Annex VI report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **Acequinocyl**

EC Number: **611-595-7**

CAS Number: **57960-19-7**

Submitted by: **Bureau REACH, RIVM, The Netherlands, bureau-reach@ rivm.nl**

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Substance Name:	**Acequinocyl**
EC Number:	611-595-7
CAS number:	57960-19-7
Other identifier:	CIPAC 760 (collaborative international pesticides analytical council)
Purity:	≥95.5% w/w acequinocyl
Impurities:	Based on the available environmental and (eco)toxicological information, there are no relevant impurities

Proposed classification based on Directive 67/548/EEC:

Phys/Chem hazards:

-

Health hazards:

Xi; R37, R43

Environment:

N; R50/53

Eventual classification of acequinocyl for developmental effects using read-across from warfarin should be discussed together with the other coumarines.

Proposed classification based on Regulation EC 1272/2008:

Phys/Chem hazards:

-

Health hazards:

Skin Sens. 1	H317
STOT SE 1	H370
STOT RE 2	H373

Environment:

Aquatic acute 1	H400

Eventual classification of acequinocyl for developmental effects using read-across from warfarin should be discussed together with the other coumarines.

Proposed labelling:

Directive 67/548/EEC:

Symbol : Xi, N

Risk phrases : 37-43-50/53

Safety phrases : (2-)24-37-60-61

Regulation EC 1272/2008:

Pictogram : GHS08, GHS09

Signal word : Danger

Hazard statement codes : H317: May cause an allergic skin reaction

H370: Causes damage to organs (lung) after inhalatory exposure

H373: May cause damage to organs through prolonged or repeated exposure.

H400: Very toxic to aquatic life

Precautionary statements : Not required as PS are not included in Annex VI.

Proposed specific concentration limits (if any):

M-factor according to Directive 67/548/EEC and Regulation EC 1272/2008:

The M-factor is 1000 based on an IC50 value of 0.93 µg/L obtained for the marine crustacean *Mysidopsis bahia* in a 96-h flow-through study.

Proposed notes (if any):

None

JUSTIFICATION

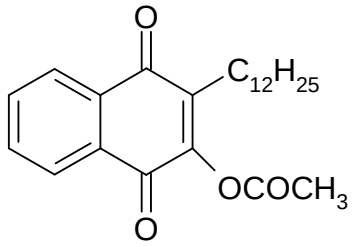
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	2-(acetyloxy)-3-dodecyl-1,4-naphtalenedione
EC Name:	611-595-7
CAS Number:	57960-19-7
IUPAC Name:	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
ISO name:	Acequinocyl
Other identifier	CIPAC 760
Manufacturer's development code number	AKD-2023

1.2 Composition of the substance

Main constituent:

Chemical Name:	2-(acetyloxy)-3-dodecyl-1,4-naphtalenedione
EC Number:	611-595-7
CAS Number:	57960-19-7
IUPAC Name:	3-dodecyl-1,4-dihydro-1,4-dioxo-2-naphthyl acetate
Molecular Formula:	$C_{24}H_{32}O_4$
Structural Formula:	
Molecular Weight:	384.5
Typical concentration (% w/w):	
Concentration range (% w/w):	≥ 95.5%

Impurities:

The identity of the impurities is confidential. The impurities are considered not relevant for classification and labelling.

Testmaterial

All studies were performed with the technical form of acequinocyl which had a purity of 95.5% or

higher unless stated otherwise.

1.3 Physico-chemical properties

Table 1.3.1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	Light brown flakes/solid (98.25%) Soft yellow crystals (99.9%)
VII, 7.2	Melting/freezing point	3.2	59.6 °C
VII, 7.3	Boiling point	3.3	It was concluded that the substance has no boiling point below 200°C, as decomposition takes place above 200°C
VII, 7.4	Relative density	3.4 density	D420 = 1.13 (98.25%, isopropyl alcohol used as immersion liquid) D420 = 1.154 (99.5%, sodium lauryl sulphate used as immersion liquid)
VII, 7.5	Vapour pressure	3.6	1.69 x 10^{-6} Pa at 25°C (extrapolated)
VII, 7.6	Surface tension	3.10	Not determined (solubility in water is < 1 mg/L)
VII, 7.7	Water solubility	3.8	6.69 x 10^{-6} g/L at 25°C in distilled water
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	Log K_{ow} > 6.2 at 25°C
VII, 7.9	Flash point	3.11	no data
VII, 7.10	Flammability	3.13	Not highly flammable
VII, 7.11	Explosive properties	3.14	No explosive properties
VII, 7.12	Self-ignition temperature		No self ignition up to 450°C
VII, 7.13	Oxidising properties	3.15	Non-oxidising
VII, 7.14	Granulometry	3.5	no data
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	solvent: solubility at 20°C (g/L) acetone: > 250 1,2-dichloroethane: > 250 ethyl acetate: > 250 n-heptane: 36.0 methanol: 6.1 n-octanol: 29.2 xylene: > 250
XI, 7.16	Dissociation constant	3.21	No dissociation constant could be determined
XI, 7.17	Viscosity	3.22	no data
	Reactivity towards container material	3.18	no data
	Thermal stability	3.19	no data
	Hydrolytic stability (DT_{50})		pH: Hydrolysis 1.2: DT_{50} = 19 days (37°C) 4: DT_{50} = 74 days (25°C) 7: DT_{50} = 53 hours (25°C) 9: DT_{50} = 76 minutes (25°C)

	Volatility, Henry's law constant		9.7×10^{-2} Pa m^3 mol^{-1}
	Photostability (DT_{50}) (aqueous, sunlight, state pH)		Xenon lamp with 290 nm cut off filter Acetate buffer at pH 5, at 25°C DT_{50} = 14 minutes
	Quantum yield		Φ = 0.065 (Xenon lamp with 290 nm filter, pH 5, p-nitroacetophenone/pyridine actinometer
	UV/VIS absorption (max.)		λmax (nm) / ε ($L.mol^{-1}.cm^{-1}$) Acidic (0.1 M HCL in methanol/ water 90/10): 242 / 16524; 248 / 16989; 270 / 13905; 335 / 2836 Neutral (methanol/ water 90/10): 242 / 16582; 248 / 16873; 270 / 13207; 271 / 2851 Basic (0.1 M NaOH in methanol/water 90/10): 232 / 19055; 245 / 13149; 275 / 2172; 362 / 8999

The above data are obtained from the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of acequinocyl in Annex I of Council Directive 91/414/EEC (DAR September 2007 + addendum January 2008, RMS The Netherlands).

2 MANUFACTURE AND USES

Not relevant for this type of report.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of EC 1272/2008

Acequinocyl is not included in Annex VI of EC 1272/2008.

4 ENVIRONMENTAL FATE PROPERTIES

The environmental fate properties assessment for acequinocyl is based on the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of acequinocyl in Annex I of Council Directive 91/414/EEC (DAR September 2007 + addendum January 2008, RMS The Netherlands) on the inclusion of acequinocyl in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

All tables in the present assessment are copied from the DAR or the addendum to the DAR. The tables are renumbered in accordance with the paragraph numbers.

4.1 Degradation

4.1.1 Stability

Hydrolysis

Hydrolysis studies were carried out at two temperatures (15 and 25ºC) and at several pH levels. Hydrolysis increased in the order of pH 4 < pH 7 < pH 9. Under acid conditions the hydrolysis is slower. The most significant hydrolysis product was 2-dodecyl-3-hydroxy-1,4-naphathalenedione (referred to R1 in Table 4.1). The half life times for hydrolysis are given in Table 4.1.

Table 4.1: Half life times for the hydrolysis of acequinocyl at 15ºC and 25ºC.

	First hydrolysis test (carried out at 15℃) [1)]			Second hydrolysis test (carried out at 25℃)	
pH value	**Max. proportion of metabolite R1**	**Calculated DT50**	**Corrected for 20℃**	**Calculated DT50**	**Corrected for 20℃**
pH = 1.2	29% (after 12 d)			19 days[2)]	73 days[2)]
pH = 4	23% (after 30 d)	102 days[2)]	70 days[2)]	74 days[2)]	110 days[2)]
pH = 7	55% (after 96 h)	53 hours	36 hours	52 hours	77 hours
pH = 9	49% (after 90 min)	78 minutes	53 minutes	67 minutes	99 Minutes

1) The test at pH 1.2 was carried out at a temperature of 37ºC.

2) Extrapolated value

Photolysis in water

In one study photolytic half life was determined in two parallel buffer systems exposed in a Suntest apparatus. Half lives of 38 and 46 minutes were found. The contribution of dark reactions was negligible. In a second study in a sterilised buffer system the estimated half life in the dark control was 16 days. As a result of exposure to the xenon lamp system half life was about 14 minutes in the sterilised buffer system. In the river water system (pH 7.8) the estimated half life in the dark control was 8 hours. For the irradiated river water system the half life was calculated to be 12 minutes.

Photolysis in soil

A photolysis study was carried out with [^{14}C-phenyl] acequinocyl, applied at a sandy loam soil and subsequently incubated for 13 days under irradiated and non-irradiated conditions at a temperature of 25 ± 2°C. Table 4.2 presents the calculated DT50 and DT90 values that were calculated for acequinocyl. The DT50 values of irradiated and non-irradiated soil are within the same range, which indicates that photolysis is not a major process in the degradation of acequinocyl.

Table 4.2: DT50 values and DT90 values for [^{14}C-phenyl] acequinocyl in irradiated and non-irradiated soil (analysed by HPLC and TLC).

	Irradiated soil		**Non-irradiated soil**	
	DT50 (d)	**DT90(d)**	**DT50 (d)**	**DT90 (d)**
HPLC	2.6	8.5	1.8	6.0
TLC	1.8	5.8	1.4	4.7

Photo-oxidative degradation in air

A theoretical calculation of the photo-oxidation of acequinocyl in the atmosphere, using the method of Atkinson (1989), updated by Kwok & Atksinson (1995), gave a DT50 of 1.21 hours, assuming 12 hour light per day, suggesting that the concentrations of acequinocyl in air are negligible.

4.1.2 Biodegradation

4.1.2.1 Screening test

Readily biodegradability

In a 28d ready biodegradability CO_2 evolution test (Modified Sturm test), the maximum biodegradability of acequinocyl in duplicate flasks was 31.0 and 22.3%. The results from this test demonstrate that the inoculum that was used for this test had sufficient activity. In the sterile control, less CO_2 than in the blank control flask was released, which indicates that under abiotic conditions, no mineralization of acequinocyl had taken place. It is concluded that acequinocyl can be regarded as not readily biodegradable.

4.1.2.2 Simulation tests

Biodegradation in water/sediment systems

Aerobic water/sediment system

An aerobic water/sediment study with two sediment systems using labelled acequinocyl was conducted at 20°C with two water sediment systems. The sediments were classified according to the German soil classification (DIN) as clay loam soil and clay soil, respectively. The DT50 values in the whole systems were 0.47 and 0.42 days, respectively. The summarized results are given in Table 4.3. In the water phase, 2-dodecyl-3-hydroxy-1,4-naphtalenedione and CBAA (2-carboxy-α-oxo-benzene acetic acid) were identified as major metabolites. In sediment extracts, AKM-18 (2-(1',2'-dioxotetradecyl) benzoic acid) was identified as the major metabolite and 2-dodecyl-3-hydroxy-1,4-naphtalenedione as a minor metabolite. From the results it is concluded, that [^{14}C-phenyl] acequinocyl and its metabolites degrade rapidly in both systems.

Table 4.3: DT50 values and DT90 values for [^{14}C-phenyl] acequinocyl in water/ sediment systems (estimated/ calculated from the results of the main experiment).

	Bury Pond system (clay loam soil)		**Houghton Meadow system (clay soil)**	
	DT50 (d)	**DT90(d)**	**DT50 (d)**	**DT90(d)**
Water	< 0.25[1)]	< 2[1)]	< 0.75[1)]	< 2[1)]
System	0.47	1.6	0.42	1.4

1) Reliable kinetic calculations were not considered possible.

Biodegradation in soil

Aerobic degradation

In the studied soils, acequinocyl was rapidly degraded. The DT50 values at 20°C ranged between 1.1 and 2.8 days. The DT50 value at 10°C in Clay loam soil was 1.9 days compared to a DT50 of 1 days at 20°C. The summarised results are presented in Table 4.4. The major degradation product was CO_2, indicating that the dissipation of acequinocyl is due to biodegradation. This is confirmed by the slow degradation in sterile soils. In the non-sterile soils two major metabolites are formed: acequinocyl-OH and AKM-18 at a maximum of 33.8% and 43.4%, respectively. Also these metabolites degraded very rapidly with an estimated mean DT50 value of around 7 days and 3.5 days, respectively.

Table 4.4 DT50 values and DT90 values (in days) for with [^{14}C-phenyl] acequinocyl and [^{14}C-dodecyl] acequinocyl under aerobic conditions[1)].

Temperature	**Radiolabelled form**	**Clay loam**		**Sand**		**Sandy loam**		**Silt loam**	
		DT50	**DT90**	**DT50**	**DT90**	**DT50**	**DT90**	**DT50**	**DT90**
20°C	[^{14}C-dodecyl] acequinocyl	n.d.	n.d.	n.d.	n.d.	2.1	6.8	2.6	8.6
	[^{14}C-phenyl] acequinocyl	1.1	3.6	2.0	6.8	2.2 (86)	7.4 (285)	2.8	9.2
10°C	[^{14}C-phenyl] acequinocyl	1.9	6.2						

Values between parentheses represent the DT50 value and DT90 value in sterile soil.

n.d. = not determined

Anaerobic degradation

In an anaerobic soil degradation study with [^{14}C-phenyl] acequinocyl, applied at a flooded sandy loam soil and subsequently incubated for 365 days under anaerobic conditions, the first order DT50 and DT90 values of acequinocyl in anaerobic soil are 1.8 and 5.8 days, respectively.

Field dissipation test

A soil dissipation study with acequinocyl was carried out over a period of 240 days at three sites. Following application, residues of acequinocyl declined rapidly with time. No residues were found below 15 cm. Due to the rapid degradation, for the estimation of the DT50/DT90, an adjusted time line was used. Therefore, to all time events, 3 hours was added (0 hours DALT then becomes 3 hours DALT, etc.). The residue at time 0 was taken the sum of acequinocyl and metabolites. Doing so, the DT50 and DT90 values are considered to represent more closely the actual situation. The summarised results are presented in Table 4.5.

Table 4.5: Calculated first order DT50 and DT90 values[1)]

Location		DT50 (hours)	DT90 (hours)	Regress. Coeff.
California	Acequinocyl	2.9	9.5	0.945
	Acequinocyl-OH	2.8	9.2	
New York	Acequinocyl	2.2	7.3	0.903
	Acequinocyl-OH	7.2	24	
Georgia	Acequinocyl	6.2	21	0.937
	Acequinocyl-OH	3.5	12	

1) Simple first order fit

4.1.3 Summary and discussion of persistence

Biodegradation in water

Acequinocyl is considered rapidly degradable based on the results of the simulation tests.

The water sediment test showed that acequinocyl disappears very rapidly from the water phase.

Degradation in soil

The aerobic and anaerobic degradation studies, the field test and the photolysis study show a rapid degradation in various soil types. In the laboratory the DT50 values at 20°C varied between 1.0 and 2.8 days. In the field, DT90 values ranged between 2.2 – 7.2 hours.

Overall, even not readily biodegradable, regarding its stability/degradability, acequinocyl is rapidly disappears in the main compartments and the main mechanism of degradation is biodegradation. It can be concluded that acequinocyl is considered rapidly degradable in water, sediment and soil.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

Acequinocyl can be classified as immobile in soil, according to the Koc values determined according to the OECD Guideline no. 106. In four different types of soil, adsorption Koc values of acequinocyl varied between 3390 and 123000 dm^3/kg.

The leaching behaviour of acequinocyl was studied in a soil column leaching study. The test was performed with four soils (sandy loam, silty clay loam, sand and loamy sand). In both ‘unaged’ and ‘aged’ column leaching tests, the major part of the radioactivity (95%) remained in the top section of the soil columns, whereas 5% leached not more than 5 cm. It is concluded that acequinocyl has a very low potential for leaching in soil.

4.2.2 Volatilisation

Based on the vapour pressure of 1.69×10^{-6} Pa (25^oC), acequinocyl is not considered as a volatile substance.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

A fish bioconcentration study is available for acequinocyl. A bioconcentration test with the radio-labelled compound [^{14}C-phenyl] acequinocyl (97.1% purity) was carried out with carp (*Cyprinus carpio*) according to the OECD Guideline 305E and in compliance with GLP. The bioaccumulation and depuration of the test compound was determined in a 28d exposure period, followed by a 14d depuration period to study the elimination. The major observed degrade in the test water was acequinocyl-OH. This account for 24-32% of the radioactivity. Bioconcentration factors (BCFs) were calculated as a ratio of concentrations of total radioactivity in fish, compared to total radioactivity in the exposure medium. The BCF for whole fish was 366 at 0.17 µg/L and 288 at 1.7 µg/L and thus similar at both exposure levels with an overall average BCF (21 - 28 days) of 327. The fish homogenate did not contain any acequinocyl or acequinocyl-OH, indicating that both compounds are metabolized to more polar water soluble metabolites.

4.3.2 Terrestrial bioaccumulation

Adult earthworms (adults 5.5-8 months old, 300 – 600 mg wwt, acclimatisation period five days) were exposed for 56 days to loamy sand soil that received a single application of [^{14}C-phenyl] acequinocyl (15% SC). Next, the elimination of the test substance in clean soil was monitored for another 35 days. The Biota/Sediment Accumulation Factor (BSAF)-value of 22.1 is expressed in soil dry weight and in earthworm dry weight; the BSAF value of 1.86 is expressed in soil dry weight and in earthworm wet weight.

4.3.3 Summary and discussion of bioaccumulation

BCF values of 327 in fish and 22.1 in earthworm were obtained. These data suggest that acequinocyl has limited potential for bioconcentration and bioaccumulation. The value of 327 fufils the criterion for bioaccumulating potential conform Directive 67/548/EEC, since it exceeds the value of 100. However, it does not fulfil the criterion for Regulation EC1272/2008, since it does not exceed the value of 500.

4.4 Secondary poisoning

Not relevant for this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

The summaries included in this proposal are partly copied from the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of acequinocyl in Annex I of Council Directive 91/414/EEC (DAR September 2007 + addendum January 2008, RMS The Netherlands). Some details of the summaries were not included when considered not important for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR and its addenda. No references to individual mammalian studies are included to protect the privacy and integrity of the individual (Article 14 of directive 91/414). These references are available in the DAR and its addenda.

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Absorption

Based on experiments with intact rats, oral absorption 120 h after low dose administration of acequinocyl (10 mg/kg bw) was between 11 and 15%, based on radiolabel recovered from urine, cage wash and carcass. After single high dose (500 mg acequinocyl/kg bw), absorption was at least 8.5%. There were neither major differences in absorption between single and repeated doses nor between males and females. Furthermore, comparison of the plasma AUC's (area under the curve) showed only a 20-fold increase after high dose administration as compared to low dose administration, indicating lower relative absorption after high dose administration (saturation).

Based on experiments with bile duct cannulated rats and including radiolabel recovered from bile into the absorbed pool, absorption after a single oral low dose was between 25 and 29% for males and between 33 and 42% for females, 48 h after administration. As there are distinct indications for a sizeable biliary first-pass effect (see section below on excretion), a significant part of the biliary radiolabel may not represent systemically available parent compound and/or metabolites. However, based on the critical effect of acequinocyl (see introduction above), the extent of absorption that should be taken into account for the subsequent risk assessment is 28% (based on urinary excretion, biliary excretion, cage wash and res. carcass after 48 h).

Excretion

In intact animals, total excretion within 120 h after single oral high and low dose administration was similar with ca. 95% of the administered dose excreted. After single or repeated oral low dose ca. 13% of the administered radioactivity was eliminated in urine and ca. 87% in faeces, irrespective of sex. Some retardation was observed in faecal excretion after high dose: within the first 24 h after low dose approximately 75% of the administered dose had been excreted, while after high dose this was ca. 40%.

In the bile excretion study 46-65% of the administered low dose was eliminated in the first 24 h and 92% of the administered high dose. Thus, at low dose total excretion within the first 24 h was considerably lower in bile cannulated animals than in intact animals. At high dose, total excretion within the first 24 h was higher in bile cannulated rats. It can be hypothesized that in the bile duct cannulated animals the rate of excretion is somewhat altered during the first 24 h and that it is normalized after 48 h.

There are distinct indications for sizeable biliary first-pass elimination:

1. Parent compound and two of the five identified metabolites appear in bile and faeces, but not in urine (see metabolism below).

2. AKM-05 (2-hydroxy-3-dodecyl-1,4-naphtalenedione) is the major biliary metabolite (conjugated and unconjugated about half of the total metabolites present in bile) and is much less prominent in urine (less than 20%) (see metabolism below).

3. Excretion of radiolabel occurs predominantly via faeces, even after single low dose (see above).

Distribution

Concentrations of radioactivity measured in tissues were generally highest in animals sacrificed at the time of peak plasma concentrations (Cmax), and decreased steadily in tissues obtained at later times. Twenty-four hours after single oral low dose administration (10 mg/kg bw), highest concentrations were in the gastrointestinal tract (3-6 mg eq/kg bw) and contents (9-41 mg eq/kg), and intermediate concentrations were found in the fat, kidneys, liver, lungs, lymph nodes and pancreas (0.3 to 1 mg eq/kg bw at 24 h). There was no relevant difference in the concentrations between male and female tissues or in tissues of animals dosed with different radiolabelled forms of acequinocyl.

Metabolism

Repeated low dose administration changed the metabolite pattern in faeces (higher percentage of AKM-18 (2-(1,2-dioxotetradecyl)-benzoic acid) and lower percentage of AKM-05 compared to other dosing levels). Furthermore, an increase of the percentage of parent compound and a decrease of AKM-18 in faeces was found after single oral high dose administration. These effects are indications for saturation of the intestinal metabolism of the test substance. Acute toxicity tests with AKM-05 and AKM-18 indicated low toxicity of these metabolites (LD50 > 5000 mg/kg bw).

AKM-14 (2-hydroxy-3-butanoic acid-1,4-naphtalenedione) and AKM-15 (2-hydroxy-3-hexanoic acid-1,4-naphtalenedione) were identified as the main metabolites in urine, while no parent compound was detected. The main faecal metabolites were AKM-05 (ca. 42% of faecal radioactivity) and AKM-18 (ca. 33%). AKM-15 was found as well (ca. 6%), while parent compound represented only ca. 2% of the radiolabel recovered from faeces. The main metabolite in bile was identified as a glucuronide conjugate of AKM-05. In the two biliary metabolism experiments submitted, this metabolite accounted for about 35% of total radioactivity in bile. In the first experiment also AKM-14 and -15 were identified in bile, while in the second experiment their presence in bile could not be demonstrated. The other metabolites identified in bile were AKM-05 (ca. 10% of biliary radiolabel) and AKM-18 (ca. 5%). About 2.5% of the biliary radioactivity consisted of parent compound. No marked differences between the sexes were observed in the profile of metabolites in the bile and there were no qualitative differences between low and high dose level.

Unidentified metabolites accounted for 30% of the urinary radiolabel, 10% of the faecal radiolabel and 20% of the biliary radiolabel.

Besides toxicokinetic experiments with intact animals, two different experiments with bile duct cannulated rats were submitted. Acequinocyl and most of its identified metabolites are structure analogues of vitamin K. Therefore, its mechanism of toxicity is probably competitive inhibition of the vitamin K dependent prothrombin synthesis. Although the amount of acequinocyl excreted via bile is probably not systemically available, it should also be taken into account in the subsequent hazard assessment, since the critical effect on prothrombin synthesis occurs in the liver.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Two oral studies were available (limit tests) and resulted not in any deaths. Both studies were performed in accordance with OECD 401. Watery faeces (containing test article-like material), observed up to 2 hours after administration was the only finding in the studies.

Table 5.1: Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle
Acequinocyl	> 5000 mg/kg bw	rat	oral	0.5% aqueous solution of methylcellulose
Acequinocyl	> 5000 mg/kg bw	mouse	oral	0.5% aqueous solution of methylcellulose

5.2.2 Acute toxicity: inhalation

One study was available in which rats were exposed to acequinocyl at concentrations of 0, 0.62, 0.69, 0.84 mg/L (MMAD (± gsd): 2.1-2.5µm (± 2.0)); vehicle aceton. The high dose group was exposed to the maximum attainable concentration. The study was performed in accordance with OECD 403. One female rat of the 0.69 mg/L group died immediately following exposure and one male rat 0.84 mg/L was found dead on day 3 of the observation period.

Symptoms of toxicity during the observation period were exaggerated respiratory movements, brown staining around snout and jaws, red/orange staining on the tail, and red/orange staining on the tray paper in all test substance treated groups. Rats exposed to 0.62 mg/L of air had wet fur around the snout on the day of exposure and had wet fur on the underbody. All surviving animals had recovered from the effects of exposure by day 3 of observation.

All treated rats showed pulmonary lesions (aggregates of alveolar macrophages; thickening of alveolar walls; apparent alveolar collapse; bronchiolar epithelial erosion or necrosis; hyperplasia or squamous metaplasia of bronchiolar epithelium; peribronchiolar inflammatory cells; and bronchiolar obliteration/obstruction with recanalisation or giant cells with mineralisation).

Table 5.2: Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle
Acequinocyl	> 0.84 mg/L	rat	inhalation	acetone

5.2.3 Acute toxicity: dermal

One dermal study (performed in accordance with OECD 402) was available (limit test) and resulted not in any deaths. There were no treatment-related findings.

Table 5.3: Acute toxicity, LD_{50}/LC_{50} values

Test substance	LD_{50}/LC_{50}	Species	Route	Vehicle
Acequinocyl	> 2000 mg/kg bw	rat	dermal	water

5.2.4 Acute toxicity: other routes

No data available.

5.2.5 Summary and discussion of acute toxicity

Acequinocyl does not need to be classified on the basis of its acute oral and dermal toxicity in rats. Although one of the high dose animals in the inhalation study died at a concentration of 0.84 mg/L, it was the highest attainable concentration. Therefore no classification for acute inhalatory toxicity (lethality) is required. However, in this test all treated rats showed pulmonary lesions starting at a dose of 0.62 mg/l. This effect is probably caused due to the soap like properties of acequinocyl. Most effects (excluding lethality) observed in the acute inhalatory study are expected to be reversible. Therefore classification with R39/23 according to 67/548/EEC is not proposed. However, the effects of acequinocyl could be considered as an irritating effect on the lungs (see 5.3.3).

In the acute inhalation test all treated rats showed pulmonary lesions starting at a dose of 0.62 mg/l. This is below the guidance value of 1 mg/L for STOT SE category 1. The effects are also considered severe because there was lethality in some exposed rats and severe effect on the lung in all exposed rats. Therefore, according to EC 1272/2008 acequinocyl should be classified as STOT-SE Cat 1: H370: Causes damage to organs (lung) after inhalatory exposure.

5.3 Irritation

5.3.1 Skin

A skin irritation study was performed mostly in accordance with OECD 404, although the test substance was applied occlusive without moistening. However, this deviation was considered of no influence on the results of the study. Only (very) slight erythema was observed after 1 and 24 h.

Table 5.4: Results skin irritation study

Scores observed after	1 hour	24 hours	48 hours	72 hours
Erythema	0, 1, 1, 1, 0, 0	0, 2, 1, 1, 1, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Oedema	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

Acequinocyl does not need to be classified as irritating to the skin.

5.3.2 Eye

An eye irritation study in rabbits was performed in accordance with OECD 405. The treated eyes were washed 24 hours after instillation of the test substance in the ‘unwashed group’ (6 rabbits) and in the ‘washed group’ (3 rabbits) the treated eyes were washed 2 to 3 minutes after application. According to OECD 405 an eye wash may be executed 24 hour after application of a solid test substance. Therefore, only the ‘unwashed’ group was treated according to OECD 405, hence only the results of this group are reported here.

Table 5.5: Results eye irritation study

Scores observed after	1 hour	24 hours	48 hours	72 hours
Cornea/opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Iris	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva redness	0, 1, 0, 1, 1, 0	0, 1, 0, 1, 1, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva chemosis	1, 1, 1, 1, 1, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva discharge	2, 2, 2, 2, 2, 2	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

After 24 h only slight conjunctival redness was observed in 3 out of 6 rabbits.

Acequinocyl does not need to be classified as irritating to the eyes.

5.3.3 Respiratory tract

In the acute inhalation test all treated rats showed pulmonary lesions (aggregates of alveolar macrophages; thickening of alveolar walls; apparent alveolar collapse; bronchiolar epithelial erosion or necrosis; hyperplasia or squamous metaplasia of bronchiolar epithelium; peribronchiolar inflammatory cells; and bronchiolar obliteration/obstruction with recanalisation or giant cells with mineralisation) (see section 5.2.2 and 5.2.5).

This inflammatory reaction observed in the lungs is very likely being caused by the physico-chemical properties of the soap like compound. The incidences reported in the study are low. The effects are probably not dose related, but this can not be excluded at the moment, since the dose intervals were small. The effect is probably related to an effect on the surface tension in the alveoli and could be considered as irritation of the respiratory tract.

There is no sub-chronic inhalation study to verify the results.

5.3.4 Summary and discussion of irritation

Acequinocyl is considered not irritating to skin and eyes according to Directive 67/548/EEC and Regulation EC 1272/2008. However, in an acute inhalation test all treated rats showed pulmonary lesions starting at a dose of 0.62 mg/l. These effects are considered to be the result of respiratory tract irritation. Therefore, acequinocyl should be classified as irritating to the respiratory tract: Xi; R37 according to Directive 67/548/EEC.

For Directive EC 1272/2008, these effects are considered as STOT SE category 1.

5.4 Corrosivity

The skin irritation study (5.3.1) shows no need for classification for corrosion.

5.5 Sensitisation

5.5.1 Skin

A GPMT test was performed in accordance with OECD 406. Intradermal injection of 5% w/v acequinocyl in propylene glycol caused moderate erythema in a single animal. Topical induction of 50% w/v acequinocyl in propylene glycol caused exfoliation and pink staining in all treated animals

and a single incidence of eschar formation. Following, challenge with 10% w/v acequinocyl in propylene glycol, caused a significant response in six out of 20 test animals and in no control animals.

Acequinocyl is sensitising to the skin of guinea pigs in this Maximisation test because 30% of the guinea pigs showed a positive reaction.

5.5.2 Respiratory system

No data available.

5.5.3 Summary and discussion of sensitisation

Six out of 20 guinea pigs showed a positive response in a Maximisation test. Therefore, acequinocyl should be classified sensitising to the skin: R43 according to Directive 67/548/EEC and Skin Sens Cat 1: H317 according to Regulation EC 1272/2008.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

The results of the relevant subacute, semichronic and chronic toxicity studies are summarised in Table 5.6.

Table 5.6: Subacute /Semichronic/Chronic oral studies

Duration	Species	guideline	NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects
28 days	rat	?	44	220	haematological effects, mortality
13 weeks	rat	OECD 408	30	120	haematological and haemorrhagic effects
104 weeks	rat	OECD 453	2.3	9.0 (MOAEL)	increased platelet levels no increases of neoplastic lesions
13 weeks	mouse	OECD 408	-	16 (MOAEL)	liver effects
80 weeks (carcino-genicity study)	mouse	OECD 451	2.7	7.0	histopathological liver effects and associated clinical chemical changes no increases of neoplastic lesions
13 weeks	dog	OECD 409	40	160	effects on body weight, haematological effects
52 weeks	dog	OECD 452	5	20	increased platelet levels

In a 28-day oral rat study clear systemic toxicity (*i.e.* mortality in both sexes and a statistically significant increase in platelet levels, prothrombin time (PT) and activated partial prothrombin time (PTT) in females) was observed from 2500 mg acequinocyl/kg food (~ 220 mg/kg bw) onwards. Also two 28-day oral studies in mice and dogs, respectively, were submitted as range-finding studies. In the oral mouse study with dose levels of 0, 100, 500, 2500, 5000 mg/kg food (up to ~ 715 mg/kg bw/day), clear systemic toxicity (*i.e.* decreased haematocrit and haemoglobin values in males, increased white blood cell counts in females, and increased liver weight in both sexes) was observed from 500 mg/kg food (~71.5 mg/kg bw/day) onwards. In the dog study, dose levels of 0,

30, 100, 300, 1000 mg/kg food were used, and clear systemic toxicity (*i.e.* decreased body weight and food consumption, and total protein) was observed from 1000 mg/kg food (25 mg/kg bw/day) onwards. However, the mouse and dog studies did not meet OECD guidelines and were not relevant for the overall toxicity profile.

A 13-week oral toxicity study was performed on rats administered up to 3200 mg acequinocyl/kg food (~ 253 and 286 mg/kg bw/day for males and females, respectively). The study was performed in accordance with OECD 408 (1981), with some minor deviations that were not considered to have major influence on the study results (clinical observations only 6 days per week and no histopathological examination of the bone marrow). All the animals in the highest dose group died during week 1-3, exhibiting pallor, swelling and haemorrhages. The animals had numerous haemorrhages, and atrophic or necrotic organs. In the 1600 mg/kg food group, haematological effects and a single case of haemorrhage in the eye was observed. In addition, urine bilirubin levels and blood PTT were significantly increased in both sexes, while the PT, white blood cell count (WBC) and platelet values were significantly increased in the males. A NOAEL of 30 mg/kg bw/day was determined.

A combined 104 week toxicity/carcinogenicity study in rats was performed in accordance with OECD 453. It should be noted that the stability of the test substance in the diet at the highest dose was somewhat low (87% left of the original concentration four days after admixture to the diet). For the lowest dose the stability was >90% four days after admixture, while no data on the intermediary doses were provided. However, it is considered not to have had an undue influence on the outcome of the study. Oral administration of acequinocyl during 104 weeks to rats mainly affected the coagulation system and the eye. Effects were most pronounced at dose levels of 800 and 1600 mg/kg (36 and 74 mg/kg bw/day, respectively), although the increases in platelet number and coagulation time were neither high nor always very consistent over time. In females, increased spleen weights accompanied with slight histopathological changes were observed in the two highest dose groups (800 and 1600 mg/kg). Only in week 26 these changes were statistically significant. In view of the haematological effects of acequinocyl, these changes are probably test substance related, although not very pronounced and limited to one interim kill and one sex. Slight effects on the eyes of males (corneal opacity and pale to unclear fundus oculi) were observed at 1600 mg/kg (74 mg/kg bw/day) and on platelet numbers in males and females were observed at 200 mg/kg (9.0 and 12 mg/kg bw/day in males and females, respectively) and higher (in males significant at 1600 mg/kg, in females at 800 mg/kg). Unfortunately, no ophthalmoscopy was performed on the low and mid dose groups (50, 200 and 800 mg/kg). Despite the minimal nature of the effects at 200 mg/kg (increase in platelets, hypertrophy of eyeballs and intra-ocular haemorrhage), corresponding to 9.0 mg/kg bw/day, they are considered treatment-related, since they are consistent with the overall effects in the study.

Oral exposure of mice to acequinocyl at dose levels up to 1500 mg/kg food (~ 231 mg/kg bw/day) for 13 weeks (study performed in accordance with OECD 408 (1981)), resulted in the death or sacrifice of all animals at the highest dose level by week 2, and 8/20 females and 1/20 males administered 1000 mg/kg food. The animals exhibited poor clinical condition, and had numerous haemorrhages, pale organs and heart degeneration. Haematological and haemorrhagic effects were observed from doses of 500 mg acequinocyl/kg food onwards. In the 500 mg/kg food group, one male died from treatment-related effects. Increased liver weights and hepatocyte vacuolation were noted from doses of 100 mg acequinocyl/kg food onwards.

An 80 week carcinogenicity study in mice was performed in accordance with OECD 451. Chronic oral administration of acequinocyl to mice at doses of 150 mg/kg food (20 and 26 mg/kg bw/day in males and females, respectively) and higher provoked increased relative liver weights in males and

females, and increased relative kidney weights in males. The changes in kidney weights were accompanied by glomerular accumulation of amyloid. The changes in liver weight were accompanied by increases in plasma liver enzymes and macroscopic and microscopic liver pathology. Most prominent in this respect and clearly dose related were the incidence of brown pigmented cells and associated inflammatory cells in the liver. Significant increases in brown pigmented liver cells were already observed from the lowest dose level upward in females (20 mg/kg food, ~ 3.5 mg/kg bw/d) and from the next lowest dose level upward in males (50 mg/kg food, ~ 7.0 mg/kg bw/d). The level of the liver enzymes Glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) are also significantly increased from the lowest dose level upward, although not in a dose related fashion. In absence of other liver pathology (e.g. associated inflammatory cells, generalised fat), the changes at the lowest dose level were not considered adverse. Furthermore, there were two males in the 20 ppm group (~ 2.7 mg/kg bw/d) which showed high values for alkaline phosphatase (AP), GPT and GOT in comparison with controls. These two animals were noted histopathologically to have both lung and liver tumours, probably contributing to their high AP, GPT and GOT values which unduly influenced the group mean. If the values for these animals and animals from all other groups (including controls) which were noted to show both liver and lung tumours are excluded from the analysis, the group mean values for AP, GPT and GOT in the 20 ppm group are considerably lower. Finally, the increases in GPT and GOT levels were inconsistent during the study: in week 55 the GPT and GOT levels in males were comparable to the concurrent controls and the GPT and GOT levels in females were – not dose-related – increased. In contrast, in week 81 the GPT and GOT levels in males were – not dose-related – increased, and the GPT and GOT levels in females were only affected at the highest dose levels. Taking this all into account, the changes in GPT and GOT at 20 ppm were considered not adverse. The observed decreases in plasma globulin concentrations were not very consistent, and therefore not considered to be treatment-related.

In a 13-week oral toxicity study, performed in accordance with OECD 409, Beagle dogs were administered up to 1000 mg acequinocyl/kg bw/day. The animals in the highest dose group and 2 females in the 640 mg acequinocyl/kg bw/day group were sacrificed after three weeks of exposure, due to poor clinical condition. The necropsy revealed histopathological effects in several organs (red discoloration of the gastrointestinal tract and thymal cortical atrophy). A significant dose-related decrease in body weight gain was observed from 160 mg acequinocyl/kg bw/day, in addition to haematological effects increased white blood cell and platelet counts) and incidental histopathological damage.

A 52-week oral toxicity study was performed (in accordance with OECD 452) on Beagle dogs administered up to 320 mg acequinocyl/kg bw/day. One male and one female in the highest dose group were sacrificed due to bad physical condition, probably as a result of the treatment. In sacrificed animals of the highest dose group, reduced food consumption caused a reduced body weight gain. Males in the highest dose group also had increased PT. An increase in platelet levels was observed in both sexes in the two highest dose groups, and in males administered 20 mg/kg bw/d. However, in males the changes can be regarded as normal variation. The females seem to be more sensitive. Although the increase of platelets is no adverse effect, it is considered the reaction on an (unknown) adverse effect. In the control groups, the platelets decrease throughout the study, which is normal. However, in the females, an increase in platelets was observed from a dose level of 20 mg/kg bw/d, and therefore, the NOAEL of the 1-year dog study is considered to be 5 mg/kg bw/d

5.6.2 Repeated dose toxicity: inhalation

No studies available.

5.6.3 Repeated dose toxicity: dermal

In a 28-days dermal toxicity study (in accordance with OECD 410) with rats administered 0, 40, 200 or 1000 mg acequinocyl/kg bw/day, a statistically significant increase in PTT in both sexes was observed in the top dose group, and statistically significantly increased PT and fibrinogen levels in males, in addition to heart changes (enlarged heart, inflammatory cell foci) observed in several males. No effects were observed at lower doses, with the exception of 1 male with an enlarged heart in the 40 mg/kg bw/day group. Haematological parameters were not analysed in this study.

5.6.4 Other relevant information

No data available.

5.6.5 Summary and discussion of repeated dose toxicity:

All short term and chronic studies executed with acequinocyl in rats or dogs showed haematological effects (increase in plate number and/or prolongation of blood clotting time) as main effect of exposure to acequinocyl. Acequinocyl and most of its identified metabolites are structure analogues of vitamin K. Therefore, its mechanism of toxicity is probably competitive inhibition of the vitamin K dependent prothrombin synthesis. A reduction in prothrombin results in an increase in blood clotting and an increase in haemorrhages and related haematological effects. An increase in blood clotting time plus an increase in haemorrhages is considered sufficient for classification for repeated dose toxicity.

In an oral rat 28-d range finding study, increase in platelet levels, PT and PTT in females were observed from 2500 mg acequinocyl/kg food (~ 220 mg/kg bw/d) onwards. Also in a 13-week oral toxicity study performed on rats administered up to 286 mg acequinocyl/kg bw/day, haematological effects and ocular haemorrhage were reported. In rats, chronic exposure (104 weeks) causes an increased number of platelets from doses of 9.0 mg acequinocyl/kg bw/day onwards and a prolongation of clotting time from doses of 36 mg/kg bw/d onwards. Ocular effects were also observed from doses of 36 mg acequinocyl/kg bw/day onwards.

In an oral toxicity study with mice exposed to doses of acequinocyl of up to 1500 mg/kg food (~ 231 mg/kg bw/day) for 13 weeks, dose related liver effects (increased liver weights and hepatocyte vacuolation) were observed from 100 mg/kg food (16 mg acequinocyl/kg bw/day) onwards. Haematological and haemorrhagic effects were observed from doses of 500 mg acequinocyl/kg (81 mg/kg bw/d) food onwards. In mice, chronic (80) week oral exposure to acequinocyl provoked hepatotoxicity (increased relative liver weights, increases in the liver enzymes GPT and GOT, increases in brown pigmented liver cells and associated inflammatory cells in the liver) and renal toxicity (increased relative kidney weights, by glomerular accumulation of amyloid) at doses of 150 mg/kg food and higher (~ 20 and 26 mg/kg bw/d in males and females, respectively).

In a 13-week oral toxicity study, in which Beagle dogs were administered up to 1000 mg acequinocyl/kg bw/d, a dose-related decrease in body weight gain was observed from 160 mg/kg bw/d onwards, in addition to haematological effects and incidental histopathological damage. In a 52-week oral toxicity study performed on Beagle dogs administered up to 320 mg acequinocyl/kg bw/d, haematological effects were observed from 20 mg/kg bw/d onwards.

In all species, high oral exposure (160 mg/kg bw/day in mice, 286 mg/kg bw/day in rats and 320 mg acequinocyl/kg bw/d in dogs) resulted in such bad clinical conditions that animals were sacrificed.

In a 28-days dermal toxicity study with rats administered up to 1000 mg acequinocyl/kg bw/day, a statistically significant increase in PTT in both sexes, and statistically significantly increased PT and fibrinogen levels in males were found, in addition to heart changes in several males.

Table 5.7: Overview of the guidance values for classification versus the oral dose levels with effects warranting classification (effective dose).

Species	Duration	R48/22	R48/25	STOT RE Cat 2	STOT RE Cat 1	Non-effective dose	Effective dose	Effects	Resulting classification
Rat	28 days	150 mg/kg bw/day	15 mg/kg bw/day	300 mg/kg bw/day	30 mg/kg bw/day	88 mg/kg bw	440 mg/kg bw	Mortality and haematotoxicity	Between no classification and STOT RE 2 for CLP. No classification for 67/548
Rat	90 days	50 mg/kg bw/day	5 mg/kg bw/day	100 mg/kg bw/day	10 mg/kg bw/day	30 mg/kg bw/day (NOAEL)	120 mg/kg bw/day	Reduced clotting, heamatotoxicity and haemorrhages	Between no classification and STOT RE 2 for CLP. No classification for 67/548
Rat	104 weeks	6.25 mg/kg bw/day	0.625 mg/kg bw/day	12.5 mg/kg bw/day	1.25 mg/kg bw/day	2.3 mg/kg bw/day (NOAEL)	9.0 mg/kg bw/day (MOAEL)	Intra-ocular haemorrhage, hypertrophy eyeballs	STOT RE 2 for CLP. No classification for 67/548
Mouse	90 days	50 mg/kg bw/day	5 mg/kg bw/day	100 mg/kg bw/day	10 mg/kg bw/day	16 mg/kg bw/day (MOAEL)	81 mg/kg bw/day	Death, haemorrhages in several organs	STOT RE 2 for CLP. No classification for 67/548
Mouse	80 weeks	8.3 mg/kg bw/day	0.83 mg/kg bw/day	16.7 mg/kg bw/day	1.67 mg/kg bw/day	7.0 mg/kg bw/day	20 mg/kg bw/day	Increased liver weight, liver pathology (brown pigmented cells, inflammatory cells, fatty liver)	Between no classification and STOT RE 2 for CLP. No classification for 67/548
Dog	90 days	50 mg/kg bw/day	5 mg/kg bw/day	100 mg/kg bw/day	10 mg/kg bw/day	160 mg/kg bw/day (LOAEL)	640 mg/kg bw/day	Mortality (sacr due to poor clinical condition), cortical atrophy thymus, reduced bm cellularity, discolouration gastrointestinal tract	No classification
Dog	52 weeks	12.5 mg/kg bw/day	1.25 mg/kg bw/day	25 mg/kg bw/day	2.5 mg/kg bw/day	80 mg/kg bw/day	320 mg/kg bw/day	Mortality (sacr due to bad physical condition)	No classification

In conclusion, based on the effects in the longest studies in rats (104 weeks) and mice (80 weeks), classification for repeated dose oral toxicity at the effective dose level in comparison to the guidance levels according to Directive 67/548/EEC seems not necessary. In addition, neither of the

shorter repeated dose toxicity studies provides reasons for classification according to Directive 67/548/EEC.

Based on mortality, liver effects, haemorrhages and haematological effects (including effects on clotting) observed in several studies and several species at dose levels at or below the guidance levels, according to Regulation EC 1272/2008 acequinocyl should be classified for specific target organ toxicity / repeated exposure as STOT RE Cat 2: H373 May cause damage to organs (blood) through prolonged or repeated exposure.

Based on the dermal toxicity study in rats, classification for the dermal route according to Directive 67/548/EEC seems not necessary.

5.7 Mutagenicity

5.7.1 *In vitro* data

Ames tests, pre-incubation method, were performed on various *S. typhimurium* and *E. coli* strains according to OECD 471. The test substance did not induce point mutations in *S. typhimurium* and *E. coli* strains.

Table 5.8: Bacterial mutagenicity assays

Indicator cells	Endpoint	Res. - act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	
B: *S. typh.* TA 98 TA 100 TA 1535 TA 1537 B : *E.coli* WP2uvrA	 point mut. point mut. point mut. point mut. point mut.	 - - - - -	 - - - - -	rat liver	PB[1] and BF[2]	0, 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate (- S9-mix) 0, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500, 5000 µg/plate (+ S9-mix) vehicle: DMSO

[1] Phenobaribital
[2] 5,6-Benzoflavone
Test substance: AKD-2023 technical, lot no. AK23921T, purity 96.5%, yellow powder
Cytotoxicity observed at dose levels ≥ 313 µg/plate (- S9 and + S9-mix)
Precipitation observed at dose levels ≥ 156 µg/plate (- S9-mix) and 625 µg/plate (+ S9-mix)

A cytogenic assay was performed on Chinese hamster lung cells according to OECD 473. The test substance did not induce chromosome aberrations.

Table 5.9: *In vitro* Chromosome aberration test

Indicator cells	Endpoint	Res. -act.	Res. +act.	Activation		Dose range
				Tissue	Inducer	
Chinese hamster lung (CHL) cells	chromosome aberration	-	-	rat liver	PB[1] and BF[2]	24 and 48 h (-S9): 150, 300, 600, 1200 µg/mL 6 h (+S9): 481, 963, 1925 and 3850 (10 mM) µg/mL vehicle: 1 % CMC[3]

[1] Phenobarbital
[2] 5,6-Benzoflavone
[3] Carboxymethylcellulose sodium solution
Test substance: AKD-2023 technical, lot no. AK23921T, purity 96.5%, yellow powder
Cytotoxicity observed at dose level: 1200 µg/mL (48h; -S9)
Precipitation observed at dose level: -

A gene mutation test according to OECD 476 was performed on mouse lymphoma cells L51/8Y. In the table below the study is summarized. Furthermore, additional tables with actual values for mutation frequencies are added in order to facilitate interpretation of the results.

Table 5.10: *In vitro* gene mutation assay

Indicator cells	Endpoint	Res. –act.	Res. +act.	Activation		Dose range[2]
				Tissue	Inducer	
mouse lymphoma cells L5178Y	gene mutations (TK)	-	-	rat liver	Arochlor 1254	Experiment 1 0, 10, 20, 40, 80, 160 µg/mL (-S9) 0, 10, 20, 40, 80, 160 µg/mL (+S9)
		-[1]	-			Experiment 2 0, 10, 20, 40, 80, 120 µg/mL (-S9) 0, 10, 20, 40, 80, 100, 120 µg/mL (+ S9)
		-[1]	[3]			Experiment 3 0, 10, 20, 40, 80 µg/mL (-S9) vehicle: DMSO

[1] Small but statistically significant increases in mutant frequencies were observed at the top four doses of experiment 2 and at two of the four top doses of experiment 3. A linear trend was obtained in experiment 2, not in the other two independent experiments performed in the absence of S9. The increases of mutant frequencies were small (< 2 fold over the concurrent negative control value) and were not reproducibly dose-related.
[2] The values in this column present the actual concentrations analysed for viability and TFT resistance. Higher doses were included in the tests but were considered too toxic for useful analysis. The survival was circa 16 - 17%, 36 - 33% and 34% at the highest dose level analysed in experiment 1, 2, and 3, respectively.
[3] Experiment 3 was only performed without S9.
Test substance: AKD-2023 technical, lot no. AK23931T, purity 97.1%, yellow powder
Precipitation observed at dose level: -

From the actual values for mutation frequencies it could be seen that the increases of mutant frequencies were small (< 2 fold over the concurrent negative control value) and were not reproducibly dose-related. Therefore, it was concluded that the test substance did not induce reproducible gene mutations in mouse lymphoma cells L5178Y.

5.7.2 *In vivo* data

A mouse micronucleus test was performed according to OECD 474 on mouse CD-1 (SF) bone marrow cells.

Table 5.11: *In vivo* micronucleus assay

Species	Endpoint	Result	Dose range
mouse, CD-1 (SF) 15/sex/dose[1]	micronuclei (bone marrow)	-[2]	0, 1250, 2500 and 5000 mg/kg bw/d, administered once orally by gavage, sacrifice at 24, 48 h and 72 h after treatment vehicle: aqueous 0.5% carboxymethylcellulose

[1] In positive control group 5/sex
[2] At the 72 hour sampling time a statistically significant decrease in the p/n ratio ($p < 0.01$) was obtained for the three groups treated with the test substance. This decrease is not considered of biological relevance, since there were no signs of a dose-response relationship. The decrease was merely a consequence of the relatively high p/n ratios in the vehicle controls at the 72 hour sampling time. Moreover the p/n ratios in the animals treated with the test material were fully comparable with the ratios found in test animals 24 and 48 h after treatment.
Test substance: AKD-2023 technical, lot no. AK23921T, purity 96.5%, yellow powder
Toxicity observed at dose level: there were no indications of treatment-related bone marrow toxicity.

The test substance did not induce micronuclei in mouse bone marrow cells. There were no indications of treatment-related bone marrow toxicity. However, the ADME study showed uptake of the test substance at about 12-14% after oral administration.

5.7.3 Human data

No data available.

5.7.4 Other relevant information

No data available.

5.7.5 Summary and discussion of mutagenicity

The results from the *in vitro* and *in vivo* genotoxicity studies are summarised in the Tables 5.12 and 5.13.

Table 5.12: *In vitro* genotoxicity studies

Test substance	Type of study		Result	
	Indicator cells	Endpoint	without activation	with activation
Acequinocyl	S. typhimurium E. coli	point mutation point mutation	- -	- -
Acequinocyl	Chinese hamster lung (CHL) cells	chromosome aberrations	-	-
Acequinocyl	Mouse lymphoma cells L5178Y	gene mutations (TK)	-	-

Table 5.13: *In vivo* genotoxicity studies

Test substance	Type of study		Result
	Species	Endpoint	
Acequinocyl	mouse	micronuclei (bone marrow)	-

Acequinocyl was found to be negative in the *in vitro* Ames test, negative in the *in vitro* chromosome aberration test, negative in the *in vitro* TK gene mutation test and negative in the *in vivo* micronucleus test. On the basis of the above results, acequinocyl is not considered genotoxic.

Therefore, acequinocyl does not need to be classified for mutagenicity.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

A combined toxicity study/carcinogenicity study in rat was performed in accordance with OECD 453. It should be noted that the stability of the test substance in the diet at the highest dose was somewhat low (87% left of the original concentration four days after admixture to the diet). For the lowest dose the stability was >90% four days after admixture, while no data on the intermediary doses were provided. However, it is considered not to have had an undue influence on the outcome of the study. Oral administration of acequinocyl during 104 weeks to rats did not increase tumour incidence.

In addition, a carcinogenicity study in mice was performed in accordance with OECD 451. Doses of 0, 2.7, 7.0, 20 and 66 mg/kg bw (males) or 0, 3.5, 8.7, 26 and 86 mg/kg bw (females) were administered via the diet (0, 20, 50, 150 and 500 mg/kg food). In this study no carcinogenic potential of acequinocyl was observed.

5.8.2 Carcinogenicity: inhalation

No data available.

5.8.3 Carcinogenicity: dermal

No data available.

5.8.4 Carcinogenicity: human data

No data available.

5.8.5 Other relevant information

No data available.

5.8.6 Summary and discussion of carcinogenicity

Neither in rats nor mice, acequinocyl showed carcinogenic potential. Thus, acequinocyl does not need to be classified for carcinogenicity.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

A 2-generation study was performed in accordance with OECD 416.

Treatment related effects on mating, fertility or gestation were not observed at any dose levels in the F0 and F1 animals. Developmental effects observed in the 2-generation study are discussed in section 5.9.2.

5.9.2 Developmental toxicity

A 2-generation study was performed in rats in accordance with OECD 416. Doses of 100, 800, 1500 mg/kg food were administered (equal to 7.3, 59 and 111 mg/kg bw/d for F0-males, and to 8.7, 69 and 134 mg/kg bw/d (pre-mating) and 6.9, 56 and 107 (gestation) and 15, 120 and 226 (lactation) for F0-females; equal to 8.2, 66 and 124 mg/kg bw/d for F1-males, and to 8.9, 70 and 136 mg/kg bw/d (pre-mating); 7.0, 56 and 108 (gestation); 15.8, 125 and 230 (lactation) for F1-females). Results are summarized in Table 5.14.

Table 5.14: 2-generation study

Dose (mg/kg food)	**0**		**100**		**800**		**1500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	no mortality								
Clinical signs - pale appearance[1]	0/25	0/25	0/25	0/25	0/25	0/25	1/25	1/25	
Body weight	no treatment-related effects								
Food consumption	no treatment-related effects								
Organ weight - spleen								ic[a]	
Pathology									
macroscopy	no treatment-related effects								
microscopy	no treatment-related effects								
F1 pups									

Dose (mg/kg food)	**0**		**100**		**800**		**1500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Litter size	no treatment-related effects								
Survival index *Mortality* - day 22-56	2/173		0/162		1/174		40/175		
Clinical signs (per litter after weaning) - haemorrhages - swollen body part - pale - bad physical condition - blue nose					+		+++ +++ ++ ++ +		
Sex ratio	no treatment-related effects								
Body weight	no treatment-related effects								
Physical development	no treatment-related effects								
Functional development (days 21/35/48)	no treatment-related effects								
Pathology									
macroscopy - blood in abdomen - bleeding organs - subcutaneous bleeding - blood clots - brain cavity blood filled							i[2] i[2] i[2] i[2] i[2]		
F1 animals									
Mortality	0/25	0/25	0/25	0/25	0/25	0/25	0/25	1/25	
Clinical signs - haemorrhages - protruding eye(s) - red coloured urine					+		+ +	+	
Body weight	no treatment-related effects								
Food consumption	no treatment-related effects								
Organ weight - spleen - liver - lungs								i[a] ic[a] ic[a]	
Pathology									
macroscopy	no treatment-related effects								
microscopy	no treatment-related effects								
F2 pups									
Litter size	no treatment-related effects								
Survival index *Mortality* - day 22-35	1/179		0/172		8/185		64/178		

Dose (mg/kg food)	**0**		**100**		**800**		**1500**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Clinical signs (per litter after weaning) - blue head - blue extremities - swollen extremities - swollen head - bad physical condition - pale					+ +		+++ +++ +++ +++ +++ ++		
Sex ratio	no treatment-related effects								
Body weight	no treatment-related effects								
Physical development time of: - onset of eye opening - descending of testes - preputial separation - opening of vagina					ic	ic	ic ic ic	ic ic	
Functional development (day 21) - startle response intensity					dc	dc	dc	dc	
Pathology									
<u>macroscopy</u> - colouration of organ/area - bleeding organs - subcutaneous bleeding - blood clots - brain haemorrhage					i[2] i[2]		i[2] i[2] i[2] i[2] i[2]		

dr dose related. If no dose effect relation was determined, the cell is empty
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
[a] absolute organ weight, relative organ weight was not provided
\+ present in ≥5-30% of the animals
++ present in >30-50% of the animals
+++ present in >50% of the animals
[1] males: day 149-155, females: day 71
[2] not tested for statistically significance

In F0-animals, pallor was observed in two animals administered 1500 mg/kg food and the spleen weight was increased, but it is not clear whether the effect was treatment-related or not. In F1-animals administered 1500 mg/kg food, treatment-related clinical signs like haemorrhages and protruding eyes were observed in a few animals, in addition to increased organ weights. In the 800 mg/kg food dose group of F1-animals haemorrhages and protruding eyes were observed in some animals. Treatment-related effects were observed in F1-pups after weaning. The mortality increased in the highest dose group, where pups with haemorrhages, swollen body parts and pallor were observed in most litters. The incidence of histopathological haemorrhagic effects was high compared to the control group. A few pups administered 800 mg/kg food showed the same or similar haemorrhagic effects. Effects similar to those seen in the F1-pups were observed in F2-pups administered 800 or 1500 mg/kg food. However, the adverse effects in the F2-pups were greater, with more severe effects in more litters, and clinical symptoms starting earlier after weaning. A statistically significant delay in the physical development and functional development was observed in the two highest dose groups, indicating an effect on the offspring through the parents. The delay was found for more parameters in the highest dose group than in the 800 mg/kg food group. The adverse effects (clinical signs and mortality) in the F1 and F2 pups occur post weaning, indicating that this is probably caused by exposure to a higher dose of acequinocyl when this is consumed via

food instead of milk (starting at a dose of 800 mg/kg food, or ≥ 59 mg/kg bw/day, since pups eat more food/kg bw than adults).

A teratogenicity study in rats was performed in accordance with OECD 414 (1981). Due to the maternal toxicity observed in animals in the higher dose groups, dosing was suspended in the main study between day 10 and 13, i.e. animals in this dose group were dosed for minimally four and maximally seven days instead of the required eleven days. The surviving females were sacrificed on day 20 of pregnancy. Results are summarized in Table 5.15.

Table 5.15: Teratogenicity study in rats

Dose (mg/kg bw/day)	**0**	**50**	**150**	**500**	**750**	**dr**
Maternal effects						
Mortality (n=25)	0	0	0	1	4	
Clinical signs - occasional red vaginal discharge - pallor - hypoactivity - pale eyes - piloerection - slow/irregular breathing	 0/25 0/25 0/25 0/25 0/25 0/25	 0/25 0/25 0/25 0/25 0/25 0/25	 0/25 0/25 0/25 0/25 0/25 0/25	 1/25 1/25 0/25 1/25 1/25 1/25	 7/25 4/25 4/25 4/25 2/25 4/25	
Pregnant animals	no treatment-related effects					
Body weight gain - day 7-17					d	
Food consumption - pregnancy day 7-10					d	
Organ weight - gravid uterus weight					dc	
Pathology						
Macroscopy - blood stained fur, thin/brown blood in vaginal opening - pale kidney(s) - pale liver - pale spleen - intra-uterine haemorrhage - stomach/intestinal contents blood stained				 + + + + +	 + + + + + + +	
Litter response						
Live foetuses					dc	
Foetal weight					d	
Post implantation loss					ic	
Sex ratio	no treatment-related effects					
Examination of the foetuses						
External and visceral observations - major abnormalities					i[1]	

Dose (mg/kg bw/day)	**0**	**50**	**150**	**500**	**750**	**dr**
Skeletal findings - major abnormalities					i[1]	

dr dose related. If no dose effect relation was determined, the cell is empty
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
\+ present in a few animals
[1] 5.9%. See text below for details

In the highest dose group (750 mg/kg bw/d), three foetuses with abnormalities were observed (from three litters). One foetus had dextrocardia and single right and left lung lobes; one foetus had imperforate anus, filamentous tail, and caudal and sacral centra and neural arches absent; one foetus had interrupted aortic arch, transposition of the great blood vessels, enlarged atria, misshapen heart, hypoplastic lung lobes, anasarca, short tail, brachydactyly and a misshapen femur. OECD Guideline 414 states that the highest dose should be chosen with the aim of inducing some maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering. As treatment-related maternal mortality was observed at the highest dose of 750 mg/kg bw/d, this dose was obviously too high to properly assess the developmental effects of the test substance. Moreover, the observed abnormalities at the high dose were of a diverse nature and were limited to three foetuses from nineteen litters (246 foetuses examined).

Maternal effects were observed from 500 mg/kg bw/d onwards. One female administered 500 mg/kg bw/d and four females administered 750 mg/kg bw/d were prematurely sacrificed between days 13 and 17 of pregnancy, due to treatment-related adverse effects. The animals had red vaginal discharge, pallor, irregular breathing and were hypoactive. At necropsy, observations included intra-uterine haemorrhage, bloodstained stomach or intestinal contents, pale organ(s) and brown coloured, thin blood. As the females in the high dose group were only dosed 4-7 times, the foetuses were only exposed to the test substance during certain periods of the gestation. Therefore, the adverse effects seen in the highest dose group could have been worse or more numerous should the prescribed exposure have been implemented.

Another teratogenicity study was performed in rabbits, according to OECD 414 (1981). Dose levels were based on a range finding study, in which animals administered 240 mg/kg bw/d were sacrificed prematurely due to body weight loss and haemorrhage from the vagina, and they showed haemorrhage of internal organs at necropsy and in which in the 60 and 120 mg/kg bw/d dose groups, slight body weight loss and increased post-implantation loss was observed compared to control. In the main study, the surviving females were sacrificed on day 28 of pregnancy. Results are summarized in Table 5.16.

Table 5.16: Teratogenicity study in rabbits

Dose (mg/kg bw/day)	**0**	**30**	**60**	**120**	**dr**
Maternal effects					
Mortality	1	0	0	5	
Clinical signs - red liquid on the tray liner - faeces loose/ liquid				 + +	
Pregnant animals	no treatment-related effects				
Resorption (80-100%)				+	
Body weight gain	no treatment-related effects				
Food consumption				d[1]	
Organ weight - gravid uterus weight	no treatment-related effects				
Pathology					
macroscopy - blood stained vaginal opening - pale lungs - pale liver - fur in stomach - intra-uterine haemorrhage - discoloured amniotic fluid - blood in urine				 + + + + + + +	
Litter response					
Live foetuses	no treatment-related effects				
Foetal weight	no treatment-related effects				
Post implantation loss	no treatment-related effects				
Sex ratio	no treatment-related effects				
Examination of the foetuses					
External and visceral observations	no treatment-related effects				
Skeletal findings - extra 13th ribs group mean	 24.5 %	 19.1 %	 I 33 %	 Ic 43.8 %	

dr dose related. If no dose effect relation was determined, the cell is empty
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
\+ a few animals more than in control group
[1] statistically significant days 6-8 and 16-18 of pregnancy

Maternal effects were observed at 120 mg/kg bw/d. Five females in this dose group were sacrificed between day 15 and 20 of pregnancy as red liquid on the tray liner and loose/liquid faeces was observed. In the opinion of the reporting member state, the reason for sacrificing the animals is not justified, and more could have been learnt about the effects of the test substance on the rabbits and particularly the offspring by keeping them alive until the scheduled end of the study. The pathological findings included intra-uterine haemorrhage, pale liver and lungs, blood in the urine and resorption of foetuses. In three surviving females administered 120 mg/kg bw/d, the amniotic fluid was discoloured. A statistically significant increase in the number of 13th ribs was observed in

offspring of females in the highest dose group, and is probably a result of maternal toxicity. Although a slight, not statistically significant, increase was also observed in the 60 mg/kg bw/d dose group, it is not considered to be clearly treatment-related. The PRAPeR meeting (04; 28 Nov.- 01 Dec. 2006) agreed that classification is not necessary.

5.9.3 Human data

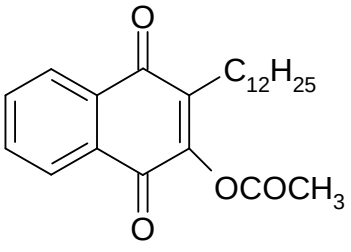
No data available.

5.9.4 Other relevant information

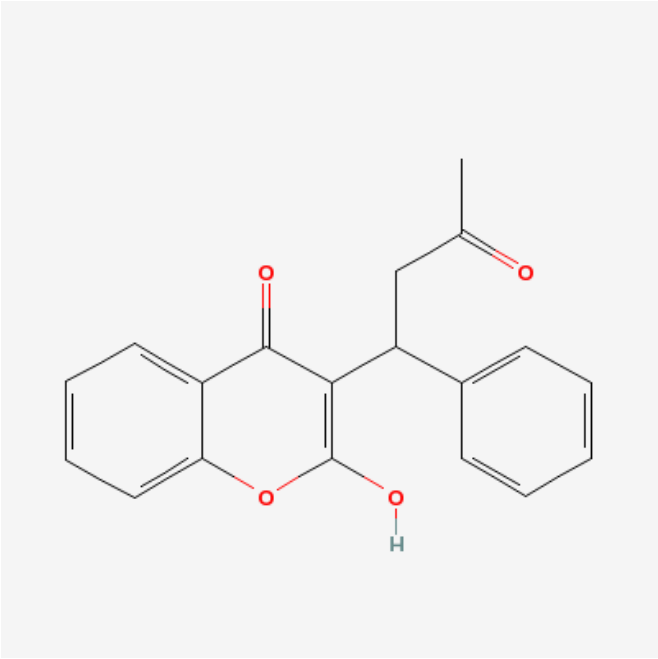
Acequinocyl and most of its identified metabolites are structural analogues of vitamin K (see structures below). Therefore, its mechanism of toxicity is expected to be competitive inhibition of the vitamin K dependent prothrombin synthesis. A reduction in prothrombin synthesis will result in a prolonged blood clotting time and an increase in haemorrhages and related haematological effects as observed in the repeated dose toxicity studies with acequinocyl.

Warfarin, another structural analogue of vitamin K (see structure below) was used in humans to reduce blood clotting. Administration of warfarin (CAS 81-81-2) to pregnant women resulted in an increase in fetal warfarin syndrome or warfarin embryopathy characterised by bone stippling (chondrodysplasia punctata) and nasal hypoplasia (C&L proposal warfarin ECBI/54/06). Warfarin is classified in Annex VI of EC 1272/2008 with Repro Cat 1; R61.

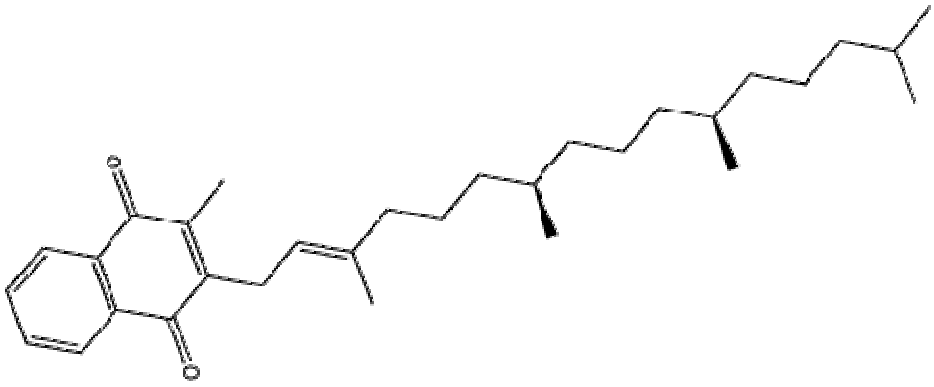
The eventual classification of several analogues of warfarin (coumarines) used as rodenticides for developmental toxicity based on read-across from warfarin is planned to be discussed in the RAC. This discussion started already in the TC-C&L but was not finalised. Coumarines have a structural similarity with vitamine K and effects on blood clotting. This also applies to acequinocyl, although the potency of acequinocyl is much lower than the potency of warfarin. As for acequinocyl, there are negative developmental studies with the coumarines. The discussion on the justification of read-across of the developmental toxicity from warfarine to the other coumarines is therefore also important for acequinocyl. Eventual classification of acequinocyl for developmental effects using read-across from warfarin should be discussed together with the other coumarines.



acequinocyl



warfarin



vitamin K

5.9.5 Summary and discussion of reproductive toxicity

The results of the reproduction toxicity and teratogenicity studies are summarised in Table 5.17.

Table 5.17: Summary of reproduction toxicity and teratogenicity studies

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects
Reproduction toxicity					
2-generation toxicity study	rat	parental	6.9	56	haemorrhages
		developmental	6.9	56	haemorrhagic effects, delayed physical and functional development before weaning
		fertility	107	-	no reproductive effects
Embryo/foetal toxicity and teratogenicity					
teratogenicity study	rat	maternal	150	500	intra-uterine haemorrhage, thin blood
		developmental	500	750	fewer live foetuses, increased post-

Type of study	Species		NOAEL (mg/kg bw/d)	LOAEL (mg/kg bw/d)	Critical effects
					implantation loss
				-	no teratogenic effects
teratogenicity study	rabbit	maternal	60	120	haemorrhage, pale liver and lungs
		developmental	60	120	increased incidence of 13^{th} ribs
				-	no teratogenic effects

No fertility effects were observed in the available studies including the 2-generation study. Therefore, no classification for effects on fertility is proposed.

In a 2-generation reproduction study in rats, post-weaning adverse clinical effects and mortality were observed. These effects are considered to be secondary to the high exposure to acequinocyl in this period (highest growth rate thus highest food uptake) and not due to a higher susceptibility of the developing pups. Therefore, these effects do not warrant classification for developmental toxicity.

In a teratogenicity study in rats, an increased number of non-consistent major abnormalities were observed (not significant, three pups with different abnormalities) at a dose that also induces marked maternal toxicity including mortality. Classification is not necessary because these effects are probably not treatment related.

In rabbits, a statistically significant increased incidence of variations (13^{th} ribs) was observed in a teratogenicity study (43.8% in the top dose group vs. 24.5% in the control group), at a dose that also induces marked maternal toxicity including mortality. Classification is not necessary based on these effects because the effects are considered to be minor and probably secondary to the maternal toxicity.

Based on the study results it can be concluded that acequinocyl does not need to be classified for effects on reproduction. However, eventual classification of acequinocyl for developmental effects using read-across from warfarin should be discussed maybe together with the other coumarines.

5.10 Other effects

No data available.

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

According to the results of the tests on explosive properties (EEC Method A14 Flame test) with the technical active substance (purity 98.25%), acequinocyl is not thermal sensitive and has no mechanical sensitivity towards shock and friction (Young, 1999a) (B.2.1.22 (IIA 2.13)). Therefore,

it can be concluded that acequinocyl has no explosive properties and does not require classification for explosivity.

6.2 Flammability

According to the results of the tests for flammability and auto-flammability (EEC Method A10 and EEC Method A16) with the technical active substance (purity 98.25%), acequinocyl is not highly flammable and has no self ignition up to 450°C (Young, 1999a) (DAR B.2.1.20 (IIA 2.11)). Therefore, classification for flammability is not required.

6.3 Oxidising potential

According to the results of the tests for oxidising properties (EEC Method A17, CIPAC MT 75.2) with the technical active substance (purity 98.25%), acequinocyl is not oxidising and does not require classification (Young, 1999a) (B.2.1.23 (IIA 2.15)).

7 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for acequinocyl is based on the Draft Assessment Report, the Addendum to the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of acequinocyl in Annex I of Council Directive 91/414/EEC (DAR September 2007 + addendum January 2008, RMS The Netherlands) on the inclusion of acequinocyl in Annex I to Directive 98/8/EC concerning the placing biocidal products on the market.

All tables in the present assessment are copied from the DAR or the addendum to the DAR. The tables are renumbered in accordance with the paragraph numbers.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

In all fish tests and the algal growth test, the test substance was dosed above the water solubility of acequinocyl of 6.69 µg/L. Such a value for water solubility can be expected on basis of the log K_{ow}, being > 6. However, no adverse effects were observed in the fish and algae tests. The tests with crustaceans were all carried out at much lower concentration levels, most likely because physical hampering by undissolved material would have been occurred otherwise. The results of the acute aquatic toxicity studies with acequinocyl are summarized in Table 7.1.

Table 7.1: Summary of acute toxicity values of acequinocyl to aquatic organisms.

Purity	Species	Endpoint	Toxicity value in µg/L	Test Guideline
Acute toxicity to fish				
97.1%	Rainbow trout *Oncorhynchus mykiss*	96h-LC50 96h-NOEC	> water solubility ≥ water solubility	OECD 203
97.1%	Sheepshead minnow	96h-LC50	> water solubility	FIFRA 72-3

	Cyprinodon variegatus	96h-NOEC	≥ water solubility	OPPTS 850.1075
97.1%	Bluegill sunfish *Lepomis macrochirus*	96h-LC50 96h-NOEC	> water solubility ≥ water solubility	FIFRA 72-3 OPPTS 850.1075
97.1%	Zebra fish *Brachydanio rerio*	96h-LC50 96h-NOEC	> water solubility ≥ water solubility	OECD 203 EC C.1
Acute toxicity to invertebrates				
99.85%	Waterflea *Daphnia magna*	48h-EC50 48h-NOEC	3.9 1.5	OECD 202 FIFRA 72-2
97.1%	Mysid *Mysidopsis bahia*	96h-EC50 96h-NOEC	0.93 0.27	OPPTS 850.1035
Toxicity to algae				
97.1%	*Pseudokirchneriella subcapitata*	72h-ErC50 -72h-E_bC50 NOEC	> water solubility ≥ water solubility	EC C.3

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to bees

Not applicable for this type of dossier.

7.2.1.2 Toxicity to soil macro organisms

Not applicable for this type of dossier.

7.2.1.3 Toxicity to soil micro-organisms

Not applicable for this type of dossier.

7.2.1.4 Toxicity to other terrestrial organisms

Not applicable for this type of dossier.

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

No data available.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

Not applicable for this type of dossier.

7.4.2 PNEC for sewage treatment plant

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

In the acute toxicity tests with the active substance acequinocyl the 48h-EC50 for mobility of Daphnia magna was 3.9 µg/L. The 48h EC50 for the marine mysid shrimp was 0.93 µg/L. In the fish tests with four species and in the algae growth test, there were no effects observed at the water solubility level. Acequinocyl hydrolyses under all pH conditions. Under neutral and alkaline conditions it degrades rapidly. Under acid conditions the hydrolysis is slower. The log Kow is > 6 and the solubility in water is 6.69 µg/L.

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

Acequinocyl produces an EC50 value in crustaceans at a concentration < 1 mg/L.

Acequinocyl is considered rapidly degradable.

The BCF value of acequinocyl (based on total radioactivity) is > 100.

Acequinocyl therefore fulfils the criteria for classification with N; R50/53.

According to directive 2006/8/EC (amending directive 1999/45/EC) an M-factor of 1000 should be applied, based on an EC50 value of 0.93 µg/L obtained for the marine crustacean *Mysidopsis bahia* in a 96-h flow-through study.

Conclusion of environmental classification according to GHS

Acequinocyl produces an EC50 value in crustaceans at a concentration < 1 mg/L.

Acequinocyl and its relevant metabolites are considered rapidly degradable.

The BCF value of acequinocyl (based on total radioactivity) is < 500.

The major metabolites (acequinocyl-OH and AKM-18) formed in soil and water/sediment are also considered rapidly degradable and not more toxic than the parent compound. Based on the information provided in the bioaccumulation study, it is considered unlikely that the major metabolites are more bioaccumulative than the parent compound.

Acequinocyl therefore fulfils the criteria for classification into aquatic environmental hazard acute category 1.

An M-factor of 1000 should be applied, based on an EC50 value of 0.93 µg/L obtained for the marine crustacean *Mysidopsis bahia* in a 96-h flow-through study.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Acequinocyl is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation EC 1272/2008 article 36.2).

OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of acequinocyl according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DAR and the 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 addenda.

REFERENCES

European Commission. Draft Assessment Report Acequinocyl, prepared by The Netherlands, revised version of September 2007.

European Commission. Draft Assessment Report Acequinocyl, prepared by The Netherlands, final addendum of January 2008.

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