Committee for Risk Assessment

RAC

Annex 1

Background document

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

Benzenamine, 2-chloro-6-nitro-3-phenoxy- (Aclonifen)

ECHA/RAC/ CLH-O-0000001543-79-03/A1

EC number: 277-704-1
CAS number: 74070-46-5

Adopted

14 September 2011

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Aclonifen

EC Number: 277-704-1

CAS number: 74070-46-5

Registration number (s): -

Purity: minimum 970 g/kg

Impurities: There are a number of impurities claimed as confidential by the producer

Proposed classification based on Regulation (EC) No 1272/2008 criteria:

RAC has concluded that the proposed additional classification of aclonifen as Skin Sens. 1 – H317 and Carc. 2 – H351 is appropriate, as is the existing classification of aclonifen as Aquatic Acute 1 – H400 and Aquatic Chronic 1 – H410. This would result in:

Carc. 2	H351
Skin Sens. 1	H317 (1A, according to 2nd ATP)
Aquatic Acute 1	H400
Aquatic Chronic 1	H410

Proposed classification based on Directive 67/548/EEC criteria:

RAC has concluded that the proposed additional classification of aclonifen as R43 and Carc. Cat. 3; R40 is appropriate, as is the existing classification of aclonifen as N; R50/53. This would result in:

Carc. Cat. 3; R40

R43

N; R50/53

Proposed labelling based on Regulation (EC) No 1272/2008:

Pictogram: GHS07, GHS08, GHS09

Signal word: Warning

Hazard statement codes: H317, H351, H410

Proposed labelling based on Directive 67/548/EEC:

Symbol: Xn, N

Risk phrases: R40-43-50/53

Safety phrases: S(2-)36/37-60-61

Proposed M-factors and specific concentration limits (if any):

HUMAN HEALTH

C≥0.1% R43

In line with the generic concentration limit for the sub-category Skin Sens. 1A – H317 under CLP according to the 2nd ATP.

ENVIRONMENT

M-factors based on Regulation (EC) No 1272/2008, taking into account the 2nd ATP

The M-factor for the short-term hazard category is determined by using the reported ErC_{50} value of 0.0069 mg/L obtained for the algae *Desmodesmus subspicatus* in a 96 hr static study. Consequently, an M-factor of 100 is assigned to the short-term hazard category.

For the long-term hazard category, the M-factor is 10, based on the NOErC of 0.0012 mg/L for *Lemna gibba*.

Specific concentration limits under Directive 67/548/EEC:

C≥0.25%	N; R50/53
0.025%≤C<0.25%	N; R51/53
0.0025%≤C<0.025%	R52/53

Proposed notes (if any): None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: Benzenamine, 2-chloro-6-nitro-3-phenoxy-

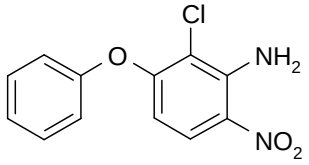
EC Name: 2-chloro-6-nitro-3-phenoxyaniline

CAS Number: 74070-46-5

IUPAC Name: 2-chloro-6-nitro-3-phenoxyaniline

1.2 Composition of the substance

There are a number of impurities stated as confidential by the producer.

Chemical Name:	Benzenamine, 2-chloro-6-nitro-3-phenoxy-
EC Number:	277-704-1
CAS Number:	74070-46-5
IUPAC Name:	2-chloro-6-nitro-3-phenoxyaniline
Molecular Formula:	$C_{12}H_9ClN_2O_3$
Structural Formula:	
Molecular Weight:	264.7 g/mol
Typical concentration (% w/w):	confidential information
Concentration range (% w/w):	≥ 970 g/kg

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	yellow powder (99.6% pure, 99.4 % technical material)	EFSA (2008)
VII, 7.2	Melting/freezing point	4.2	81.2 °C (purity 99.6 %)	
VII, 7.3	Boiling point	4.3	not detectable (purity 99.6 %)	
VII, 7.4	Relative density	4.4	1.50 at 20 °C (purity 99.6 %)	
VII, 7.5	Vapour pressure	4.6	1.6×10^{-5} Pa at 20 °C (99.3 % purity)	
VII, 7.6	Surface tension	4.10	72.0 mN/m at 20 °C (99.4 % purity)	
VII, 7.7	Water solubility	4.8	1.4 mg/L at 20 °C pH 5, 7 and 9 (99.7 % purity)	
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7	4.37	
VII, 7.9	Flash point	4.11	not relevant	
VII, 7.10	Flammability	4.13	not highly flammable (99.4 % purity)	
VII, 7.11	Explosive properties	4.14	not explosive (99.4 % purity)	
VII, 7.12	Self-ignition temperature		not detected	
VII, 7.13	Oxidising properties	4.15	no oxidising properties (99.4 % purity)	
VII, 7.14	Granulometry	4.5	not relevant	
IX, 7.15	Stability in organic solvents and identity of relevant degradation products	4.17	not detected	
IX, 7.16	Dissociation constant	4.21	not measurable, by calculation constant is -3.15	
IX, 7.17	Viscosity	4.22	not detected	
	Auto flammability	4.12	No self ignition between room temperature and melting point (99.4 % purity)	
	Reactivity towards container material	4.18	not detected	
	Thermal stability	4.19	decomposition starts at 297 °C (99.6 % purity)	

2 MANUFACTURE AND USES

2.1 Manufacture

confidential information

2.2 Identified uses

Herbicide for pre-emergence control of annual grass and broad-leaved weed species in several dicotyledonous crops.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of Regulation (EC) No 1272/2008

Table 3.1-1: Entry of aclonifen in Table 3.1 of Annex VI of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
612-120-00-6	aclonifen (ISO); 2-chloro-6-nitro-3-phenoxyaniline	277-704-1	74070-46-5	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			

Table 3.1-2: Entry of aclonifen in Table 3.2 of Annex VI of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
612-120-00-6	aclonifen (ISO); 2-chloro-6-nitro-3-phenoxyaniline	277-704-1	74070-46-5	N; R50-53	N R: 50/53 S: 60-61		

3.2 Self classification(s)

Not relevant for this dossier.

4 ENVIRONMENTAL FATE PROPERTIES

The environmental fate properties assessment for aclonifen is based on the Draft Assessment Report and Proposed Decision of Germany prepared in the context of the possible inclusion of aclonifen in Annex I of Council Directive 91/414/EEC (EFSA, 2008; DAR August 2006 + Final addendum to DAR June 2008). The references cited below are from the DAR, volume 3, parts B.8 (Environmental fate and behaviour) and B.9 (Ecotoxicology).

4.1 Degradation

4.1.1 Stability

Hydrolysis

- Godward, P.J. et al., 1991, Document No.: R007157

Under sterile aqueous conditions, at temperatures of 22 °C, 50 °C and 70 °C, aclonifen was found to be hydrolytically stable at pH 5, 7 and 9. The study was performed according to OECD 111 (1981) and BBA 55 (1980) with [^{14}C]-labelled aclonifen dissolved in sterile buffers at a nominal concentration of approximately 1 mg/L (actual concentrations pH 5, 0.91 mg/L; pH 7, 0.88 mg/L; pH 9, 0.76 mg/L).

Photolysis in water

- Oddy, A. et al., 2003, Document No.: C031518

Photolysis in buffered solution at pH 7 takes place to a certain, but not high extent. The study was performed according to EU (=EEC) 94/37/EC. The half-life for the decline of aclonifen in the irradiated experiment was calculated to be equivalent to 197 days natural summer sunlight (European Union at latitude 50°N) according to first order kinetics.

- Offizorz, P., 1993, Document No.: R007202

The quantum yield of direct photolysis of aclonifen in aqueous solution was determined to be 5.19×10^{-6}.

Based on this quantum yield ABIWAS 2.0 calculations for middle Europe (55 ° North) result in DT_{50} values of 9 days (May, Minimum) to 1040 days (December, Maximum).

Photolysis in soil

- van Dijk, A. and Burri, R., 1994, Document No.: R007084

The photolytic degradation of [U-14C-aniline]-labelled aclonifen was studied following application to a loamy sand soil under artificial sunlight for a period of 30 days. The study was performed according to USEPA (=EPA) N, 161-3. The artificial sunlight was provided by a xenon arc lamp with filters to cut off any radiation below 290 nm. The samples were incubated at 22 ºC under a 12 hour light/12 hour dark cycle (irradiated samples) or in the dark (non-irradiated samples).

The presence of light slightly enhanced the degradation rate of aclonifen on soil surfaces. The DT_{50} value for aclonifen assuming first order kinetics under irradiated conditions was 75.3 days. No degradation of aclonifen was observed in samples incubated in the dark.

Photo-oxidative degradation in air

- Maurer, T., 2000, Document No.: C010366

Based on AOP version 1.88 the half-life of aclonifen in the atmosphere was calculated as being in the range of 0.84 to 1.26 days dependent upon the mean aerial OH concentration chosen for the calculation, 0.5 x 10^6 cm^{-3} averaged over a 24 hours or 1.5 x 10^6 cm^{-3} averaged over 12 hours, respectively. It can be concluded that aclonifen will be readily degraded in the air due to its fast reaction with photolytically generated hydroxyl radicals.

Recalculation using AOP version 1.91 yields the same results.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

No data available.

4.1.2.2 Screening tests

Readily biodegradability

- Voigt, H., 1990, Document No.: R003640

The ready biodegradability of aclonifen was determined according to the Sturm test (OECD guideline 301B). Aclonifen of purity 91.3 % was incubated in the test medium, inoculated with activated sludge (from municipal purification plant at Hildesheim), at concentrations of 5 and 10 mg/L. The released carbon dioxide was monitored for a period of 28 days and quantified by precipitation as $BaCO_3$ followed by back titration of $Ba(OH)_2$ with 0.05 M HCl. A parallel experiment was performed using sodium acetate to validate the test results.

The results, expressed as a percentage of the maximum theoretical CO2 production, for both aclonifen and the reference substance (sodium acetate) are shown in Table 4.1-1. Aclonifen was found to be not readily biodegradable within 28 days.

Table 4.1-1: Ready biodegradability expressed as percentage of maximum theoretical CO_2 production

Time (days)	Aclonifen (5 mg/L)	Aclonifen (10 mg/L)	Sodium acetate (20 mg/L)
7	9 %	4 %	70 %
16	15 %	0 %	70 %
28	22 %	0 %	74 %

4.1.2.3 Simulation tests

Biodegradation in water/sediment systems

- Lowden, P. et al., 2000, Document No.: C009991

The behaviour of [^{14}C]-labelled aclonifen, uniformly labelled in the aniline ring, was investigated in two contrasting water/sediment systems, characterised as a sandy silt loam (Manningtree system) and a clay loam (Ongar system), over a period of 180 days according to the guidelines EU (= EEC) 95/36/EC, Section 7.2.1.3.2, (1995) and SETAC 1.1 (1995).

The results of the aerobic incubation are summarised in Table 4.1-2.

Table 4.1-2: Degradation of aclonifen in aerobic water/sediment system

Substance	Water / sediment system	T (^{o}C)	pH water	pH sed.	oc[1)] (%)	DT_{50} water (d)	DT_{50} sed. (d)	DT_{50} whole system (d)
[^{14}C]-labelled aclonifen	I Manning-tree	20	6.7	6.8	5.7	3.2	92	11.2
	II Ongar	20	7.5	8.4	3.8	5.6	No decline	17.3

[1)] organic carbon content of sediment

Aclonifen was metabolised in both sediment and water systems at a moderate rate with DT_{50} values of 11.2 days and 17.3 days. Aclonifen steadily partitioned to the sediment and degraded. None of the metabolites observed reached 5 % AR (applied radioactivity) in any of the phases. Mineralization was negligible in both systems (max CO_2 = 2.07 % AR) and unextractable residue in the sediment amounted up to 65.6 -76.5 % AR at the end of the experiments (180 d).

Biodegradation in soil

The rate of degradation of aclonifen was investigated in three laboratory studies in a total of nine soils according to guideline BBA IV, 4-1; BBA Merkblatt 36, EU (= EEC) 95/36/EC, Section 7.1.1.2, (1995) and SETAC 1.1, (1995). Degradation under dark aerobic conditions at 10 °C was also investigated in a study with two soils. The experiments are summarised in Table 4.1-3.

Table 4.1-3: Degradation of aclonifen in aerobic laboratory studies

Soil type/site	oc[1)] (%)	pH	T ^{o}C / % MWHC	DT_{50} /DT_{90} (d)	DT_{50} (d) 20 °C pF2/10kPa	Reference
Aldhams House (94/8/2) silty sand	1.1	6.7	20 °C / 60 % FC	134 / 443	93.6	Schanné, C. (1994), Document No.: R007085
Shelley Field (94/9/2) silty loam	1.9	7.0	20 °C / 60 % FC	73 / 242	51.0	
Westleton (94/10/2) silty loam	1.5	6.8	20 °C / 60 % FC	95 / 315	66.4	
Westleton (94/10/2) silty sand	1.5	6.8	20 °C / 30 % FC	>> 118 d		

sandy loam Arable	1.48[2)]	7.3	22 °C / 40 % MWHC	32/ 107	29.5	Schlueter, H., 1983, Document No.: R003641
sandy loam Standard soil 2.3	0.92[2)]	6.6	22 °C / 40 % MWHC	78/ 259	72.6	
loamy sand (Speyer 2.2 A)	2.64	6.0	22 °C / 40 % MWHC	93/309	83.7	Anonymous, 1982, Document No: R003643
loamy sand (Speyer 2.2 B)	2.64	6.0	22 °C / 40 % MWHC	76/ 254	68.7	
sandy loam (Speyer 2.3)	1.06	7.0	22 °C / 40 % MWHC	53/ 177	41.9	
loamy sand Westleton	6.8	6.8	10 °C / 50 % MWHC	222/ 740	86.5	England et al., 1988, Document No.: R007107
clay loam Stockland	7.2	7.2	10 °C / 50 % MWHC	218/ 723	61.8	

[1)] organic carbon, [2)] om (organic matter) value

In aerobic laboratory soil degradation studies the overall geometric mean DT_{50} value of aclonifen at 20 °C and pF2 is 62.3 days (SFO, range 41.9 – 93.6 days; n = 10).

Practically the totality of the extracted radioactivity consisted in parent compound with only very minor metabolites detected. Mineralisation was negligible or very low (CO_2: 0.7 – 5.2 % AR). Unextracted soil residue increase continuously up to 40.9 – 57.6 % AR at the end of the studies.

4.1.3 Summary and discussion of persistence

Biodegradation in water

Aclonifen was found to be not readily biodegradable in the available study.

In water/sediment systems aclonifen was metabolised at a moderate rate with DT_{50} values of 11.2 days and 17.3 days. Mineralisation was negligible in both systems (max. CO_2 = 2.07 % AR).

Biodegradation in soil

In aerobic laboratory soil degradation studies the overall geometric mean DT_{50} value of aclonifen is 62.3 days (SFO, 20 °C, pF2). Mineralisation was negligible or very low (CO_2 = 0.7 – 5.2 % AR).

Based on the findings from the screening test on ready biodegradability, water/sediment simulation test and soil aclonifen appears to be susceptible for primary degradation and not ultimate mineralisation. Considering the results of the test on ready biodegradability and levels of mineralisation in the simulation study, aclonifen is considered not rapidly biodegradable (a degradation of >70% degradation within 28 days) for purposes of classification and labeling.

4.2 Environmental distribution

Not relevant for this dossier.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Aclonifen has a log Kow of 4.37 (pH 5-6, distilled water).

4.3.1.2 Measured bioaccumulation data

In a first study [^{14}C]-aclonifen accumulated in rainbow trout with a total radioactive residue (TRR) bioconcentration factor (BCF) of 2896 L/kg ww for whole fish. Maximum BCF was reached after 16 days and then declined throughout the remaining exposure period. Based on the fitted uptake and depuration rate constants, the kinetic BCF is 2248 L/kg ww. When exposure ceased, the residues depurated with a half-life of 2.0 days. At the end of the 20 day depuration period, residues declined to a level of 1.0 % in total fish tissues, relative to those residues at the end of the uptake phase. The bioconcentration factors determined from this 28 day exposure study (considered as key study) were higher than those reported from a previous second study in which rainbow trout had been exposed continuously to [^{14}C]-aclonifen for 8 days (Maximum BCF: 1645 – 1742 L/kg ww). This lower BCF's were likely to be due to the shorter exposure duration and so the steady state was not reached. These results are only considered as additional information.

The studies are summarised in Table 4.3-1.

Table 4.3-1: Results of aquatic bioconcentration measurements

guideline/ test method	exposure	log Kow	Initial conc. [µg/L]	Steady state BCF [L/kg ww]	Kinetic BCF	Depuration time CT50(d)	Depuration time CT95(d)	Remarks	reference
OECD 305 & 305E	28 d, flow - trough	4.37	26.9 (real) 30(nom)	2896	2248	2.0	8.8	Whole fish based on TRR	Wyness, L.E. (1995), Document No.: R007430
OECD 305 & 305E	8 d, flow - trough	4.37	4.24 (real) 6 (nom) 37.7 (real) 60(nom)	1742[1)] 1645[1)]	1275 1326	0.77 0.89	3.32 3.85	Whole fish based on TRR [2)]	Wyness, L.E. (1995), Document No.: C034500

[1)] BCFmax, because steady state was not reached since only 8 days exposure was applied

[2)] Majority of the total radioactive residues (TRR) in water and fish are chromatographic analysed as parent substance (aclonifen)

4.3.2 Terrestrial bioaccumulation

No data available.

4.3.3 Summary and discussion of bioaccumulation

Aclonifen has a log Kow of 4.37. The experimentally derived steady state BCF of 2896 and kinetic BCF of 2248 are above the trigger of 100 (criterion for bioaccumulating potential conform Directive 67/548/EEC) and also above the trigger of 500 (criterion for bioaccumulating potential conform Regulation (EC) No 1272/2008). Based on the results of the bioconcentration study, aclonifen does significantly bioaccumulate.

4.4 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazard assessment for aclonifen is based on the Draft Assessment Report and Proposed Decision of Germany prepared in the context of the possible inclusion of aclonifen in Annex I of Council Directive 91/414/EEC (EFSA, 2008; DAR, August 2006 + Final addendum to DAR, June 2008). The references cited below are from the DAR, volume 3, part B.6 (Toxicology and metabolism).

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Aclonifen is rapidly absorbed when applied orally to rats (approximately 80 % within 24 hours based on urinary and biliary excretion) and widely distributed. High dose levels (1000 mg/kg bw) seem to saturate and delay the absorption process and reduce the bioavailability to slightly more than 40 % based on renal excretion data. The results indicated a sex difference in the maximum mean blood concentration, resulting in a higher systemic exposure of males. Kinetic data for whole blood indicate a terminal phase half-life of approximately 103 hours for both sexes, whereas plasma half-lives are shorter (13 and 24 hours for males and females, respectively), suggesting binding to blood cells. Residues are found mainly in liver, kidneys, lung, thyroid and skin/fur; levels in brain are very low. There is no evidence for accumulation. Bile excretion studies indicate a significant enterohepatic circulation of aclonifen-related material. Excretion is nearly complete within 48 hours. Renal excretion ranged from 38 % - 50 % in males and from 39 % - 65 % in females in the different studies available. Most of the remaining material is eliminated via the faeces. Aclonifen is extensively metabolised; more than 20 metabolites or intermediates have been identified. The main pathways are hydroxylation of the phenyl ring, cleavage of the ether bond, reduction of the nitro group and subsequent acetylation, methylation and phase II type conjugations with sulphate or glucuronic acid (Crosnier, A., Guittard, J., 2002, report no. A01255; Crosnier, A., Guittard, J., 2002, report no. A01256; Odin-Feurtet, M., 2002, report no. SA01338; Odin-Feurtet, M., 2002, report no. SA01123; Schlueter, H., 1983, report no. 127AA-651-02).

Dermal application in vivo (8 h exposure) indicated dermal absorption of up to 6 % for a concentrated (6 mg/cm^2) and 38 % for a diluted (0.015 mg/cm^2) preparation in male rats (Fitzpatrick, K., 2003, report no. BAG/355/032306). In vitro, rat and human skin (including stratum corneum) showed 4 % and 2 % dermal absorption with the concentrate, and 64 % and 15 % respectively, with the diluted formulation. (Cage, S., 2003, report no. BAG 362/032351) It is estimated that a dermal absorption of 2 % (concentrate) and 10 % (dilution) would be representative of the in vivo situation in humans.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Aclonifen was of very low acute oral toxicity in rodents. No deaths occurred in rats or mice. In rats, clinical signs consisted of reduced spontaneous activity and ataxia, which completely disappeared 24 hours after treatment. Yellow staining of the urine, indicative of ongoing excretion of the coloured test substance, persisted for 6 days. In mice, clinical signs occurred within 2 hours of dosing and consisted of reduced spontaneous activity, ataxia and piloerection. All clinical signs had

disappeared on the fourth day after administration. Yellow staining of the urine was noted in the first hours after treatment and had disappeared 48 hours later.

Table 5.2-1: Summary of acute oral toxicity

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels (mg/kg bw)	Value LD_{50} (mg/kg bw)	Remarks	Reference
OECD 401	Oral	Rat, Wistar-AF/HAN-EMD 5M+5F	5000	LD_{50} > 5000	Purity not stated Vehicle: aqueous 0.5% carboxy-methyl-cellulose	Heusener, A. and Weiße, G. (1981); report no 4/105/81
OECD 401	Oral	Mouse, NMRI-EMD 5M+5F	5000	LD_{50} > 5000	Purity not stated Vehicle: aqueous 0.5% carboxy-methyl-cellulose	Heusener, A. and Weiße, G. (1981); report no 4/105/81

5.2.2 Acute toxicity: inhalation

Aclonifen was of very low acute inhalation toxicity in rats. No deaths occurred. During the exposure period, wet fur and a decreased respiratory rate were noted. On removal from the exposure chamber additional signs of hunched posture, piloerection and yellow staining around the head and shoulders were seen in all animals. These signs were still evident one hour after exposure, with the addition of red/brown staining of the snout in three rats, but all signs had regressed by the following day in all but one animal which appeared normal on day three.

Table 5.2-2: Summary of acute inhalation toxicity

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels (mg/L)	Value LC_{50} (mg/L)	Remarks	Reference
OECD 403	Inhalative	Rat, Sprague-Dawley 5M+5F	5.06	LC_{50} > 5.06	Purity 91.3 % Dust, 4-h, nose only	Blagden, S.M. (1990); report no 282/56

5.2.3 Acute toxicity: dermal

Aclonifen was of very low acute dermal toxicity in rats. No deaths occurred. The only signs following treatment were yellow urine, indicating dermal absorption, and yellow skin which persisted up to days 7 and 14, respectively.

Table 5.2-3: Summary of acute dermal toxicity

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels (mg/kg bw)	Value LD_{50} (mg/kg bw)	Remarks	Reference
OECD 402	Dermal	Rat, Wistar-AF/HAN-EMD 5M+5F	5000 (nominal)	LD_{50} >5000	Purity not stated Vehicle: aqueous 0.5% carboxy-methyl-cellulose Solubility in water 1.4 mg/L	Heusener, A. and Weiße, G. (1981); report no 4/105/81

5.2.4 Acute toxicity: other routes

Mortalities occurred up to 3 days after i.p. administration at doses of 3200 mg/kg bw and higher, affecting all animals at 5000 mg/kg bw, two males and four females at 4000 mg/kg bw, and two males and three females at 3200 mg/kg bw. Clinical signs appeared 5-15 minutes after injection in all treated groups and consisted of reduced activity, ataxia, dyspnea and piloerection. Yellow staining of the urine was noted immediately after dosing and persisted up to day 6. Body weight was decreased up to day 3 after treatment. Gross necropsy of decedent animals showed deposition of the test material in the abdominal cavity, mucosal haemorrhages and erosions in the stomach as well as lung oedema in some animals. Gross necropsy of animals sacrificed after 15 days showed small depositions of the test material on the liver, adhesion of individual lobes of the liver as well as focal spleen capsular fibrosis.

Table 5.2-4: Summary of acute intraperitoneal toxicity

Method/ Guideline	Route	Species, Strain, Sex, No/group	Dose levels (mg/kg bw)	Value LD_{50} (mg/kg bw)	Remarks	Reference
OECD 402	Intra-peritoneal	Rat, Wistar-AF/HAN-EMD 5M+5F	0-2500-3200-4000-5000	LD_{50} (M): 3742 LD_{50} (F): 3247	Purity not stated Vehicle: aqueous 0.5% carboxy-methyl cellulose	Heusener, A. and Weiße, G. (1981); report no 4/105/81

5.2.5 Summary and discussion of acute toxicity

5.2.5.1 Dossier submitter

Aclonifen is of very low acute toxicity by the oral (LD_{50} > 5000 mg/kg bw), dermal (LD_{50} > 5000 mg/kg bw) and inhalation route (LC_{50} > 5.06 mg/L) in the rat and also by the oral route in the mouse (LD_{50} > 5000 mg/kg bw). No classification is required.

5.2.5.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for acute toxicity (or for specific target organ toxicity upon single exposure), which was not questioned during public consultation.

For assessment of oral acute toxicity one rat and one mice study, both with a reported LD_{50} of > 5000 mg/kg bw, are available. The LD_{50} is above the threshold value of 2000 mg/kg bw for both Acute Tox. 4 – H302 (CLP) and Xn; R22 (DSD).

For assessment of dermal acute toxicity one rat study with a reported LD_{50} of >5000 mg/kg bw is available. This LD_{50} is above the threshold value of 2000 mg/kg bw for both Acute Tox. 4 – H312 (CLP) and Xn; R21 (DSD).
For assessment of inhalation acute toxicity one rat study with a reported LC_{50} of >5.06 mg/L/4hr is available. This LC_{50} is above the threshold value of 5 mg/L/4hr for both Acute Tox. 4 – H332 (CLP) and Xn; R20 (DSD).

In the acute toxicity studies only slight clinical effects were observed, which were transient in nature. These effects do not fulfill the CLP criteria to classify for STOT-SE.

Based on the available data, RAC supported the conclusion of the dossier submitter that aclonifen should not be classified for acute oral, dermal or inhalation toxicity. RAC also concluded that aclonifen should not be classified for STOT-SE.

5.3 Irritation

5.3.1 Skin

Aclonifen was very slightly and transiently irritating to rabbit skin when applied for 24 hours under occlusion as moistened powder at a dose of 31.25 mg/cm^2 (500 mg on an area of 4x4 cm). A yellow discolouration of the skin was observed as well as slight erythema and, on day 3, scale formation. This effect was reversible and disappeared after 3 days.

Table 5.3-1: Summary of skin irritation

Method/ Guideline	Species, Strain, Sex, No/group	Average score* 24, 48, 72 h, 7 d		Reversibility yes/no	Results	Remarks	Reference
		Erythema	Oedema				
OECD 404	Rabbits, NZW 3M + 3F	0-0.3-0.5-0	0-0-0-0	yes	Not irritating	Purity not stated	Heusener, A. and Weiße, G. (1981); report no 4/105/81

* In the original report no individual scores were reported. The results were presented as mean scores for the six animals per timepoint.

5.3.2 Eye

Aclonifen (100 mg/eye, right eye served as a control) was not irritating to the eyes of rabbits. In 6 of these rabbits (3 male and 3 female) the eyes were not rinsed, in the remaining 3 (2 male and 1 female) the eyes were rinsed with lukewarm water 30 seconds after instillation of the test material and for a period of 60 seconds. The only observation was a yellow colouration of the hair around the eyes which persisted throughout the 7 day observation period.

Table 5.3-2: Summary of eye irritation

Method/ Guideline	Species, Strain, Sex, No/group	Average Score 24, 48, 72 h				Reversi-bility yes/no	Results	Remarks	Reference
		Cornea	Iris	Redness Conjunc-tiva	Chemo-sis				
OECD 405	Rabbits, NZW 5M + 4F	0-0-0	0-0-0	0-0-0	0-0-0	Not applicable	Not irritating	Purity not stated	Heusener, A. and Weiße, G. (1981); report no 4/105/81

5.3.3 Respiratory tract

No data are available. A slight potential for respiratory irritation may be deduced from the slight and transient findings in skin and from the reduced respiration rate in the acute inhalation toxicity study.

5.3.4 Summary and discussion of irritation

5.3.4.1 Dossier submitter

Very slight dermal and no ocular irritation was noted after application of aclonifen to the skin and eye of rabbits. Therefore no classification for irritation is required.

5.3.4.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for irritation, which was not questioned during public consultation.

For assessment of skin irritation a rabbit study is available. In this study, some slight, transient irritation was observed, with mean scores for erythema, and eschar formation or oedema formation below the threshold value of 2.3 for Skin Irrit. 2 – H315 (CLP) or 2 for Xi; R38 (DSD).

For assessment of eye irritation a rabbit study is available. In this study, no effects on the cornea, iris or conjunctiva were observed (all scores 0).

No data are available for respiratory tract irritation.

Based on the data available, RAC supported the conclusion of the dossier submitter that aclonifen should not be classified for irritation.

5.4 Corrosivity

5.4.1 Dossier submitter

In skin and eye irritation studies there was no evidence for a corrosive action of aclonifen.

5.4.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for corrosion, which was not questioned during public consultation.

In skin and eye irritation studies there was no evidence for a corrosive action of aclonifen. RAC therefore concluded that aclonifen does not fulfil the criteria for classification as Skin Corr. 1B – H314 (CLP) or C; R34 (DSD).

5.5 Sensitisation

5.5.1 Skin

In a Buehler test with guinea pigs, topical induction was carried out (0.5 mL of 75 % w/v aclonifen in arachis oil) over a period of three weeks, to give a total of nine 6-hour exposures. On day 28 a challenge dose of aclonifen (75 % w/v in arachis oil) was applied,
During the induction phase, aclonifen treatment produced isolated incidents of scattered mild, moderate and diffuse redness; desquamation and oedema were also noted in 14/20 animals and 1/20 animals, respectively. There was no adverse reaction to aclonifen noted at either the 24 or 48-hour observation time points following the challenge application. The positive control material (0.5% DNCB) produced reactions in 15/19 animals, confirming the sensitivity of the assay.

In a Magnusson & Kligman skin sensitisation test guinea pigs were given intradermal injections on day 1 with FCA (50 % v/v in 0.9% NaCl, both groups), aclonifen (1 % w/w in corn oil, treated group) or corn oil (control group) and aclonifen (1 % w/w in a mixture of 50/50 v/v FCA/0.9 % NaCl, treated group) or corn oil (50 % w/v in a mixture of 50/50 v/v FCA/0.9 % NaCl, control group). On Day 8, aclonifen at the concentration of 50 % in an 80/20 (w/w) mixture of ethanol/water (treated group) or an 80/20 (w/w) mixture of ethanol/water (control group) was applied topically, which was then covered by an occlusive dressing for 48 hours. On day 22, all animals were challenged by a cutaneous application of aclonifen at the concentration of 50 % (w/w) in an 80/20 (w/w) mixture of ethanol/water, under an occlusive dressing for 24 hours. After the challenge application, no cutaneous reactions were observed in the animals of the control group. In the treated group, at the 24-hour reading, discrete or moderate erythema was noted in 11/20 and 8/20 animals, respectively. Discrete or moderate erythema persisted at the 48-hour reading in 9/20 and 10/20 animals, respectively. Dryness of the skin was observed in almost all animals of the treated group at the 24 and 48-hour readings and an oedema was recorded in 1/20 animals of the treated group at the 48-hour reading. The cutaneous reactions observed in the animals of the treated group were attributed to delayed contact hypersensitivity.

Table 5.5-1: Summary of skin sensitisation

Method/ Guideline	Species, Strain, Sex, No/group	Number of animals sensitised/Total number of animals	Results	Remarks	Reference
OECD 406 Buehler, 9-d induction	Guinea pigs, Dunkin-Hartley 20F (treated) 10F (control)	0/20 (75 % aclonifen) 0/10 (control)	Not sensitising	Purity 91.3 % Vehicle: topical induction and challenge: arachis oil	Tuffnell, P.P. (1990); report no. 282/55

Method/ Guideline	Species, Strain, Sex, No/group	Number of animals sensitised/Total number of animals	Results	Remarks	Reference
OECD 406 GPMT	Guinea pigs, Hartley Crl (HA) BR 10M + 10F (treated) 5M + 5F (control)	19/20 (1% aclonifen) erythema 24 h: discrete 11/20; moderate 8/20 erythema 48 h: discrete 9/20; moderate 10/20 oedema 48 h: 1/20 0/10 (control)	Sensitising	Purity not stated. Vehicle: intradermal induction: 0.9 % aqueous NaCL/Freund' s Complete Adjuvant (FCA) 1:1; topical induction and challenge: ethanol/water mixture (80:20 w/w)	Griffon, B. (2002); report no. 22624TSG

5.5.2 Respiratory system

No data are available. A potential for respiratory sensitisation could be deduced from the findings in the sensitisation test.

5.5.3 Summary and discussion of sensitisation

5.5.3.1 Dossier submitter

While in a Buehler test negative results were obtained, aclonifen caused delayed contact hypersensitivity in guinea pigs in a Magnusson & Kligman skin sensitisation test. With the exception of one animal all induced guinea pigs (95 %) showed a skin reaction after challenge. Based on these data a classification as **R43 "Irritant; May cause sensitisation by skin contact"** is required.

Classification (and Labelling) for sensitisation according to Directive 67/548/EEC:

R43 (Irritant; May cause sensitisation by skin contact)

Classification (and Labelling) for sensitisation according to Regulation (EC) No 1272/2008:

Skin Sens. 1; H317 (May cause an allergic skin reaction)

5.5.3.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter to classify aclonifen for skin sensitisation with Skin Sens. 1 – H317 (CLP) or R43 (DSD). This classification proposal was not questioned during public consultation, but a sub-categorisation under CLP was asked for, in accordance with the 2nd ATP.

For assessment of skin sensitisation 2 guinea pig studies are available. In the Buehler test, 0% of the animals showed a positive response. However, in the GPMT test, 95% of the test animals showed a positive response, compared to 0% of the controls. This is above the threshold of 30% for Skin Sens. 1 – H317 (CLP) or R43 (DSD). As the response is also above the threshold of 60% at an intradermal induction dose of 1%, aclonifen can be considered a strong sensitiser, leading to sub-category 1A under CLP according to the 2nd ATP, as well as the setting of a specific concentration limit (SCL) of 0.1%.

No data are available for respiratory sensitisation.

Based on the data available, RAC supported the proposal of the dossier submitter to classify aclonifen for skin sensitisation. The appropriate classification is:

Skin Sens. 1A – H317: May cause an allergic skin reaction (CLP, taking into account the 2nd ATP)

R43: May cause sensitisation by skin contact (DSD)

SCL: 0.1%

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

Rat

In three 90-day dietary rat studies generally similar effects have been reported.

In the first 90 day rat study (dose levels 0, 50, 500 and 5000 ppm), decreased body weight gain and food consumption and increased water consumption were observed during the treatment period in the top dose group (body weight at necropsy in males and females compared to controls was 84% and 88%, respectively). In females this was reversible after 8-wk recovery, but males still had a lower body weight (94% of control value). Yellow discouloration of the urine was observed at 500 and 5000 ppm, and of the anogenital region and abdomen at 5000 ppm. The following significant changes were observed in the 5000 ppm group: in females, slight increases in alanine aminotransferase (ALT) values (130%), alkaline phosphatase (AP) activities (132%) and bilirubin concentration (142%) as well as decreases in cholesterol level (78%) and total protein (TP) serum levels (96%); in males, slight decreases in AP (88%) and TP (96%), a slight increase in bilirubin (102%) and haematuria (11/12 vs 0 in controls). Gross necropsy revealed enlarged kidneys with granulated to rough, irregular surfaces in males and to a lesser extent in females receiving 5000 ppm. Organ weight analysis revealed increased absolute and relative kidney weights in males (+40% and + 68%, resp.) and increased relative liver weight in males and females of the high dose group (+28% and +4%, resp.). Also in this group, males had significantly reduced absolute weights of heart, lungs, spleen and prostate, and females significant reductions in absolute heart, kidney and adrenal weights which is considered to be a consequence of the reduction in body weight. The only treatment related changes noted during histopathological examination were confined to the kidneys of animals in the high dose group, including tubular nephropathy with haematuria, hydronephrosis and consecutive interstitial nephritis (chronically recurring pyelonephritis). Brownish deposits were found in the tubular epithelium. These appeared to enter the tubular lumen, forming aggregates which were also observed in the renal pelvis and urinary bladder. These changes were pronounced in males of both the treated and reversibility group. Female animals showed a less pronounced and fully reversible nephropathy with moderate hydronephrosis, but no haematuria or interstitial nephritis.
Based on the kidney changes the NOAEL in this study was 500 ppm, equivalent to 26.4 and 29.4 mg/kg bw/day in males and females, respectively.

In a second 90 day rat study (dose levels 0, 50, 500 and 5000 ppm), dark urine and stained fur around the ano-genital area was observed in animals treated at 500 and 5000 ppm. Body weight gain (81%) and food consumption of the females treated at 5000 ppm were lower than those of controls. In haematology a slight but significant decrease in red blood cell count (90%; in 500 ppm

group 96%), haemoglobin (94%) and haematocrit (93%) was noted in males at 5000 ppm while mean corpuscular haemoglobin concentration (104%) was slightly increased. In clinical chemistry, urea (138%) , cholesterol (188%) and albumin (115%) were significantly increased in males at 5000 ppm. Crystals, different from those usually observed in urine were noted at 5000 ppm in 5/10 males and 1/10 female. At 5000 ppm mean urinary volume was increased in males (206%) and to a lesser extent also in females (153%); haematuria and the occurrence of white blood cells were each noted in 2/10 males. At 5000 ppm a high proportion of rats of either sex had pale kidneys; in male rats this was associated with increased absolute (114%) and relative kidney weights (116%). Three male rats showed renal cortical yellowish deposits. Histopathological examination of the kidneys revealed transitional cell hyperplasia of the pelvis in males and females, and necrosis of the papilla in two males at 5000 ppm. At 5000 ppm, liver weight was increased in both sexes (absolute: males 137%, females 112%; relative: 139% for both sexes). In addition, grossly enlarged livers were noted in seven male rats. Histopathology examinations revealed diffuse liver centrilobular hypertrophy in both sexes at 5000 ppm and centrilobular hypertrophy in one male at 500 ppm. In the thyroid there was a dose-related higher incidence of follicular cell hypertrophy in both sexes at 500 and 5000 ppm, but no clear effect was noted on thyroid weight.
Based on the changes observed in the kidney, liver and thyroid the NOAEL was 50 ppm in both sexes, equivalent to approximately 3.6 and 4.2 mg/kg bw/day in males and females, respectively.

In the third 90 day rat study (dose levels 0, 50, 500 and 5000 ppm), body weight and body weight gain were consistently reduced throughout treatment at 5000 ppm. Overall body weight was reduced by 13 % in both sexes with overall body weight gain reduced by 25 % in males and 41 % in females, compared with the controls. Food consumption was reduced throughout treatment in both sexes, about 13 % in males and 20 % in females. Clinical signs consisted of dark yellow urine and stained fur around the ano-genital area in animals at 5000 ppm. Clinical chemistry findings were restricted to the high dose groups and revealed an increase in cholesterol, urea and creatinine concentration, and a decrease in potassium and globulin concentration males. In addition, there was a slight increase in albumin concentration with a consequent increase in albumin/globulin ratio. A decrease in cholesterol concentration was observed in the females. Assessment of the thyroid hormones T_3, T_4 and TSH revealed slight changes at 5000 ppm. These changes were increases in TSH plasma levels associated with a decrease in T_4 levels at weeks 2, 6 and 13 in males and weeks 2 and 6 in females. An increase in urinary volume associated with lower pH and refractive index values was observed in males at 5000 ppm. About half the females in this group also produced an increased amount of urine. Red blood cells were found in 9/10 male urine samples and there was an increase in the number of white blood cells noted in 3/10 male urine samples. At 5000 ppm absolute kidney weight was increased by 74 % and relative kidney weight increased by 102 % in males. Macroscopically, yellow foci were observed in the kidney of 6/10 males and the kidneys of 6/10 males had an abnormal shape. Microscopic renal findings consisted of severe changes in all high dose males, including corticotubular nephrosis, acute to chronic medullary nephritis and necrosis of the papillary tip. No abnormalities were detected in the kidneys of female animals. Absolute liver weight at 5000 ppm was increased by 25 % in males and 18 % in females with relative liver weight being increased by 46 % and 39 % in males and females, respectively. In addition, the livers of 2 males had rounded borders, whilst 4/10 females had dark coloured livers. Centrilobular hepatocellular hypertrophy was noted in all high dose animals of both sexes. Thyroid weight was not affected but follicular hypertrophy of the thyroid gland was observed in 4/10 high dose males.
Based on the changes in kidney, liver and thyroid/thyroid hormones at 5000 ppm, the NOAEL of aclonifen in this 90-day study was 500 ppm in both sexes, equivalent to approximately 29.4 and 36.3 mg/kg bw/day in males and females, respectively.

Mouse

In a mouse 28-day study (dose levels 0, 780, 3125, 12500 and 50000 ppm), aclonifen caused yellow staining of the coat and/or skin and yellow colouration of urine. Clinical signs (including lethargy, body tremors, thin or hunched appearance, piloerection and pallor) were noted at 50000 ppm from week 2 of treatment, with evidence of recovery being present by the final week. Two male and three female mice of the 50000 ppm group died or were sacrificed in extremis. The cause of death could not be clarified. At 50000 ppm, a markedly lower body weight (males -17%, females -20%) and body weight gain (males -63%, females -95%) were observed. Absolute and relative liver weight was increased in both sexes at 12500 (males +19% and+25%, resp.; females +24% and +32%, resp.) and 50000 ppm (males +29% and +57%, resp.; females +9% and +36%, resp.) and there was a decrease in absolute and relative ovary weight in females receiving 50000 ppm (-55% and -45%, resp.). Histopathological examination revealed treatment related effects in the kidneys, liver and ovaries. Changes in the kidney comprised moderate to marked tubular dilation and, occasionally, proximal tubular necrosis, cystic tubules or basophilic epithelium. When considered together with the increases in relative kidney weights (males +8% and +15% at 12500 and 50000 ppm, resp.; females +10 and +33% at 12500 and 50000 ppm, resp.) these results are indicative of a nephrotoxic potential of aclonifen at 12500 and 50000 ppm. In the liver, treatment related changes were noted for all treated male groups and for females which had received 3125 ppm and higher. For animals receiving 50000 ppm these findings consisted of significant panacinar hepatocytic enlargement while the other affected groups showed periacinar hepatocytic enlargement. The ovaries of all female mice receiving 50000 ppm had fewer luteal cells than the controls. The NOAEL was 780 ppm, equivalent to 121.2 mg/kg bw/day and 143.1 mg/kg bw/day in males and females, respectively.

Dog

In dogs the main findings after a 26-week dietary exposure (dose levels 0, 100, 500 and 5000 ppm) to the high dose of 5000 ppm were a significant decrease in body weight gain (males and females lost 0.05 and 0.58 kg, respectively, whereas control males and females gained 0.73 and 1.33 kg, respectively) and increases in lymphoid cells and liver weight in males and females, and non-significant increases in alkaline phosphatase and cholesterol in males. Also the urine had a yellow colour. There were no treatment-related clinical signs, histopathology findings or behavioural changes. The NOEL was 500 ppm (approximately 15 mg/kg bw/day).

Table 5.6-1: Summary of oral repeat dose toxicity

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels ppm (mg/kg bw /d)	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results, Main effects/ Target organs	Remarks	Reference
OECD 408	Oral/diet, 90 days	Rat, Wistar Chbb:THOM; 12M+12F	0-50-500-5000 (M: 0-2.6-26.4-258; F: 0-2.9-29.4-279)	500 (M: 26.4; F: 29.4)	5000 (M: 258; F: 279)	Bw gain↓; food consumption↓; water consumption ↑; ALT, AP, bilirubin ↑ (F); liver: weight ↑ (M); kidney: weight ↑, nephropathy, hydronephrosis , haematuria (M), pyelonephritis (M)	Purity not stated Recovery group: 12 M+12F, 5000 ppm over 8 weeks Kidney toxicity was not reversible in males	Paul, W. (1982); report no 127AB-433-03
OECD 408	Oral/diet 90 days	Rat, SD, 10M+10F	0-50-500-5000 (M: 0-3.6-35.4-341; F: 0-4.2-40.8-390)	50 (M: 3.6; F: 4.2)	500 (M: 35.4; F: 40.8)	RBC ↓; liver: hepatocellular hypertrophy (M); thyroid: follicular cell hypertrophy	Purity 99.9 %	Dange , M. (1997); report no. SA 96097
OECD 408	Oral/diet 90 days	Rat, Wistar RJ:WI(IOPS AF) 10M+10F	0-50-500-5000 (M: 0-2.9-29.4-295; F: 0-3.7-36.3-323)	500 (M: 29.4; F: 36.3)	5000 (M: 295, F: 323)	Bw gain ↓; food consumption ↓; cholesterol ↑ (M), ↓ (F), urea↑ (M), creatinine↑ (M), potassium ↓ (M); liver: weight↑, hepatocellular hypertrophy; kidney: weight↑ (M), urinary volume↑ (M), haematuria (M); nephrosis, nephritis, necrosis of papilla (M); thyroid: follicular cell hypertrophy (M), T4 ↓, TSH ↑	Purity 99.2 %	Wason, S. (2001); report no. SA00458

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels ppm (mg/kg bw /d)	NO(A)EL ppm (mg/kg bw /d)	LO(A)EL ppm (mg/kg bw /d)	Results, Main effects/ Target organs	Remarks	Reference
OECD 407	Oral/diet 28 days	Mouse, Crl:CD-1 12M+12F	0-780-3125-12500-50000 (M: 0-121-481-2003-8906 ; F: 0-143-555-2335-12403)	780 (M: 121 F: 143)	3125 (M: 481; F: 555)	Bw (gain) ↓ at high dose; liver: hepatocyte hypertrophy; kidney: basophilic cortical tubules, and at high dose necrosis and dilatation; ovary: corpora lutea ↓ at high dose	Purity 81.3%	Amyes, S.J. (1988); report no. 87/RHA15 6/711
Similar to OECD 452	Oral/diet, 26 weeks	Dog, Beagle, 4M+4F	0-100-500-5000 (0-3-15-142)	500 (15)	5000 (142)	Bw gain ↓; lymphoid cells ↑; plasma: AP ↑ (M), cholesterol ↑ (M); liver: weight↑	Purity not stated	Paul, W. (1982); document no. 127AB-437-02

The effects in the three rat studies included decreased body weight gain, changes in blood chemistry indicative of liver damage, increases in urinary volume indicative of impairment of kidney function, increased liver and kidney weights, liver and kidney pathology, haematuria, and in two studies also thyroid pathology. Changes were consistently more severe in males than in females, especially in kidney. Liver and kidney were also identified as target organs in the mouse. In addition, a reduction in the number of corpora lutea was seen in mice. Non-neoplastic findings in combined chronic toxicity/carcinogenicity studies in rats and mice (see section 5.8, Table 5.8-4) also included reductions in body weight gain and effects on the liver, with LOAELs of 1600 ppm in rats (equivalent to 61-67 mg/kg bw/d) and 700 ppm in mice (equivalent to 76-80 mg/kg bw/d).

5.6.2 Repeated dose toxicity: inhalation

No data are available. Based on the results of the acute toxicity study and the physical properties of aclonifen, repeated dose inhalation toxicity studies for use as a herbicide have not been required according to the data requirements of directive 91/414/EEC.

5.6.3 Repeated dose toxicity: dermal

Dermal exposure for 4 weeks (dose levels 0, 250, 500 and 1000 mg/kg bw/d) was well tolerated by Sprague-Dawley rats at the local level as aside from yellow colouration of the skin no cutaneous reactions or histopathological findings at the application site were observed. A significant lower body weight (-9%) and body weight gain (-32%) associated with reduced food consumption and lower glucose levels in males and decreased white blood cell counts in males and females were the only findings, observed at the highest dose-level. The NOAEL was 500 mg/kg bw/day.

Table 5.6-2: Summary of dermal repeat dose toxicity

Method/ Guideline	Route of exposure, Duration	Species, Strain, Sex, No/group	Dose levels mg/kg bw/d	NO(A)EL mg/kg bw/d	LO(A)EL mg/kg bw/d	Results, Main effects/ Target organs	Remarks	Reference
OECD 410	Dermal, 28 days	Rat, SD; 10M+10F	0-250-500-1000	500	1000	Bw (gain) ↓ (M); plasma glucose ↓ (M); WBC ↓	Purity 99.2% Vehicle: aqueous 0.5% methyl-cellulose	Chevalier, G. (2002); report no 21601TSR

5.6.4 Other relevant information

No other data are available.

5.6.5 Summary and discussion of repeated dose toxicity

5.6.5.1 Dossier submitter

Liver and kidney have been identified as the main target organs. Toxic effects in these organs appear to be related to concentrations that overwhelm metabolic and/or excretional capacities. No classification for repeated dose toxicity is required.

5.6.5.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for repeated dose toxicity, which was not questioned during public consultation.

For assessment of oral repeated dose toxicity, five studies were available, among which three 90-day studies in rat. Liver and kidney have been identified as the main target organs in rats and mice. In rats, the thyroid was also affected, in mice the ovaries. The lowest NOAEL and LOAEL in the oral repeated dose studies were 3.6 and 35.4 mg/kg bw/day, respectively, in a 90-day rat study. At this LOAEL, some hyperplasia and hypertrophy was noted, without clear effects on organ weights or associated blood and urine parameters. These effects are not considered "significant and/or severe toxicity" in the sense of classification. The next higher LOAEL is 258 mg/kg bw/day. Although at this dose level (and comparable dose levels in the other studies) more significant effects were observed, the dose level is clearly above the 90-day guidance value of 100 mg/kg bw/d for STOT RE 2 – H373 (CLP) and of 50 mg/kg bw/d for Xn; R48/22 (DSD).

For the assessment of dermal repeated dose toxicity, one 28-day study in rats was available. The LOAEL in this study is 1000 mg/kg bw/day. This is clearly above the guidance value of 600 mg/kg bw/ (recalculated for 28 days) for STOT RE 2 – H373 (CLP) and of 300 mg/kg bw/d (recalculated for 28 days) for Xn; R48/21 (DSD).

No studies were available for repeated dose inhalation toxicity.

Based on the data available, RAC supported the conclusion of the dossier submitter that aclonifen should not be classified for repeated dose toxicity.

5.7 Mutagenicity

5.7.1 In vitro data

Aclonifen did not induce gene or chromosome mutations in bacterial or mammalian cell assays and did not provoke unscheduled DNA synthesis.

Table 5.7-1: Summary of in vitro mutagenicity

Method/ Guideline	Test system (Organism, strain)	Concentra-tions tested (give range)	Results		Remarks give information on cytotoxicity and other	Reference
			+ S9	- S9		
Similar to OECD 471 Bacterial Reverse Mutation	*S. typhimurium*: TA1535, TA1537, TA1538, TA98, TA100	0-5000 µg/plate	Negative	Negative	Purity >99%	Kramer, P. J. (1982); report no. 4/42/82
OECD 471 Bacterial Reverse Mutation	*S. typhimurium*: TA1535, TA1537, TA102, TA98, TA100	0-5000 µg/plate	Negative	Negative	Purity 97.3% Bacterial growth inhibition at ≥16 µg/plate; precipitation at 5000 µg/plate	Herbold, B. (2006); report no. AT02825
OECD 473 in vitro Mammalian Chromosome Aberration	Human lymphocytes	0-100 µg/mL +S9 0-30 µg/mL –S9	Negative	Negative	Purity 99.3% Reduced mitotic indices at ≥20 µg/mL –S9 and at 100 µg/mL +S9	Dance, C. A. (1992); report no. 92/RHA477/0471
Similar to OECD 476 in vitro Mammalian Cell Gene Mutation	Chinese hamster lung V79 cells	0-1000 µg/mL +S9 0-25 µg/mL –S9	Negative	Negative	Purity 95.5%	Oesch, F. (1984); report no. SP 579/VT-19
OECD 482 in vitro Mammalian Cell Unscheduled DNA Synthesis	Rat hepatocytes	0-25 µg/mL +S9	Negative	Negative	Purity >93% Cytotoxicity at 25 µg/mL	Meli, C. (1991); report no. 121009-M-03691

5.7.2 In vivo data

Aclonifen was examined for clastogenicity *in vivo* using the mouse micronucleus assay. The high dose level in this assay appeared to be a borderline effective dose for a beginning impairment of erythropoiesis in mice (haematologic variations in some animals were observed). However, aclonifen administration did not produce any increase in the number of micronuclei at any time point.

Table 5.7-2: Summary of in vivo mutagenicity

Method/ Guideline	Species, Strain, Sex, No/group	Route, Frequency of application	Sampling times	Dose levels mg/kg bw	Results	Remarks	Reference
OECD 474 (Micronucle-us assay)	Mouse, NMRI, 5M+5F	Oral, single dose	16, 24, 48 hours	0-578-1650-7260	Negative	Only 24-h timepoint investigated at 578 and 1650 mg/kg bw	Engelhardt, G. (1984); report no. 26M0286/8332

5.7.3 Human data

A test for clastogenicity in vitro was performed with human lymphocytes. Aclonifen was negative in this assay. No other human data are available regarding this endpoint.

5.7.4 Other relevant information

The binding of aclonifen (or metabolites) to liver and urinary bladder DNA and chromatin protein was determined in male CD1 mice after a single oral dose of 900 mg/kg bw (Sagelsdorff, P., 1995, report no. CB95/24). The animals excreted about 34 % of the administered radioactivity in the urine within 24 hours. This amount would have been available for binding to the analytes in bladder cells. Chromatin protein isolated from liver retained 177 - 275 pmol aclonifen-derived material/mg protein; an even higher value (319 pmol/mg protein) was obtained for the bladder. In contrast, no relevant interaction was detected with the DNA.

5.7.5 Summary and discussion of mutagenicity

5.7.5.1 Dossier submitter

Aclonifen did not induce gene mutations in procaryotes or mammalian cell cultures, chromosome aberrations in cultured human lymphocytes or in vivo in bone marrow cells from NMRI mice, nor did it lead to DNA damage in mammalian cells in the *in vitro* UDS assay. Aclonifen (or metabolites) does not bind to DNA *in vivo*, but has been shown to interact with chromatin proteins (specific interaction partners were not identified). Therefore, it may produce epigenetic changes on chromosomes and on gene expression. Taken together, the results demonstrate that aclonifen is not genotoxic and is unlikely to present a genotoxic hazard to humans. Classification for genotoxicity is not required.

5.7.5.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for mutagenicity, which was not questioned during public consultation.
For assessment of mutagenicity, several *in vitro* (bacterial and mammalian cell assays) and one *in vivo* study were available. All studies were negative with regard to mutagenicity. Consequently, RAC supported the conclusion by the dossier submitter that no classification for mutagenicity is necessary.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

Rat

In two 2-year combined chronic/carcinogenicity studies in rats using two different Wistar strains and similar dose levels, females showed reduced body weight gains and food consumption at 1600 ppm. The only clinical sign observed in these studies was a yellow staining of urine, fur and/or tails. The systemic NOAEL in both studies was 200 ppm, equivalent to approximately 8 mg/kg bw/d.

In the first study (dose levels 0, 40, 200 and 1600 ppm), a slight increase in total protein and albumin in males and females at 1600 ppm without a concomitant increase in globulin was observed, as well as reduced triglyceride values. T_4 was reduced at 1600 ppm in males. Necropsy after 24 months of treatment revealed a slight decrease in absolute liver and spleen weights, and an increase in relative liver weight for females receiving 1600 ppm. In the original report, a slightly higher incidence of thyroid C-cell carcinoma was seen in the females of the 200 and 1600 ppm dose group.,The thyroid histological sections were subsequently reviewed twice by consultant pathologists (Grasso, P., 1990; Rittinghausen, S., 1995), using somewhat different classification systems for the pre-neoplastic lesions. These two pathologists noted much higher incidence values in all groups for non-malignant hyperplastic changes while generally fewer C-cell carcinomas were identified (see Table 5.8-1). The overall conclusion from both reviews was that in the absence of any significant dose-related trend, coupled with a biological tendency of this strain of rat to develop both hyperplasia and neoplasia of C-cells with age, the observed tumour incidence should be considered a chance phenomenon and not related to aclonifen administration.

Table 5.8-1: Incidences of thyroid C-cell tumours and lesions

	Males				Females			
Dietary concentration	**0 ppm**	**40 ppm**	**200 ppm**	**1600 ppm**	**0 ppm**	**40 ppm**	**200 ppm**	**1600 ppm**
No. of animals examined	*60*	*60*	*60*	*60*	*60*	*60*	*60*	*60*
C-cell nests	0	2	2	3	1	1	1	1
Grasso (1990) ; C-cell hyperplasia	33	35	30	37	39	40	36	44
Rittinghausen (1995); C-cell diffuse hyperplasia	53	52	46	51	56	54	48*	52
C-cell focal/multifocal hyperplasia	15	15	17	10	15	25*	23*	14
Thyroid C-cell adenoma	1	2	4	2	2	2	0	3
Grasso (1990)	1	5	11#	7	10	9	8	7
Rittinghausen (1995)	2	11**	12**	7	6	7	6	10
Thyroid C-cell carcinoma	1	4	5	2	1	2	7*	6*
Grasso (1990)	1	3	1	2	1	3	1	3
Rittinghausen	1	3	1	2	1	2	5	2

(1995)								

*) $p < 0.05$ and **) $p < 0.01$ (Fisher's exact test)
#) $p < 0.01$ (IARC Peto test)

In the second study (dose levels 0, 20, 40, 200 and 1600 ppm), a slight tendency towards a higher number of urinary crystals was seen at 1600 ppm in males at 18 months, compared with the control group. A slight diffuse centrilobular hepatocellular hypertrophy was found in 4/9 females at 1600 ppm at the 12-month sacrifice. This finding correlated to the higher mean liver weights observed in this group. At the 24-month terminal sacrifice, centrilobular hepatocellular hypertrophy was found in 28/60 males and 17/60 females of the high dose group, the severity ranging from slight to mild in males and slight to moderate in females. A few cases of hepatocellular hypertrophy were also observed in males (4/60) and females (2/60) at 200 ppm. No evidence of an oncogenic effect on the thyroid was seen; there was a tendency towards a higher incidence of C-cell adenomas in treated females but without dose-relationship, and an increase in C-cell carcinomas, as originally reported for the first rat study, was not found (see Table 5.8-2). However, in this study malignant astrocytomas were observed in the brains of 4/60 females at the high dose (1600 ppm) at final necropsy (see Table 5.8-2). Statistically, the incidence of astrocytoma showed a positive trend; however, the incidences were not significantly greater when pairwise comparisons with control females were conducted. This incidence rate was outside the small laboratory data base (7/240 in males and 0/240 in females) and the Registry of Industrial Toxicology Animal Data (4/4061 in males and 1/3963 in females).

Table 5.8-2: Incidences of thyroid C-cell tumours and lesions and of malignant astrocytomas

	Males					Females				
Dietary concentration	**0 ppm**	**20 ppm**	**40 ppm**	**200 ppm**	**1600 ppm**	**0 ppm**	**20 ppm**	**40 ppm**	**200 ppm**	**1600 ppm**
No. of animals examined	*60*	*60*	*60*	*59*	*60*	*60*	*60*	*60*	*60*	*60*
Thyroid										
C-cell focal hyperplasia	12	15	7	7	5	15	7	15	11	7
C-cell adenoma	5	2	5	3	5	1	9	9	7	6
C-cell carcinoma	1	0	0	0	0	2	1	2	1	2
Brain										
Malignant astrocytoma	1	0	1	1	2	0	0	0	1	4*

*) Statistically significant (Trend test $p < 0.01$)

Mouse

In an 80-wk mouse combined chronic/carcinogenicity study (dose levels 0, 70, 700 and 7000 ppm), clinical signs were confined to yellow staining of the coat. Lower body weight gains were observed in male and female mice of the top dose group, as well as in males at the intermediate dose level. Moderate liver enlargement was observed in males and females at 7000 ppm; this was reversible and not associated with any hepatic lesion. Histopathological examination revealed that a specific target organ was the urinary bladder with male mice appearing to be more susceptible than females (see Table 5.8-3). A statistically significant higher incidence of transitional cell hyperplasia was observed in males from the 7000 ppm dosage group after 39 weeks of treatment and in males from both the 700 and 7000 ppm dosage group after 80 weeks of treatment, compared with their respective controls. After the six week reversibility period this change was only occasionally seen.

Similar findings were infrequent or absent in females. A low incidence of chronic inflammation of the urinary bladder was observed among animals from the 7000 ppm dosage group after both 39 weeks and 80 weeks of treatment, with no inflammation observed after 6 weeks recovery following 39 weeks of treatment. At the highest dose (7000 ppm) urinary bladder tumours were found in two males and one female.
The systemic NOAEL was 7.1 mg/kg bw/d based on decreased body weight gain and urinary bladder transitional cell hyperplasia.

Table 5.8-3: Incidences of urinary bladder tumours and lesions

	Males				Females			
Dietary concentration	**0 ppm**	**70 ppm**	**700 ppm**	**7000 ppm**	**0 ppm**	**70 ppm**	**700 ppm**	**7000 ppm**
39 weeks								
No. of animals examined	*12*	*12*	*12*	*12*	*12*	*12*	*11*	*12*
Transitional cell hyperplasia	0	0	1	8*	0	0	0	2
Chronic inflammation	0	0	0	2	0	0	0	4
39 weeks + 6 weeks recovery								
No. of animals examined	*11*	*12*	*10*	*11*	*9*	*9*	*11*	*12*
Transitional cell hyperplasia	0	1	0	2	0	0	0	0
Chronic inflammation	0	0	0	0	0	0	0	0
80 weeks								
No. of animals examined	*48*	*49*	*52*	*50*	*51*	*51*	*52*	*51*
Transitional cell hyperplasia	2	3	10*	31*	1	0	2	2
Chronic inflammation	0	0	0	4	1	2	1	6
Transitional cell papilloma	0	0	0	1	0	0	0	0
Transitional cell carcinoma	0	0	0	1	0	0	0	0
Sarcoma	0	0	0	0	0	0	0	1

*) Statistically significant ($P < 0.05$; Fisher's exact test, Cochran-Armitage trend test)

Upon review of the urinary bladder findings, a consultant pathologist (Grasso, P., 1994) reported an angular outline of some of the urothelial hyperplasia and the presence of crystal-like inclusions within granulomas which may be considered evidence that the response was caused by crystals or calculi. The urothelial hyperplasia mostly resolved after the 6-week reversibility period, but in a few animals they persisted. Although no data are available on the urinary excretion of aclonifen-related material in mice, based on measurements in rats it was suggested that at high doses the concentration in urine can be close to or even above the maximum amount soluble in water. This could indicate that the tumours likely resulted from an inflammatory non-genotoxic mechanism limited to a high dose range which is not relevant for humans.

Table 5.8-4: Summary of oral carcinogenicity

Method/ Guideline Route of exposure	Route of exposure, duration	Species, Strain, Sex, No/group	Dose levels ppm (mg/kg bw/d)	Results Main effects/ Target organs/ Tumors	NO(A)EL ppm (mg/kg bw/d)	LO(A)EL ppm (mg/kg bw/d)	Remarks	Reference
OECD 453	Oral/diet 12 and 24 months	Rat, Wistar Chbb:THOM 10M + 10F 60M + 60F	0-40-200-1600 (M: 0-1.6-8.1-66.9; F: 0-1.7-8.5-67.1)	1600 ppm: bw ↓ (F); total protein ↑, albumin ↑; triglycerides ↓; T4 ↓ (M); abs. liver wt ↓	200 (8)	1600 (67)	combined chronic toxicity/ carcinogenicity study Purity: 95.5%	Kirsch, P., Kühborth, B. (1989); report no. 71S0001/8401 Grasso, P. (1990); report no, 423593 Rittinghausen, S. (1995); report no. 440954/448864
OECD 453	Oral/diet 12 and 24 months	Rat, Wistar WI-IOPS 10M + 10F 60M + 60F	0-20-40-200-1600 (M: 0-0.8-1.5-7.6-61;F: 0-1.1-2.1-11-86)	1600 ppm: bw ↓ (F); liver hypertrophy; malignant astrocytoma in 4/60 (F)	200 (7.6)	1600 (61)	combined chronic toxicity/ carcinogenicity study Purity: 99.2%	Wason, S. (2004); report no. SA 00591
US-EPA Guideline No 83-5	Oral/diet 39 and 80 weeks	Mouse, Crl:CD-1 12M + 12F 24M + 24F for T3, T4 measurement and reversibility 52M + 52F	0-70-700-7000 (M: 0-7.1-75.6-892; F: 0-8.3-80.1-984)	700 ppm: bw gain ↓ (M), urinary bladder transitional cell hyperplasia (10M) 7000 ppm: bw gain ↓; liver wt ↑, urinary bladder transitional cell hyperplasia (31M), urinary bladder inflammation (4M, 6F), bladder tumours (2M, 1F)	70 (M: 7.1; F: 8.3)	700 (M: 75.6; F: 80.1)	combined chronic toxicity/ carcinogenicity study Purity 97.8%	Amyes, S.J. (1991); report no. 89/RHA157/1047 Grasso, P. (1994); report no, 440943

5.8.2 Carcinogenicity: inhalation

No data are available.

5.8.3 Carcinogenicity: dermal

No data are available.

5.8.4 Carcinogenicity: human data

No data are available.

5.8.5 Other relevant information

As described in 5.7.4, aclonifen did not bind to DNA in cells of the urinary bladder and liver of mice *in vivo*. Binding to chromatin proteins, however, was observed, indicating an opportunity to exert effects on epigenetic markers (histone modifications, DNA methylation).

5.8.6 Summary and discussion of carcinogenicity

5.8.6.1 Dossier submitter

In the carcinogenicity study in mice, urinary bladder tumours were found in two males and one female at the highest dose (7000 ppm). Taking into account the lack of genotoxicity and that the kidney is responsible for the excretion of a major part of the dose, these tumours are attributed to the continuous irritation of the tissue at high doses of aclonifen. A similar mechanism can be excluded with respect to the astrocytomas seen in four out of sixty female rats in the high dose group. According to the toxicokinetic data aclonifen/metabolite levels in male and female rat brains are low, even at time points with the highest blood and plasma concentrations; unless astrocytes have a mechanism of concentrating the test substance or unless the blood-brain barrier becomes leaky with age or prolonged treatment, very little exposure should occur. In addition, male rats experience higher blood, plasma and brain levels of aclonifen-related material than females and should therefore be at a larger risk for a tumourigenic effect on astrocytes. Thus no mechanistic explanation could be found. However, due to the rarity of this tumour type in control groups, the finding in female rats remains a concern and is considered as limited evidence of carcinogenicity. Consequently, a classification of aclonifen as a carcinogen is proposed.

Classification (and Labelling) for carcinogenicity according to Directive 67/548/EEC:

Carc. Cat. 3 R40 (Harmful; Limited evidence of a carcinogenic effect)

Classification (and Labelling) for carcinogenicity according to Regulation (EC) No 1272/2008:

Carc. 2; H351 (Suspected of causing cancer)

5.8.6.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter to classify aclonifen for carcinogenicity as Carc. 2 – H351 (CLP) or Carc. Cat. 3; R40 (DSD), based on a low incidence of unusual brain tumours in female rats. There was support for this proposal during public consultation, aside from one Industry association that referred to a position paper. In this position paper (dated February 2006), Industry commented during the peer review consultation of the aclonifen DAR on a similar proposal for classification by the RMS Germany, and considered the brain tumours observed in high dose females to be unlikely related to the administration of

aclonifen. The Industry comments however did not change the opinion of EFSA: EFSA concluded in their final opinion of 2008 that the brain tumours remained of concern, and therefore kept the classification proposal for Carc. Cat. 3; R40.

For assessment of the carcinogenic potential of aclonifen, three combined toxicity/carcinogenicity studies are available, two in rats and one in mice. In the study in mice, urinary bladder tumours were found in two males and one female at the highest dose (7000 ppm). Neither in males or in females the incidence was statistically significantly increased, nor was there a positive trend. Moreover, at 7000 ppm also chronic inflammation was observed in the urinary bladder, as well as transitional cell hyperplasia. Upon review of the urinary bladder histological lesions, signs suggestive of crystal formation were noted that had not been reported in the original study. In rats, where the kidney is responsible for the excretion of a major part of the aclonifen dose, at high doses of 5000 ppm crystals have been observed in urine and aggregated brownish deposits in kidney and urinary bladder. If these data on urinary excretion are applicable for mice as well, crystal formation can be expected at high doses where the urinary concentration of test substance derived material approaches or exceeds the limit of solubility in aqueous media. Taking further into account that aclonifen is not genotoxic, the urinary bladder tumours likely resulted from a persistent irritation/inflammation of the tissue following crystal formation at high doses of aclonifen. All in all, RAC concluded that the urinary bladder tumours observed at 7000 ppm are not relevant for classification.

In one of the rat studies, a slightly higher incidence of thyroid C-cell carcinomas was seen in females without a dose-response relationship. This finding was not confirmed upon two separate histological re-evaluations of the thyroid sections, nor was there evidence of an oncogenic effect on the thyroid in the second rat study. Therefore RAC considered the finding probably unrelated to aclonifen treatment.

In the second rat study, an increased incidence (positive trend) of astrocytomas was observed in brains of females of the high dose group. The incidence in the high dose females (4/60, as compared to 0/60 for the controls) was above the reported historical control incidences. Also in the high dose males, where the incidence of astrocytomas (2/60) was not statistically significantly increased compared to the controls (1/60), the incidence was slightly above the reported historical control incidences. There is no mechanistic explanation for the astrocytoma findings. The toxicokinetic data on aclonifen indicate that aclonifen/metabolite levels in male and female rat brains are low. So, unless astrocytes have a mechanism of concentrating the test substance or unless the blood-brain barrier becomes leaky with age or prolonged treatment, very little exposure should occur. In addition, male rats experience higher blood, plasma and brain levels of aclonifen-related material than females and should therefore be at a larger risk for a tumourigenic effect on astrocytes. But that was not the case in this study. Due to the rarity of this tumour type and the absence of a mechanistic explanation, the finding in female rats remains a concern and is considered as limited evidence of carcinogenicity. Consequently, RAC supported the proposal of the dossier submitter to classify aclonifen for carcinogenicity. The appropriate classification is:

Carc. 2 – H351: Suspected of causing cancer

Carc. Cat. 3; R40: Limited evidence of a carcinogenic effect

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

In a two-generation study only very minor signs of toxicity were seen. In the parental generations slight but statistically significant decreases in body weight and food consumption were noted in F0 males at 2000 and 500 ppm (91-95% of controls) and in F1 males at 2000 ppm (88-89% of controls) during prepairing and postpairing, and in F0 and F1 females at 2000 ppm during prepairing, pregnancy and lactation until necropsy (82-88% of controls). Fertility was normal at all dose levels, and there were no pathological findings. Pup weights at birth and throughout weaning were decreased (by 7-22%) for both the F1- and the F2-generation in the 2000 ppm group. At this dose, also body weight in the dams was reduced. A very small decrement in pup weight (up to minus 6%) was also observed in offspring at 125 and 500 ppm, but this was not regarded adverse. The NOAELs were 125 ppm (equivalent to approximately 8 mg/kg bw/d) for parental toxicity, 500 ppm (equivalent to approximately 35 mg/kg bw/d) for offspring toxicity, and 2000 ppm (equivalent to approximately 120-140 mg/kg bw/d) for reproductive effects.

Table 5.9-1: Summary of effects on fertility

Method/ Guideline	Route of exposure	Species, Strain, Sex, No/group	Dose levels ppm	Critical effect Parental, Offspring (F1, F2)	NO(A)EL Parental toxicity ppm (mg/kg bw/d)	NO(A)EL reproductive toxicity ppm (mg/kg bw/d)	NO(A)EL offspring toxicity ppm (mg/kg bw/d)	Reference
OECD 416	Oral/diet	Rat, Wistar KFM-Han, 25M, 25F	0-125-500-2000 purity 95.5%	P: bw gain ↓ F1, F2: birth wt ↓, bw gain ↓	125 (M: 8; F: 10)	2000 (120-140)	500 (35)	Becker, H. (1985); report no. 030644

5.9.2 Developmental toxicity

Aclonifen was not teratogenic in the rat developmental toxicity study. The NOAEL for maternal and developmental effects of 60 mg/kg bw/d was derived from decreased bw gain of the dams (10% lower body weight at termination compared to controls) and reduced foetal weights (93% compared to controls) at 600 mg/kg bw/d. In rabbits the highest dose level of 25 mg/kg bw/d did not exert any maternal toxicity and as aclonifen was also devoid of any embryotoxicity the maternal and developmental NOAEL were set at this dose.

Table 5.9-2: Summary for developmental toxicity

Method/ Guideline	Route of exposure, Duration	Species, Strain, No/group	Dose levels mg/kg bw	Critical effects 1) dams 2) fetuses	NO(A)EL Maternal toxicity mg/kg bw/d	NO(A)EL Teratogenicity Embryotoxicity mg/kg bw/d	Remarks	Reference
OECD 414	Oral, pregnancy day 6-20	Rat, Wistar Chbb:THOM, 25F	0-6-60-600	1) Bw gain ↓ 2) Bw ↓	60	60	purity not stated Vehicle: 0.5% aqueous Tween 80-0.9% NaCl	Niggeschulze, A. et al. (1982); document no. 127AB-451-02
OECD 414	Oral, pregnancy day 6-18	Rabbit, Chinchilla Hybrid 16F	0-1-5-25	1) - 2) -	25	25	purity 93.2% Vehicle: 2% aqueous carboxy-methyl cellulose	Becker, H. (1984); report no. 025525

5.9.3 Human data

No data are available.

5.9.4 Other relevant information

No other relevant information is available.

5.9.5 Summary and discussion of reproductive toxicity

5.9.5.1 Dossier submitter

Aclonifen did not affect reproduction and influenced developmental parameters only at a dose that also induced systemic effects in the dams. The decrease in the number of corpora lutea observed in the 28-day mouse study at a dose of 12 g/kg bw/day is not considered a specific effect on reproduction. As no specific impairments of fertility and embryo-foetal development have been observed a classification for fertility effects or developmental toxicity is not required.

5.9.5.2 RAC opinion

The evaluation by RAC relates to the proposal of the dossier submitter not to classify aclonifen for reproductive toxicity, which was not questioned during public consultation.

For assessment of the reproductive toxic potential of aclonifen, a 2-generation study in rats and two developmental toxicity studies (one in rat, one in rabbit) are available. Aclonifen did not affect reproductive parameters in the rat, nor was it teratogenic in the rat and rabbit. The only effect observed in these studies was a reduction in foetal (minus 7%) and pup (up to 22%) body weight in the rat developmental toxicity and 2-generation study, respectively, at doses that also induced maternal toxicity (reduced body weight gain of 10% and 12-18%, respectively). The decrease in the number of corpora lutea observed in the 28-day mouse study at 50000 ppm (approximately 12 g/kg

bw/d) is not considered a specific effect on reproduction when occurring at such a high dose level. As no specific impairments of fertility and embryo-foetal development have been observed, RAC supported the conclusion of the dossier submitter that aclonifen should not be classified for fertility effects or developmental toxicity.

5.10 Other effects

5.10.1 Neurotoxicity

Based on the chemical structure and the mode of action of aclonifen no neurotoxicity studies are necessary. Evaluation of neurotoxicity endpoints (various reflex tests) in a 90-day dietary study in rats (Wason, S., 2001, report no. SA00458) did not reveal any evidence of a neurotoxic potential.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

Aclonifen is not sensitive to heat, shock or friction.

6.1.1 Dossier submitter

Aclonifen (technical) is not explosive in the sense of EEC method A14.

6.1.2 RAC opinion

Based on the available information on explosivity, RAC concluded that aclonifen does not need to be classified as explosive.

6.2 Flammability

On contact by the hot wire, technical aclonifen melted, but no flame was observed. Technical aclonifen melted at about 85 °C, no autoinflammation occurred.

6.2.1 Dossier submitter

Aclonifen (technical) is not highly flammable in the sense of EEC method A10.

6.2.2 RAC opinion

Based on the available information on flammability, RAC concluded that aclonifen does not need to be classified as flammable.

6.3 Oxidising potential

A mixture of 40/60 % aclonifen/cellulose gave reproducibly higher burning rates than $BaNO_3$/ cellulose. When cellulose was replaced by silica, the flame rapidly extinguished.
Under nitrogen the test mixture did not burn.

6.3.1 Dossier submitter

Aclonifen (technical) has no oxidising properties in the sense of EEC method A17.

6.3.2 RAC opinion

Based on the available information on oxidising potential, RAC concluded that aclonifen does not need to be classified as oxidising.

7 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard assessment for aclonifen is based on the Draft Assessment Report and Proposed Decision of Germany prepared in the context of the possible inclusion of aclonifen in Annex I of Council Directive 91/414/EEC (EFSA, 2008; DAR August 2006 + Final addendum to DAR June 2008. The references cited below are from the DAR, volume 3, part B.9 (Ecotoxicology).

7.1 Aquatic compartment

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

The acute toxicity of aclonifen to fish is summarised in Table 7.1-1.

Table 7.1-1: Acute toxicity of aclonifen to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 203	*Oncorhynchus mykiss*	flow through	96	LC_{50}	0.67 nom	Douglas M.T. et al. (1991), Document No: R007151
OECD 203	*Cyprinus carpio*	flow through	96	LC_{50}	1.7 m.m.[1)]	Douglas, M.T. et al. (1991), Document No: R007155

[1)] m.m. … mean measured

Long-term toxicity to fish

The long term toxicity of aclonifen to fish is summarised in Table 7.1-2.

Table 7.1-2: Long-term toxicity of aclonifen to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 204	*Oncorhynchus mykiss*	flow through	21	NOEC	0.01 nom	Douglas M.T. et al. (1991), Document No: R007156
OECD 204	*Oncorhynchus mykiss*	flow through	21	NOEC	0.009 nom	Jenkins, C.A. (1993), Document No.: R007413
OECD 210, USEPA 72-4	*Pimephales promelas*	ELS, flow through	35	NOEC	0.005 nom growth 0.011 nom hatch	Mc Elligott, A. (1997), Document No.: R007440

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The acute toxicity of aclonifen to invertebrates is summarised in Table 7.1-3

Table 7.1-3: Acute toxicity of aclonifen to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 202, part 1	*Daphnia magna*	static	48	EC_{50}	1.2 nom	Douglas M.T. et al. (1991), Document No: R007149

Long-term toxicity to aquatic invertebrates

The long-term toxicity of aclonifen to invertebrates is summarized in Table 7.1-4.

Table 7.1-4: Long-term toxicity of aclonifen to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 202, part 2	*Daphnia magna*	Semi-static	21	NOEC	0.016 m.m.[1)]	Douglas M.T. et al. (1991), Document No: R007153

[1)] m.m. … mean measured

7.1.1.3 Algae and aquatic plants

The toxicity of aclonifen to algae and aquatic plants is summarised in Table 7.1-5

Table 7.1-5: Toxicity of aclonifen to algae and aquatic plants

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 201	*Desmodesmus subspicatus*	static	96	E_rC_{50} NOEC	0.0069 nom 0.0025 nom	Handley, J.W. et al. (1990), Document No: R007145
OECD 201	*Navicula pelliculosa*	static	72	E_rC_{50} NOErC	1.2 m.m.[1)] 0.23 m.m.[1)]	Hoberg, J.R. (1998), Document No.: R005692
USEPA (= EPA) 122-2, USEPA (= EPA) 123-2	*Lemna gibba*	static	14 d	E_rC_{50} NOErC	0.012 m.m.[1)] 0.0012 m.m.[1)]	Hoberg, J.R. (1998), Document No.: R005693

[1)] m.m. … mean measured

The study with algae *Desmodesmus subspicatus* can be regarded as the key study for the acute aquatic toxicity of aclonifen and hence for classification and labelling. The study with *Lemna g*ibba gives the lowest toxicity value for chronic toxicity, the concentration being close to the NOEC value from the *Desmodesmus subspicatus* test. Therefore the studies are presented in more detail below:

Toxicity of aclonifen to *Desmodesmus subspicatus*

Author: Handley, J.W. et al. (1990)

Report: The algistatic activity of aclonifen CME 127. Rhone-Poulenc; Safepharm Laboratories Limited, Derby U.K.
Report No.: 423883; 282/53; AT282/001; unpublished report
Document No: R007145
Guidelines: OECD 201
Deviations: None
GLP/GEP: Yes
Validity: Acceptable

Material and methods:

Test substance: aclonifen, CME 127 (batch n° DA 618), appearance: yellow powder, purity: 91.3 %.
Algal cultures of *Desmodesmus subspicatus* were exposed to nominal concentrations of aclonifen equal to 1.25, 2.5, 5, 10 and 20 µg/L plus a control and a solvent control (100 acetone µL/L), each in triplicate, over a 96 hour period. The test was conducted in 250 mL conical flasks containing 100 mL test solution. At initiation of the study the culture contained a nominal cell density of 6.47 x 104 cells/mL. Test chambers were held at a temperature of 24 °C. The target light intensity was approximately 8000 lux. The test media were not aerated.

Measurements of growth were performed at 0, 24, 48, 72 and 96 hours. Mean cell density of the controls was determined at test initiation and termination. pH values were recorded at 0 and 96 hours. The nominal concentrations of aclonifen were verified by chemical analysis (HPLC method) at 0 and 96 hours.

Findings:

The measured pH at initiation was 7.9 and 8.0 to 8.9 at test termination. Analytical measurements showed actual test levels to be equal or somewhat in excess of the nominal values (average mean measured concentration over the study period = 106 % of the nominal values). Measured concentrations ranged 97.3 % to 124 % of the nominal concentrations at study initiation and 90.8 to 109.0 % after 96 hours, showing the substance to be stable in water under the conditions of the test.

All results are expressed in terms of nominal rather than actual measured test levels.

The percent of inhibition after 96 hours of incubation were indicated in the following table:

Table 7.1-6: Percent of inhibition after 96 hours of incubation

Nominal concentration (mg/L)	Percent inhibition based on (%)	
	Area under the growth curve	Growth rate
Control	-	-
Solvent control	-	-
0.00125	1	1
0.0025	4	9
0.0050	29	24
0.010	72	68
0.020	93	83

Algal growth was inhibited at levels of 5 µg/L and above. Microscopical examination of algal cells revealed no aberrations indicating aclonifen to exert an algistatic rather than an algicidal effect.

Conclusion:

Based on nominal concentrations, confirmed by chemical analysis, the 96 hour E_bC_{50} (based on biomass) and E_rC_{50} (based on growth rate) values in *Desmodesmus subspicatus* were highly similar at 6.7 and 6.9 µg/L, respectively. The 96-hour NOEC was determined to be 2.5 µg/L.

Toxicity of aclonifen to *Lemna gibba*

Author: Hoberg, J.R. (1998)
Report: ACLONIFEN - Toxicity to the duckweed, *Lemna gibba*. Rhone-Poulenc; Springborn Laboratories, Inc. (SLS), USA
Report No.: R005693; 603258; 10566.0398.6485.410; 98-7-7411; unpublished report
Doc ID: WAT1999-114
Guidelines: USEPA (= EPA) 122-2, USEPA (= EPA) 123-2
Deviations: None
GLP/GEP: Yes
Validity: Acceptable

Material and methods:
Test substance: Aclonifen (lot n°97013/03) in the form of yellow powder, purity: 994 g/kg.
A total of 5 plants with three fronds each were exposed to six concentrations of aclonifen (0.00078, 0.0016, 0.0030, 0.0063, 0.013 and 0.025 mg/L), plus a solvent control (dimethylformamide, DMF, 0.1 mL/L) and a dilution water control for a period of 14 days. Solution renewals were performed on Days 3, 6, 9 and 12. The test was conducted in an environmental chamber controlled to maintain a temperature of 25 ± 1 °C and continuous lighting with an intensity of 3200 to 5400 lux. On days 3, 6, 9, 12 and 14 fronds were counted and observations were made. At test termination, frond densities for each replicate treatment, control and solvent control were determined. Dry weight of the fronds per treatment level was also determined on Day 14. Effect criteria for EC_{50} calculations were reduction in frond density and biomass (dry weight) after 14 days of exposure (relative to the pooled control). Temperature was measured continuously in an additional flask adjacent to the test flask. Light intensity was measured at test initiation and daily during the exposure period. pH of all exposure solutions was measured at test initiation, at each 3-day interval (in both old and new exposure solutions), and at test termination. At the beginning and end of one renewal period (i.e., day 0 and day 3), one sample from each treatment, control and solvent control solution was analysed for aclonifen concentration by gas chromatography method.

Findings:
Exposure test conditions were recorded as follows: pH of the new and aged exposure solutions ranged from 4.9 to 6.6, temperature ranged from 24 to 25 °C and light intensity ranged from 3200 to 5400 lux. Mean measured test concentrations ranged from 66 to 90 % of the nominal concentrations and defined the treatment levels tested as 0.00070, 0.0012, 0.0020, 0.0049, 0.011 and 0.020 mg/L.

The 14-day percent inhibition, based on frond density and biomass (dry weight), were indicated in the following table:

Table 7.1-7 14-day percent inhibition, based on frond density and biomass (dry weight)

Mean measured concentration (mg as/L)	14-day inhibition (%) based on	
	Frond density	Biomass
Control	-	-
Solvent control	-	-
Pooled control	-	-
0.00070	-1.5	-24
0.0012	-4.1	-48
0.0020	6.8	19
0.0049	18	56
0.011	50	87
0.020	86	90

At test termination, slightly chlorotic to chlorotic small fronds and fronds with less root formation were observed in the 0.011 and 0.020 mg as/L test solutions. Small fronds were observed on the 0.0049 mg as/L treatment level. Fronds exposed to the remaining treatment levels, the control and solvent control were observed to be normal. A significant reduction in frond density was detected in treatment levels ≥ 0.0020 mg as/L as compared to the pooled control.

The reduction in frond biomass at the 0.002 mg as/L and at higher concentrations, represented an inhibition of 19 to 90 %, as compared to pooled control.

Conclusion:
Based on mean measured concentrations, the 14-day E_rC_{50} value for frond density was calculated to be 0.012 (95 % confidence limits: 0.010 to 0.014) mg/L. The 14-day NOEC was determined to be 0.0012 mg as/L.
Based on mean measured concentrations, the 14-day E_bC_{50} value for biomass was calculated to be 0.0060 (95 % confidence limits: 0.0022 to 0.017) mg/L. The 14-day NOEC was empirically estimated to be 0.0012 mg as/L.

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

Not relevant for this type of dossier.

7.3 Atmospheric compartment

Not relevant for this type of dossier.

7.4 Microbiological activity in sewage treatment systems

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

7.6.1 *Dossier submitter*

Aclonifen is hydrolytically stable. Aclonifen was found to be not readily biodegradable within 28 days in the Sturm test (OECD guideline 301B).

Aclonifen has a log Kow of 4.37. In a BCF study, a BCF value of 2896 was obtained based on plateau total radioactive residue in whole fish and average total radioactive residue in water, whereas a BCF value of 2248 was obtained based on uptake and elimination rate constants.

Aclonifen shows a high toxicity to algae (ErC_{50} = 0.0069 mg/L) and aquatic plants (ErC_{50} = 0.012 mg/L). The lowest endpoints in long- term studies were observed with fish (35-d early life stage study NOEC = 0.005 mg/L). The toxicity of aclonifen to fish and invertebrates is in the mg/L range with a toxicity of LC_{50} = 0.67 mg/L to fish and of EC_{50} = 1.2 mg/L to invertebrates.

Conclusion of environmental classification according to Directive 67/548/EEC

In aquatic toxicity studies, ErC_{50} values for algae and aquatic plants and LC_{50} value for fish were obtained at aclonifen concentrations < 1 mg/L. Aclonifen is not readily biodegradable according to the Sturm test (OECD 301B) and the simulation tests (EU (=EEC) 95/36/EC (1995) and SETAC 1.1 (1995). Aclonifen has a log Kow of 4.37. The experimentally derived steady state BCF of 2896 and kinetic BCF of 2248 are above the trigger of 100 (criterion for bioaccumulating potential conform Directive 67/548/EEC). Aclonifen therefore fulfils the criteria for classification with N; R50/53.

Based on the toxicity data for *Desmodesmus subspicatus* (ErC_{50} 0.0069 mg/L) the following specific concentration limits should be applied:

Concentration	Classification
$C \geq 0.25\%$	N; R50/53
$0.025\% \leq C < 0.25\%$	N; R51/53
$0.0025\% \leq C < 0.025\%$	R52/53

where C is the concentration of aclonifen in the preparation.

Conclusion of environmental classification according to Regulation (EC) No 1272/2008

In aquatic toxicity studies, ErC_{50} values for algae and aquatic plants and LC_{50} value for fish were obtained at aclonifen concentrations < 1 mg/L. Aclonifen is not readily biodegradable according to the Sturm test (OECD 301B) and the simulation tests (EU (=EEC) 95/36/EC (1995) and SETAC 1.1 (1995). The experimentally derived steady state BCF of 2896 and kinetic BCF of 2248 are above the trigger of 500 (criterion for bioaccumulating potential conform Regulation (EC) No 1272/2008). Aclonifen therefore fulfils the criteria for classification as aquatic environmental hazard acute category 1, H400 and aquatic environmental hazard chronic category 1, H410.

The M-factor for aclonifen is 100. This value is based on ErC_{50} value of 0.0069 mg/L obtained for the algae *Desmodesmus subspicatus* in a 96-h static study.

7.6.2 *RAC Opinion*

The evaluation by RAC relates to the classification proposal of the dossier submitter to keep unchanged the existing harmonised classification for aquatic acute and chronic toxicity, but to add an M-factor of 100 and corresponding SCLs. This classification proposal was not questioned during public consultation, except for the M-factor where comments suggested M-factors of 100 and 10 for the short-term and long-term hazard category, respectively. RAC concluded the following.

Aclonifen is hydrolytically stable. Aclonifen was found to be not readily biodegradable within 28 days in the Sturm test (OECD guideline 301B). In a water/sediment study aclonifen is metabolised at a moderate rate (DT50s of 11.2 and 17.3 days) but there was neglible mineralisation. In a soil degradation study the DT50s for aclonifen ranged from 41.9 days to 93.6 days. Mineralisation was negligible or very low. There is no information on the degradation products in either study. Aclonifen has a log Kow of 4.37. In a BCF study, a BCF value of 2896 was obtained based on plateau total radioactive residue in whole fish and average total radioactive residue in water, whereas a BCF value of 2248 was obtained based on uptake and elimination rate constants.

Aclonifen shows a high acute toxicity to algae (ErC_{50} = 0.0069 mg/L) and aquatic plants (ErC_{50} = 0.012 mg/L). The acute toxicity of aclonifen to fish and invertebrates is in the mg/L range with an LC_{50} = 0.67 mg/L to fish and an EC_{50} = 1.2 mg/L to invertebrates. The lowest toxicity values in chronic studies were a 35-day NOEC to fish of 0.005 mg/L, a 21-day NOEC to *Daphnia* of 0.016 mg/L, a 96-h NOEC to algae of 0.0025 mg/l, and a 14-day NOErC to the aquatic plant *Lemna* of 0.0012 mg/L.

According to the CLP Regulation the aquatic plant growth inhibition tests are normally considered as chronic tests but the EC50s are treated as acute values for classification purposes.

Conclusion of environmental classification according to Regulation (EC) No 1272/2008, taking into account the 2nd ATP

In aquatic toxicity studies, ErC_{50} values for algae and aquatic plants and LC_{50} value for fish were obtained at aclonifen concentrations < 1 mg/L. The chronic toxicity values for the three trophic levels vary from 0.0012 to 0.016 mg/L aclonifen, and are below the cut-off value of 0.1 mg/L. Aclonifen is not rapidly biodegradable. The experimentally derived steady state BCF of 2896 and kinetic BCF of 2248 are above the trigger of 500. Aclonifen therefore fulfils the criteria for classification as hazardous to the aquatic environment, acute category 1, H400 (Very toxic to aquatic life) and chronic category 1, H410 (Very toxic to aquatic life with long lasting effects).

The M-factor for aclonifen for the short-term hazard category is 100. This value is based on ErC_{50} value of 0.0069mg/L obtained for the algae *Desmodesmus subspicatus* in a 96-h static study.

The M-factor for long-term hazard is 10, based on the NOErC to *Lemna gibba* of 0.0012 mg/L.

Conclusion of environmental classification according to Directive 67/548/EEC

In aquatic toxicity studies, ErC_{50} values for algae and aquatic plants and LC_{50} value for fish were obtained at aclonifen concentrations < 1 mg/L. Aclonifen is not readily biodegradable. Aclonifen has a log Kow of 4.37. The experimentally derived steady state BCF of 2896 and kinetic BCF of 2248 are above the trigger of 100. Aclonifen therefore fulfils the criteria for classification with N; R50/53 (Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment).

Based on the toxicity data for *Desmodesmus subspicatus* (ErC_{50} 0.0069 mg/L) the following specific concentration limits should be applied:

Concentration	Classification
$C \geq 0.25\%$	N; R50/53
$0.025\% \leq C < 0.25\%$	N; R51/53
$0.0025\% \leq C < 0.025\%$	R52/53

where C is the concentration of aclonifen in the preparation.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Aclonifen is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation (EC) No 1272/2008 article 36.2).

OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of the active substance aclonifen according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DAR and the final addendum to the DAR. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR and its addendum.

REFERENCES

DAR (2006). Draft Assessment Report on Aclonifen (RMS Germany). Volume 3, parts B.6 (Toxicology), B.8 (Environmental fate and behaviour) and B.9 (Ecotoxicology), August 2006.

DAR (2008). Final addendum to the Draft Assessment Report on Aclonifen (RMS Germany), June 2008.

EFSA (2008). Conclusion regarding the peer review of the pesticide risk assessment of the active substance Aclonifen. *EFSA Scientific Report* 149, 1-80.