108.2	>21	10

Bees

Aminopyralid showed low contact toxicity to bees (LD50: >100 µg/bee) and low oral toxicity to bees (LD50: >120 µg/bee). Hazard quotients for contact (Qhc) and oral exposure (Qho) are estimated to be 0.6 and 0.5, respectively. These do not exceed the trigger value (>50).

The formulated product GF-839 showed low contact toxicity to bees (LD50: >200 µg/bee) and low oral toxicity to bees (LD50: >100 µg/bee). Hazard quotients for contact (Qhc) and oral exposure (Qho) are estimated to be 10 and 20, respectively. These do not exceed the trigger value (>50).

Non-target arthropods
In Tier 1 laboratory acute contact toxicity studies, aminopyralid showed low effects on predatory mites (*T. pyri*) and parasitoids (*A. rhopalosiphi*). Although precise LR50 values can not be calculated, it can be seen that LR50 values for both species are >6 g/decare. Hazard quotients are below the trigger (>2) both in-field and off-field.

Crop	Grassland
MAF	1
LR50 (g a.s./ha)	>6 g/decare
Application rate (g a.s./ha)	6 g/decare

	Distance (meters)	Drift value (%)	Hazard quotient (HQ)	HQ trigger
In-field	0	100	<1	2
Off-field	1	2.77	<0.03	2
	3	0.95	<0.01	2
	5	0.57	<0.01	2
	10	0.29	0.00	2
	20	0.15	0.00	2
	30	0.10	0.00	2

The formulated product GF-839 was tested in extended lab studies with *A. rhopalosiphi* and *T. pyri*, and in a first tier study with *C. carnea*. The tests did not show effects above the trigger effect level of 50 %.

Earthworms
Aminopyralid showed low acute toxicity (LC50: >1000 mg/kg d.w. soil). TER is estimated to be >25000. The formulated product GF-839 showed moderate acute toxicity (LC50: 710 mg/kg d.w. soil). TER is estimated to be 534. These values do not exceed the trigger (<10). Since the log Pow for aminopyralid is <2.0 the toxicity is not corrected to take account of the relatively high organic matter content of the artificial test soils. 50 % crop interception was used in the calculations.

Other soil macroorganisms
No studies were submitted.

Soil microorganisms
Soils treated with up to 100 times the normal application rate of aminopyralid deviated less than 25 % (trigger) from untreated controls with respect to carbon mineralisation/respiration and nitrogen mineralisation.

Soils treated with up to 4.2 times the normal application rate of GF-839 showed negligible deviation (<2 %) from untreated controls with respect to carbon mineralisation/respiration and nitrogen mineralisation.

8.5.2 Aquatic organisms

Aminopyralid
The TER calculations below are based on maximum PEC-values from FOCUS surface water modelling and the lowest acute (LC50 or EC50) or chronic (NOEC) values for the different organism groups. All calculations are based on an application rate of 6 g a.s./decare.

The following table presents the relevant endpoints from the toxicity tests, the FOCUS PEC estimations and the associated TERs. All TER calculations for aminopyralid pass the EU triggers based on Step 1 FOCUS surface water scenarios.

Species	Applications (number x rate)	FOCUS Step	Time scale	Endpoint mg/l	PEC mg/l	Buffer (m)	TER	Trigger
Oncorhynchus mykiss	1 x 6 g/decare	1	Acute	>100	0.0204	1	>4902	100
Daphnia magna	1 x 6 g/decare	1	Acute	>100	0.0204	1	>4902	100
Navicula pelliculosa	1 x 6 g/decare	1	-	18	0.0204	1	882	10
Lemna gibba	1 x 6 g/decare	1	-	>88	0.0204	1	>4313	10
Pimephales promelas	1 x 6 g/decare	1	ELS	1.3	0.0204	1	64	10
Daphnia magna	1 x 6 g/decare	1	Repro	100	0.0204	1	4902	10
Chironomus riparius	1 x 6 g/decare	1	Full life-cycle	130 (water phase)	0.0204	1	6373	10
Chironomus riparius	1 x 6 g/decare	1	Full life-cycle	46.7 mg/kg (sediment)	0.0008 mg/kg	1	58375	10

Simplex

For the formulated product Simplex, it should be safe to assume that the runoff and drain flow will not move the intact formulation into the aquatic environment, due to the varying adsorption and degradation properties of the formulation's constituents. Spray drift is the only realistic source of contamination of the aquatic environment by intact Simplex. The following table presents the relevant endpoints from the toxicity tests, the spray drift estimations with 1 meter buffer zone (based on Rautmann et al. 2001) and the associated TERs. All TER calculations pass the EU triggers.

Species	Applications (number x rate)	Time scale	Endpoint mg/l	PEC mg/l	Buffer (m)	TER	Trigger
Oncorhynchus mykiss	1 x 6 g/decare	Acute	7.6	0.018	1	412	100
Daphnia magna	1 x 6 g/decare	Acute	35.0	0.018	1	1895	100
Navicula pelliculosa	1 x 6 g/decare	-	1.51	0.018	1	82	10

Bioconcentration

The log Pow for aminopyralid is given as -2.87 in pH 7 buffered solution. Bioconcentration studies are therefore not submitted, and aminopyralid is not expected to bioaccumulate.

9. Dossier quality and completeness

The dossier is complete and is adequate as a basis for an evaluation of the active substance, metabolites and product.

References

T1: Draft Report and Proposed Decision, August 2006, Volume 3, Annex B.6.
T2: JMPR report, 2007
E1: Draft Report and Proposed Decision, August 2006, Volume 3, Annex B.9.