CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: BIFENAZATE

EC Number: **442-820-5**

CAS Number: **149877-41-8**

Index Number: **none**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1.1: Substance identity

Substance name:	bifenazate
EC number:	442-820-5
CAS number:	149877-41-8
Annex VI Index number:	-
Degree of purity:	950 g/kg minimal
Impurities:	no relevant impurities

1.2 Harmonised classification and labelling proposal

Table 1.2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	None	None
Current proposal for consideration by RAC	Skin sensitisation 1B; H317 STOT RE 2; H373 Aquatic Acute 1 with an M-factor of 1; H400 Aquatic Chronic 1 with an M-factor of 1; H410	R43 N;R50/53 with SCL of Cn ≥ 25% N; R50/53, 2.5% ≤ Cn <25% N; R51/53 0.25% ≤ Cn <2.5%; R52/53

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin sensitisation 1B; H317 STOT RE 2; H373 Aquatic Acute 1 with an M-factor of 1; H400 Aquatic Chronic 1 with an M-factor of 1; H410	R43 N;R50/53 with SCL of Cn ≥ 25% N; R50/53, 2.5% ≤ Cn <25% N; R51/53 0.25% ≤ Cn <2.5%; R52/53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none			Conclusive but not sufficient for classification
2.2.	Flammable gases	none			Conclusive but not sufficient for classification
2.3.	Flammable aerosols	none			Conclusive but not sufficient for classification
2.4.	Oxidising gases	none			Conclusive but not sufficient for classification
2.5.	Gases under pressure	none			Conclusive but not sufficient for classification
2.6.	Flammable liquids	none			Conclusive but not sufficient for classification
2.7.	Flammable solids	none			Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none			Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none			Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none			Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none			Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none			Conclusive but not sufficient for classification
2.13.	Oxidising liquids	none			Conclusive but not sufficient for classification
2.14.	Oxidising solids	none			Conclusive but not sufficient for classification
2.15.	Organic peroxides	none			Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none			Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	none			Conclusive but not sufficient for classification
	Acute toxicity - dermal	none			Conclusive but not sufficient for classification
	Acute toxicity - inhalation	none			Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	none			Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	none			Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	none			No data
3.4.	Skin sensitisation	Skin Sens. 1B			-

3.5.	Germ cell mutagenicity	none			Conclusive but not sufficient for classification
3.6.	Carcinogenicity	none			Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	none			Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	none			Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 2			
3.10.	Aspiration hazard	none			Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 Aquatic Chronic 1	M=1 M=1		
5.1.	Hazardous to the ozone layer	none			Conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Pictogram: GHS07, GHS09

Signal word: warning
Hazard statements: H317 May cause an allergic skin reaction
H373 may cause damage to organs (blood) through prolonged or repeated exposure
H410 Very toxic to aquatic life with long lasting effects
Precautionary statements: not necessary

Proposed notes assigned to an entry:

None

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	None			conclusive but not sufficient for classification
Oxidising properties	None			conclusive but not sufficient for classification
Flammability	None			conclusive but not sufficient for classification
Other physico-chemical properties	None			conclusive but not sufficient for classification
Thermal stability	None			conclusive but not sufficient for classification
Acute toxicity	None			conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	None			conclusive but not sufficient for classification
Repeated dose toxicity	None			conclusive but not sufficient for classification
Irritation / Corrosion	None			conclusive but not sufficient for classification
Sensitisation	R43	none		
Carcinogenicity	None			conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	None			conclusive but not sufficient for classification
Toxicity to reproduction – fertility	None			conclusive but not sufficient for classification
Toxicity to reproduction – development	None			conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None			conclusive but not sufficient for classification
Environment	N;R50/53	Cn ≥ 25% N; R50/53, 2.5% ≤ Cn <25% N; R51/53 0.25% ≤ Cn <2.5%; R52/53		

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger:	Xi, N
R-phrases:	R43-50/53
S-phrases:	S(2)-24-37-46-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Bifenazate is a new active substance for plant protection products and as such has not been discussed before for a CLH proposal.

2.2 Short summary of the scientific justification for the CLH proposal

Classification for human health hazards.

In a well performed Guinea Pig Maximization test with an intradermal induction concentration of 6%, 85% of the induced guinea pigs showed a positive reaction. This requires classification as Skin Sens. 1B and R43.

STOT RE 2 is required as there is clear evidence of haemolytic anaemia in several species and study duration at dose levels relevant for this hazard class. R48/22 is not required as the dose guidance values are lower and there is a preference for longer studies in the DSD criteria.

Classification for environmental hazards.

In accordance with the 2nd ATP, a classification and an M-factor based on the chronic aquatic toxicity is proposed in addition to a classification and an M-factor based on the acute aquatic toxicity.

According to Directive 67/548/EEC and Directive 1999/45/EC as amended by Directive 2006/8, no distinction between acute and chronic SCLs can be made since only acute aquatic toxicity data are allowed for deriving classifications and SCLs. Therefore, only one set of SCL are proposed for classification of bifenazate according to DSD criteria.

The lowest L(E)C50 obtained for bifenazate are 0.36, 0.42 and 0.76 mg/l in algae, invertebrates and fish, respectively. Bifenazate therefore fulfils the criteria for classification as Aquatic Acute Cat. 1 with an M-factor of 1.

Bifenazate is considered not rapidly degradable (see section 5.1.3). NOEC values for bifenazate are available for all trophic levels. The lowest NOEC is 0.017 mg/l obtained for fish. Bifenazate therefore fulfils criteria for classification as Aquatic Chronic Cat. 1 with an M-factor of 1.

The lowest acute aquatic toxicity values for bifenazate are 0.36, 0.42 and 0.76 mg/l in algae, invertebrates and fish, respectively. Bifenazate is not readily degradable (see section 5.1.3). Furthermore, the log Kow value of bifenazate is 3.4. Bifenazate therefore fulfils the criteria for classification with N;R50/53. The specific concentration limits (SCL) are Cn ≥ 25% N; R50-53, 2.5% ≤ Cn <25% N; R51-53 and 0.25% ≤ Cn <2.5%; R52-53.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

None

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

None

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification according to the inventory of notified classification and labelling on 10 January 2013 was:

Table 2.1 Self-classification according to the C&L inventory.

Classification		Labelling			Specific Concentration limits, M- Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms Signal Word Code(s)			
Skin Sens. 1	H317	H317		GHS07 GHS09 Wng			23
Eye Irrit. 2	H319	H319					
Aquatic Acute 1	H400	H400					
Skin Sens. 1	H317	H317		GHS07 GHS09 GHS08 Wng			20
STOT RE 2	H373	H373					
Aquatic Acute 1	H400						
Aquatic Chronic 1	H410	H410					
Aquatic Acute 1	H400	H400		GHS09 Wng			1

2.4.2 Current self-classification and labelling based on DSD criteria

The inventory of notified classification and labelling does not contain the self-classification according to the DSD criteria. There is no registration (10 January 2013).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Bifenazate is an active substance for plant protection products and was included in Annex I of Directive 91/414/EEC via Commission Directive 2005/58/EC. Bifenazate is included in the Annex to Regulation (EC) No 1107/2009 via Commission Implementing Regulation (EU) No 540/2011. Bifenazate is therefore subject to harmonised classification and labelling according to article 36.2 of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

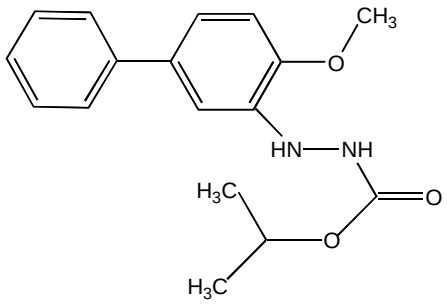
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1.1: Substance identity

EC number:	442-820-5
EC name:	isopropyl 2-(4-methoxybiphenyl-3-yl)hydrazinoformate
CAS number (EC inventory):	
CAS number:	149877-41-8
CAS name:	hydrazinecarboxylic acid, 2-(4-methoxy[1,1'-biphenyl]-3-yl)-, 1-methylethyl ester
IUPAC name:	Acording to ECHA: isopropyl 2-(4-methoxybiphenyl-3-yl)hydrazinecarboxylate According to Commision Directive 2005/58/EC: isopropyl 2-(4-methoxybiphenyl-3-yl)hydrazinoformate
ISO name	bifenazate
CLP Annex VI Index number:	-
Molecular formula:	$C_{17}H_{20}N_2O_3$
Molecular weight range:	300.4

Structural formula:



1.2 Composition of the substance

Table 1.2.-1: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
bifenazate	980 g/kg	Minimal 950 g/kg	

Table 1.2-2: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			

The impurities are not expected to affect the classification and labelling.

Table 1.2-3: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
confidential				

1.2.1 Composition of test material

The purity of the batches used as test material ranged from 90.2% to 92.4% purity. This is clearly below the typical purity of 98.0% and the minimal purity of 95.0% of the substance as put on the market. This was mainly due to an impurity in the test material which is no longer present. Most impurities present in the substance as put on the market were also present in the test material. If the toxicity is only determined by bifenazate and not by the impurities present in the test material than the actual toxicity of the substance put on the market may be underestimated by 5 to 10% due to the difference in purity. This is considered small and acceptable. If the toxicity of the test material was caused or partly caused by the impurity no longer present in the substance as put on the market, than this is considered an over prediction of the toxicity. No difference is expected due to other impurities as the concentration is small and comparable between the test material and the substance

as put on the market. Overall, the composition of the test material is considered acceptable for the prediction of the toxicity of bifenazate as placed on the market.

1.3 Physico-chemical properties

Table 1.3-1: Summary of physico - chemical properties

Property	Value (purity substance)	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Crystalline solid	Tutty, 1995[a] and Friedlander, 1998aa	Visual observation
Melting/freezing point	123-125˚C (99.7%)	Riggs, 1998 [a]	measured
Boiling point	Decomposition before boiling (98.1%)	Riggs, 2002 [a]	
Relative density	1.19 g/cm^3 (99.7%)	Stevenson, 1998[a]	measured
Vapour pressure	at 25 ˚C < 1.33 x 10^{-5} Pa (99.5%)	Penny, 1996 [a]	measured
Surface tension	64.9 mN/m for concentration of 2.09x10-^{3} g/L at 22 ˚C (technical).	Cuthbert, 2000 [a]	measured
Water solubility	pH (neutral): 2.06 mg/L at 20 ˚C (99.7%)	Riggs, 1998 [a]	measured
Partition coefficient n-octanol/water	log Pow 3.4 (99.9%) (HPLC method, non-buffered, temp. 40 ˚C)	Riggs, 2001b [a]	measured
Flash point	-	[a]	Not required, melting point > 40˚C
Flammability	Non flammable	Donnelly, 1996a, [a]	measured
Explosive properties	Non explosive	Tremain, 2000 [a]	measured
Self-ignition temperature	none	Tremain, 2000 [a]	
Oxidising properties	none	Donnelly, 1996b [a]	measured
Granulometry	No data		
Solubility in organic solvents and identity of relevant degradation products	Solubility at 20 ^{0}C for substance (purity 99.7%): ethyl acetate: 102 g/L toluene: 24.7 g/L methanol: 44.7 g/L acetonitrile: 95.6 g/L hexane: 0.232 g/L 1-octanol: 8.91 g/L Solubility at 20 ˚C, for technical substance (purity 93.4%): ethyl acetate: 113 g/L toluene: 26.2 g/L methanol: 50.7 g/L acetonitrile: 111 g/L hexane: 0.232 g/L 1-octanol: 9.54 g/L	Riggs, 1997, 1998 and 2001a [a]	measured

	acetone: 210.7 g/L 1,2-dichloroethane: 189.8 g/L dichloromethane: 331.8 g/L		
Dissociation constant	pKa = 12.94 at 23˚C	Yu, 1997 [a]	measured
Viscosity	Viscosity at 20 ^{0}C: 424 cP at 6 rpm and 83 cP at 60 rpm	McKelvie, 2000 [a]	Measured (product Floramite 240 SC, suspension containing 240 g/l bifenazate)

[a]As summarised in DAR_2003_vol3 B1-B5

The above data refer to Bifenazate. The data are obtained from the Draft Assessment Report, prepared in the context of the inclusion of the active substance in Annex 1 of Council Directive 91/414/EEC.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

Bifenazate is a specific acaricide against a wide range of phytophagous mites and will be used in crops and ornamentals.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 3-1: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point	Not required, melting point > 40˚C		[a]
Flammability	Non flammable		Donnelly, 1996a, [a]
Explosive properties	Non explosive		Tremain, 2000 [a]
Self-ignition temperature	-		Tremain, 2000 [a]
Oxidising properties	none		Donnelly, 1996b [a]

[a]As summarised in DAR_2003_vol3 B1-B5

3.1 Physical chemical properties

3.1.1 Summary and discussion of physical chemical properties

Bifenazate is solid without flammability and explosive or oxidising properties.

3.1.2 Comparison with criteria

Bifenazate does not fulfil the criteria for flammability and explosive or oxidising properties.

3.1.3 Conclusions on classification and labelling

The technical substance need not to be classified as flammable, auto-flammable, explosive or oxidising.

4 HUMAN HEALTH HAZARD ASSESSMENT

No registration of bifenazate was available in the ECHA database on 10 January 2013. However, the substance is notified in accordance with Directive 67/548/EEC. The information from this notification dossier has been assessed but does not contain additional information.

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 the active substance bifenazate in Annex I of Council Directive 91/414/EEC (DAR (March 2003), with the first addenda dated September 2004, the second revised addendum 2 dated March 2005 and the third addenda dated February 2005. 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.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption

Within 72 h in a bile excretion study, gut absorption of radiolabelled (U-ring 1-^{14}C) bifenazate was 88-86% (m-f) in the single oral low dose groups (10 mg/kg bw) and 37-33% in the high dose groups (1000 mg/kg bw), based on the radiolabel recovered from urine, bile, cage wash, tissues and residual carcass. Based on radiolabel recovered from urine, cage wash, tissues and residual carcass only of intact animals, oral "systemic" absorption was at least 28-30% in the single oral low dose groups, at least 37-34% in the repeated oral low dose groups and at least 12-15% in the single high dose groups, 168 h after administration.

Distribution

In rats, less than 1% of the administered radiolabel was retained in tissues (including residual carcass), 168 h post dosing, in all dose groups. In the 10 mg/kg bw dose group, most of the radiolabel was retained in liver, kidney, whole blood and red blood cells (□0.4 mg eq./kg). All other tissues contained less than 0.1 mg eq./kg.

Metabolism

Bifenazate was extensively metabolised in rats dosed with 10 mg/kg bw (7.2-4.8% of the administered dose excreted as parent compound) in contrast to rats dosed with 1000 mg/kg bw where metabolism was not extensive with a larger proportion of the administered dose (61.3 and 47.9%) excreted in the faeces as unchanged bifenazate. A total of eight metabolites were identified in faeces, six metabolites in bile and three metabolites were identified in urine. The metabolite pattern in faeces and urine after repeated oral doses was comparable to that of a single oral low dose. As the amount of unchanged bifenazate in faeces was decreased after repeated oral low doses as compared to single oral low dose, repeated administration does not seem to have saturated metabolism. On the contrary, some induction might have occurred.

Excretion

In rats, 96 h after administration around 90% or more of the administered label had been excreted, irrespective of sex and dose regimen. Most radiolabel was excreted in faeces, in all dose regimens. There were no sex-related differences in excretion.

4.1.2 Human information

No data.

4.1.3 Summary and discussion on toxicokinetics

The oral absorption of bifenazate is at least 28% after a single low dose (10 mg/kg bw), based on radiolabel recovered from urine, cage wash, tissues and residual carcass, measured 168h after administration. The radiolabel was mainly distributed to liver, kidneys, whole blood and red blood cells. The parent compound is extensively metabolised in a network of pathways of which the main steps were: dehydrogenation, hydroxylation, conjugation with glucuronic acid or sulphate and elimination of the hydrazine carboxylic acid moiety. Excretion was rapid as within 96 hours 90% or more of the administered radiolabel had been excreted irrespective of dosing regimen or sex. Most radiolabel was excreted in faeces, in all dose regimens. There were no sex-related differences in excretion.

4.2 Acute toxicity

Table 4.2-1: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD 401 (oral toxicity), rat	LD50 > 5000 mg/kg bw	Limit test	Hoffman, 1996a [a]
OECD 401 (oral toxicity), mouse	LD50 > 5000 mg/kg bw	Limit test	Hoffman, 1996b [a]
OECD 402 (Dermal toxicity), rat	LD50 > 5000 mg/kg bw	Limit test	Hoffman, 1996c [a]
OECD 403 (Inhalation toxicity), rat	LC50 > 4.4 mg/L	Limit test	Hoffman, 1996d [a]

[a] As summarised in DAR_2003_vol3 B6

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Bifenazate (purity 90.4%) was tested in an acute oral test with rats at a dose level of 5000 mg/kg bw (limit test). No treatment related effects were observed (Hoffman, 1996a). In the acute oral test with mice, tested with bifenazate (purity 90.4%) at a dose level of 5000 mg/kg bw, one female died. This female exhibited lacrimation, lethargy, irregular gait, laboured breathing and decreased faecal volume at 7 days after dosing.

4.2.1.2 Acute toxicity: inhalation

In an acute inhalation study with rats exposed to bifenazate (purity 90.4%), at a dose level of 4.4 mg/L (limit test) as a dust no mortality was observed. A few treatment related observations were noted immediately following the exposure including respiratory (moist rales) and secretory (chromodacryorrhea, red/brown nasal discharge) responses. Similar signs were exhibited by animals for up to a week following exposure. During the remainder of the 14-day post-exposure observation period, test animals were generally unremarkable.

4.2.1.3 Acute toxicity: dermal

Bifenazate (purity 90.4%) was tested in an acute dermal test with rats at a dose level of 5000 mg/kg bw (limit test). No mortality and no treatment related effects were observed.

4.2.1.4 Acute toxicity: other routes

No data.

4.2.2 Human information

No human data available.

4.2.3 Summary and discussion of acute toxicity

No mortality was observed in acute oral, dermal, and inhalation studies in rats at the limit dose and in an oral mouse study only 1 animal died at the limit dose.

4.2.4 Comparison with criteria

Classification is required when 50% or more of the test animal die at or below 2000 mg/kg bw (oral and dermal) or 5 mg/L (inhalation of dust). No such effect occurred. Therefore, bifenazate does not meet the criteria for classification based on the acute toxicity studies.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity is required.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the acute toxicity studies no specific effects on target organs were observed.

4.3.2 Comparison with criteria

The substance does not meet the criteria for classification.

4.3.3 Conclusions on classification and labelling

No classification is needed.

4.4 Irritation

4.4.1 Skin irritation

Table 4.4.1-1: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404	Not irritating to skin		2003Hoffman, 1996e [a]

[a]As summarised in DAR_2003_vol3 B6

4.4.1.1 Non-human information

Table 4.4.1-2 Results of the skin irritation test

Scores observed after	0.5 hours	24 hours	48 hours	72 hours
Erythema	1, 0, 1, 0, 0, 0	0, 0, 0, 0, 0, 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

In a well performed skin irritation study, rabbits (m/f) were exposed during 4 h to bifenazate (purity 90.4%) and 2/6 rabbits showed very slight erythema (score 1) only half an hour after application. No signs of irritation were observed at later time points (Hoffman, 1996e).

4.4.1.2 Human information

No human data available.

4.4.1.3 Summary and discussion of skin irritation

In a well performed skin irritation study with rabbits (m/f), very slight erythema (score 1) was observed half an hour after application of bifenazate in 2/6 rabbits and no signs of irritation were observed at later time points.

4.4.1.4 Comparison with criteria

Classification is required when a score at or above 2.3 is observed in 2 or more out of 3 animals. According to the guidance such score is required in at least 4 animals if the test is performed with 6 animals. Classification is also required if persistent effects are observed or very definite positive effects. No such effects were observed. The substance does not meet the criteria for classification.

4.4.1.5 Conclusions on classification and labelling

No classification is needed.

4.4.2 Eye irritation

Table 4.4.2-1: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405	Not irritating to the eyes	-	Hoffman, 1996f [a]

[a]As summarised in DAR_2003_vol3 B6

4.4.2.1 Non-human information

Table 4.4.2-2 Results of the eye irritation test

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	1, 1, 1, 1, 1, 1	0, 1, 1, 1, 1, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva chemosis	0, 0, 1, 1, 1, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctiva discharge	1, 0, 1, 1, 1, 1	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

In a well performed eye irritation study, rabbits received a single instillation of bifenazate (purity 90.4%) in the eye. One hour after exposure slight conjunctival redness, chemosis and discharge (score 1) was observed. Conjunctival redness was still observed in 4/6 animals at 24 h. No effects were observed at later time points.

4.4.2.2 Human information

No human data available.

4.4.2.3 Summary and discussion of eye irritation

In a well performed eye irritation study with 6 rabbits, slight conjunctival redness (score 1) was observed in all six rabbits; slight chemosis and discharge (score 1) was observed after 1 hour of exposure in 4 respectively 5 animals. Only conjunctival redness was still observed in 4/6 animals at 24 h. No effects were observed at later time points.

4.4.2.4 Comparison with criteria

Classification as eye irritant is required when a score at or above 1 (corneal opacity or iritis) or 2 (conjunctival redness or conjunctival oedema) is observed in 2 or more out of 3 animals. According to the guidance such score is required in at least 4 animals if the test is performed with 6 animals. No such effects were observed. The substance does not meet the criteria for classification.

4.4.2.5 Conclusions on classification and labelling

No classification is needed.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In the animal studies available there are no indications that the substance has adverse effects on the respiratory tract.

4.4.3.2 Human information

No human data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

There are no indications that the substance has adverse effects on the respiratory tract.

4.4.3.4 Comparison with criteria

The substance does not meet the criteria for classification.

4.4.3.5 Conclusions on classification and labelling

No classification is needed.

4.5 Corrosivity

Table 4.5-1: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
OECD 404	Not corrosive to the skin	-	Hoffman, 1996e [a]

[a]As summarised in DAR_2003_vol3 B6

4.5.1 Non-human information

No corrosive properties were observed in a skin irritation study (see above, para. 4.4.1).

4.5.2 Human information

No human data available.

4.5.3 Summary and discussion of corrosivity

No corrosive properties were observed in a skin irritation study.

4.5.4 Comparison with criteria

The substance does not meet the criteria for classification.

4.5.5 Conclusions on classification and labelling

No classification is needed.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 4.6-1: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406; Buehler test	Not sensitizing to skin		Hoffman, 1996g [a]
OECD 406; Maximisation test	Sensitizing to skin		Rakhra and Donald, 2001 [a]

[a] **As summarised in DAR_2003_vol3 B6**

4.6.1.1 Non-human information

In a Buehler test guinea pigs were induced topically with 100% w/v (weight/volume) of the bifenazate (purity 90.4%). The induction caused no dermal responses. Following challenge with 100% w/v, no dermal responses were observed in any of the test and negative control animals. Sensitisation of this strain of animals was positively tested with DNCB.

Bifenazate (purity 90.4%) was tested in a maximisation test. Intradermal injection (6% w/v) caused discrete or patchy erythema in all animals. After topical application of bifenazate at a concentration of 60% w/v, no erythema was noted in any of the animals. Mild scabbing was noted at the test site in 2 control group and 3 test group animals. Following challenge with 60% w/v, positive responses were noted in 17 out of 20 (85%) test group animals. Discrete or patchy erythema was seen in 15 animals. Moderate or confluent erythema was noted in 2 animals. No positive responses were noted in any control group animal. Sensitisation of this strain of animals was positively tested with MBT.

4.6.1.2 Human information

No human data available.

4.6.1.3 Summary and discussion of skin sensitisation

In a well performed Buehler test none of the animals (0%) showed a sensitising effect to the skin. This in contrast to a well performed maximisation test, in which 85% of the animals showed a positive effect.

4.6.1.4 Comparison with criteria

When more than 30% of the animals show a sensitising effect in the maximisation test, the substance should be labelled as skin sensitizing. The results in the maximisation test fulfil the criteria as a sensitising effect was observed in 85% of the animals. The maximisation test is

considered more sensitive than the Buehler test which was negative. Therefore, the classification is based on the results of the maximisation test.

Sub-classification as Skin Sens 1B is required according to the 2e ATP of CLP as more than 30% of the animals responded in a GPMT after intradermal induction with a dose containing more than 1% bifenazate.

There is no need to set a Specific Concentration Limit (SCL) for the substance: Although the substance scored a high percentage of sensitised animals (85%), the intradermal induction concentration used in the study was above 1%. Thus the compound is considered a moderate sensitizing substance not needing a SCL.

4.6.1.5 Conclusions on classification and labelling

According to Annex VI of Commission directive 93/21/EEC, Bifenazate needs to be classified as "may cause sensitisation by skin contact" (R43) based on the results of a Maximisation test.

According to the criteria mentioned in the 'Guidance to Regulation No 1272/2008 on CLP' the substance should be classified for skin sensitisation, category 1B: H317 May cause an allergic skin reaction.

4.6.2 Respiratory sensitisation

No specific data available.

4.6.2.1 Non-human information

None

4.6.2.2 Human information

None

4.6.2.3 Summary and discussion of respiratory sensitisation

No data available.

4.6.2.4 Comparison with criteria

Not possible as no data are available.

4.6.2.5 Conclusions on classification and labelling

No classification is needed.

4.7 Repeated dose toxicity

Table 4.7-1: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
28 day oral (dietary) toxicity study OECD 407 (1981), rat	NOAEL: < 500 mg/kg food, equal to 33.3 mg/kg bw per day	decreased body weight gain, effects on haematology and histopathological changes in the spleen	Trutter, 1997a [a]
28 day oral (dietary) toxicity study OECD 407 (1981), mouse	NOAEL: < 200 mg/kg food, equal to 33.9 mg/kg bw per day	decreased body weight gain, effects on haematology and histopathological changes in the thymus and spleen	Trutter, 1997b [a]
21 day dermal toxicity study OECD 410, rat	NOAEL: 80 mg/kg bw per day LOAEL: 400 mg/kg bw per day	decreased body weights and food consumption, effects on haematology and urinalysis, increased spleen weights and histopathological changes in the spleen	Goldenthal, 1998 [a]
90 day oral (dietary) toxicity study OECD 408, rat	NOAEL: 40 mg/kg food, equal to 2.7 mg/kg bw per day LOAEL: 200 mg/kg food, equal to 13.8 mg/kg bw per day	decreased body weights, effects on haematology, increases in relative weights of liver, kidneys, spleen and adrenals; histopathological changes in liver and spleen	Trutter, 1997c [a]
90 day oral (dietary) toxicity study OECD 408, mouse	NOAEL: 50 mg/kg food, equal to 8.0 mg/kg bw per day LOAEL: 100 mg/kg food, equal to 16.2 mg/kg bw per day	increased liver weight and pigment in the spleen	Trutter, 1997d [a]
90 day oral toxicity study OECD 409, dog	NOAEL: 40 mg/kg food, equal to 0.9 mg/kg bw per day LOAEL: 400 mg/kg food, equal to 10.4 mg/kg bw per day	changes in haematological parameters, changes in urinalysis (m), increased liver weights and histopathological changes in the liver	Goldenthal, 1997b [a]
12 months oral toxicity study OECD 452, dog	NOAEL: 40 mg/kg food, equal to 1.0 mg/kg bw per day LOAEL: 400 mg/kg food, equal to 8.9 mg/kg bw per day	changes in haematological parameters and urinalysis, histopathological changes in the bone marrow, kidneys and	Goldenthal, 1999 [a]

Method	Results	Remarks	Reference
		liver	
104 week toxicity and carcinogenicity study in rats OECD 453	NOAEL: 20 mg/kg food, equal to 1.0 mg/kg bw per day LOAEL: 80 mg/kg food, equal 3.9 mg/kg bw per day	Decreased body weight (gain), decreased total food consumption, effects on haematology and increased severity of haemosiderin pigment in the spleen. No carcinogenicity at doses up to the higest dose level tested (200 mg/kg food, equal to 9.7 mg/kg bw/day)	Ivett, 1999a [a]

[a]As summarised in DAR_2003_vol3 B6

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Exposure of rats to bifenazate (purity 91%) at concentrations of 0, 500, 1000, 5000 and 10000 mg/kg food for 28 days resulted in dose-related effects in all groups, which were generally more pronounced in females than in males. The dose levels were equal to 33.3, 66.4, and 319.4 mg/kg bw/day for males and 0, 35.3, 81.6, and 396.5 mg/kg bw/d for females of the 0, 500, 1000, and 5000 mg/kg food groups, respectively and 410.4 mg/kg bw/d for the surviving male of the 10000 mg/kg food group. The results are presented in Table 4.7-2.

Table 4.7-2 Summary of results from a 28 day diet study in rats with bifenazate

Dose (mg/kg food)	0		500		1000		5000		10000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10	9/10	10/10	m, f
Clinical signs[1]							++	++	++	++	m, f
Body weight gain[2]			dc	dc	dc	dc	dc	dc	dc	-[4]	m, f
Food consumption			dc	dc	dc	dc	dc	dc	dc	-[4]	m, f
Ophthalmoscopy	no treatment related findings										
Haematology											
- RBC				dc	dc	dc	dc	dc	d[3]	-[4]	
- Hb				dc	dc	dc	dc	dc	d[3]	-[4]	
- Ht				dc		dc	dc	dc	d[3]	-[4]	
- polychromasia									i[3]		
Clinical chemistry											
- ALP			dc	dc	dc		dc	dc	d[3]	-[4]	m
- inorganic phosphorus									d[3]		
- ALAT									i[3]		
- ASAT								ic	i[3]		

Dose (mg/kg food)	0		500		1000		5000		10000		dr
	m	f	m	f	m	f	m	f	m	f	
Organ weights	no treatment related findings										
Organ weights											
- liver					ic^r	ic^r	ic^r	ic^r	$i^{r,3}$	-[4]	m, f
- kidney						ic^r	dc^a,ic^r	ic^r	$i^{r,3}$	-[4]	m, f
- adrenal				dc^a	ic^r	ic^r	ic^r	dc^a,ic^r	$i^{r,3}$	-[4]	m, f
Pathology											
macroscopy											
liver											
- dark appearance[5]								++	+	+	
kidneys											
- dark appearance[5]								+	+	+	
glandular stomach mucosa											
- darkened areas[5]								+	++	+	
microscopy											
spleen											
- congestion			++	++	++	++	++	++	++	++	
- pigment			++	++	++	++	++	++	++	++	
- lymphoid depletion					++	++	++	++	++	++	m, f
brain and brainstem											
- vacuolisation							++	++	++	++	m, f
- haemorrhage								+	++	++	f
liver											
- atrophy								+	++	+	f
- necrosis (individual cell)							+	+	++	++	m, f
- pigment							+	+	++	++	m, f
- oval cell hyperplasia									++		
thymus											
- lymphoid necrosis		+				+	+	++	++	++	m, f
- lymphoid depletion							+	++	++	++	m, f
lymph nodes											
- lymphoid necrosis	+	+			+	+	+	++	++	++	
- lymphoid depletion								++	++	++	f
bone marrow											
- hypocellular								+	++	+	
- haemorrhage							++	++	++	++	m, f
mandibular salivary gland											
- necrosis								+	+	+	
- atrophy								+	+	+	f
seminal vesicle											
- reduced secretion							+		++		m

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative
\+ present in one/a few animals
++ present in most/all animals

[1] Clinical findings included, but were not limited to ataxia, thin appearance, perinasal crust, hypersensitivity, pale appearance, rough haircoat, hunched posture, hypoactivity, and cold to touch. Less-frequent observations included dyspnoea and/or abnormal respiratory sound, anorexia, few faeces, prostration, head tilt, partial closure of eyes, urine stains, distended abdomen, circling, and tremors.
[2] At week 5, total body weight gain was 41, 47, and 97% below control value for the 500, 1000, and 5000 mg/kg food males and 79 and 58% below control for the 500 and 1000 mg/kg food females. Mean total weight losses occurred in the 10000 mg/kg food males (51 g), and 5000 mg/kg food females (19 g).
[3] only 1 surviving animal
[4] no animals surviving
[5] in unscheduled deaths

At dose levels of 500 mg/kg food and higher, decreased body weight gain, alkaline phosphatase levels and histopathological changes in the spleen (congestion and increased pigment) were observed in both male and female rats. In addition decreased RBC, Ht, and Hb levels were observed

in females at dose levels of 500 mg/kg food and higher. No information on the level of decrease is available. At dose levels of 1000 mg/kg food and higher, histopathological changes in the spleen also included lymphoid depletion. Furthermore, increased relative liver, kidney and adrenal weights, and histopathological changes in the thymus were observed (necrosis and depletion). At 5000 mg/kg food and higher, unscheduled deaths were found, and histopathological changes in brain and brain stem, liver, sternal and femoral bone marrow, mesenteric and mandibular lymph nodes, mandibular salivary gland and seminal vesicles were observed. Gross pathology findings in the unscheduled death included dark appearance of liver, kidneys and areas on the glandular stomach mucosa.
In conclusion, the NOAEL is found to be < 500 mg/kg food, which is equal to < 33.3 mg/kg bw/d. The study was in accordance with OECD 407 (1981), however the dose levels were too high.

Administration of bifenazate (purity 91%) at concentrations of 200, 1000, 2500 and 5000 mg/kg food (equal to 33.9 and 46.7 mg/kg bw/d for the 200 mg/kg food males and females, respectively, and 154.8 mg/kg bw/d for the 1000 mg/kg bw/d males) to mice for 28 days resulted in dose-related effects in all groups, which were generally more pronounced in females than in males. The results are presented in Table 4.7-3

Table 4.7-3 Summary of results from a 28 day diet study in mice with bifenazate

Dose (mg/kg food)	**0**		**200**		**1000**		**2500**		**5000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	0/10	0/10	0/10	0/10	2/10	10/10	10/10	10/10	10/10	10/10	m, f
Clinical signs[1]					++	++	++	++	++	++	
Body weight gain[2]			dc	dc	dc	dc	dc	dc	-[4]	-[4]	m, f
Food consumption			d	i	dc	d	d[3]	d[3]	-[4]	-[4]	m
Ophthalmoscopy	no treatment related findings										
Haematology											
- total leukocyte count				dc		-[4]	-[4]	-[4]	-[4]	-[4]	
- corrected leukocyte				dc		-[4]	-[4]	-[4]	-[4]	-[4]	
- lymphocyte count				dc		-[4]	-[4]	-[4]	-[4]	-[4]	
- eosinophil counts				dc		-[4]	-[4]	-[4]	-[4]	-[4]	
- RBC					dc	-[4]	-[4]	-[4]	-[4]	-[4]	m
Clinical chemistry											
- ALAT			ic	ic	ic	-[4]	-[4]	-[4]	-[4]	-[4]	m
Urinalysis	no treatment related findings										
Organ weights											
- liver/gallbladder			ic[r]		ic[a,r]	-[4]	-[4]	-[4]	-[4]	-[4]	m
Pathology											
macroscopy											
glandular stomach											
- dark areas[5]					+	+	+	++	++	++	m
liver											
- enlarged liver[5]						+			++	++	

Dose (mg/kg food)	0		200		1000		2500		5000		dr
	m	f	m	f	m	f	m	f	m	f	
microscopy											
spleen											
- congestion					+	++	+	++	++	++	m
- lymphoid depletion					+	++	++	++	++	++	m
- lymphoid necrosis						+	+	++	+	++	m, f
- increased pigment				+	++	++	+	+			
thymus											
- lymphoid depletion					+	++	++	++	++	++	m, f
- lymphoid necrosis			+	+		+		++	+	++	f
liver											
- centrilobular necrosis							+	+	+	+	
- necrosis (individual cell)					+				+		
- pigment					++	++					
- fatty change									+	+	
- hypertrophy hepatocyte					+	+	+		+	+	
lymph nodes											
- lymphoid depletion					+	++	++	++	++	++	m
bone marrow											
- hypocellular						+			+	+	
- haemorrhage		+			+	+	++	++	++	++	m, f
brain and stem											
- vacuolisation					+	++					
- haemorrhage					+	+					

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
\+ present in one/a few animals
++ present in most/all animals
a/r absolute/relative
1 Clinical findings included, but were not limited to ataxia and/or limited use of front and/or hindlimb(s), hypoactive behaviour, hunched posture, rough haircoat, urine stains, head tilt, partial closure of eyes, tremors, circling, polypnoea, dyspnoea, pale appearance, thin appearance, hypersensitivity, and prostration.
2 At week 5, total body weight changes were 36 and 60% below control value for the 200 mg/kg food males and females, respectively. Mean total weight losses occurred in the 10000 mg/kg food males (1.8 g). Marked body weight losses were observed among the unscheduled death at 1000 and 2500 mg/kg food animals. The timing of mortality among the 5000 mg/kg animals precluded obtaining relevant information on weight change.
3 only data of week 2-3 from a few surviving animals
4 no data due to deaths
5 in unscheduled deaths

At a dose level of 200 mg/kg food, decreased body weight gain, increased ALAT serum levels, and histopathological changes in the thymus (lymphoid necrosis) were observed in males and females. In addition, decreased haematological parameters (no information on the level of the effects) and histopathological changes in the spleen (increased pigment) were observed in females, and changes in food consumption were observed in males of this dose group. At a dose level of 1000 mg/kg food and higher, increased mortality, dark areas in glandular stomach, clinical signs, and histopathological changes in liver, sternal and femoral bone marrow, mesenteric and mandibular lymph nodes, and brain and brain stem were observed in males and females. Histopathological changes in the spleen also included congestion, lymphoid depletion, and lymphoid necrosis, and thymus histopathological changes also included lymphoid depletion. In addition, decreased RBC and increased liver weights were observed in males and enlarged livers in females of the 1000 mg/kg food groups. In conclusion, the NOAEL is found to be < 200 mg/kg food, which is equal to < 33.9 mg/kg bw/d. The study was in accordance with OECD 407 (1981), however the dose levels were too high and only a limited number of clinical chemistry data were determined.

Administration of bifenazate (assumed purity 100%), at concentrations of 300, 1000, 2000 and 3000 mg/kg food to dogs (2 dogs/sex/dose group) for 28 days resulted in decreased body weights, haematological changes, and histopathological changes in the liver. The dose levels were equal to 0, 7.3, 27.9, 48.7, and 58.4 mg/kg bw/d for males and 0, 8.5, 25.3, 46.3, and 67.1 mg/kg bw/d for females of the 0, 300, 1000, 2000, and 3000 mg/kg food group, respectively. The results are presented in Table 4.7-4

Table 4.7-4 Summary of results from a 28 day diet study in dogs with bifenazate

Dose (mg/kg food)	**0**		**300**		**1000**		**2000**		**3000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none										
Clinical signs	no treatment related findings										
Body weight gain						d	d	d	d	d	m, f
Food consumption			i		i	d	i	d	d	d	
Ophthalmoscopy	not performed										
Haematology											
- RBC			d	d	d	d	d	d	d	d	
- Hb			d	d	d	d	d	d	d	d	
- Ht					d	d	d	d	d	d	
- platelet count				i	i	i	i	i	i	i	
- reticulocyte count				i	i	i	i	i	i	i	
- MCV					i	i	i	i	i	i	
- nucleated erythrocytes					i	i	i	i	i		
- leukocyte count				i		i		i		i	
- segmented neutrophils				i		i		i		i	
- band neutrophils				i		i		i		i	
Clinical chemistry	no treatment related findings										
Urinalysis	no treatment related findings										
Organ weights	not reported										
Pathology											
macroscopy	no treatment related findings										
microscopy											
liver											
- pigment					+	+	+	+	+	+	
lymph nodes											
- erythrophagocytosis	+		+	+	+	+	+		+		

dr dose related

d/i decreased/increased, but not statistically significantly since only 2 animals/sex/dose were used

\+ present in one or two animals

At a dose level of 300 mg/kg food, decreased RBC and erythrophagocytosis in mesenteric and tracheobronchial lymph nodes were observed in both sexes, and mild increases in platelet, reticulocyte, and leukocyte count and segmented and band neutrophils were observed in females only. The study is considered acceptable as a range finding study only, due to the limited number of animals used and the limited number of parameters investigated. Therefore, this study cannot be used to derive a NOAEL or to evaluate the short-term toxic effects of repeated oral exposure of dogs to bifenazate.

Rats were orally exposed to bifenazate (purity 91%) at concentration levels in the food of 0, 40, 200 or 400 mg/kg food (during 90 days). The dose levels were equal to to 0, 2.7, 13.8 and 27.7 mg/kg bw/d for males and 0, 3.2, 16.3, and 32.6 mg/kg bw/d for females. This study, which was in accordance with OECD 408, was extended with a battery of behavioural tests and observations (FOB). The results are shown in Table 4.7-5.

Table 4.7-5 Summary of results from a 90 day diet study in rats with bifenazate

Dose (mg/kg food)	0		40		200		400		dr
	m	f	m	f	m	f	m	f	
Mortality	no treatment related findings								
Clinical signs	no treatment related findings								
Functional Observation Battery	no treatment related findings								
Body weight						dc	dc	dc	m,f
Body weight gain[1]					d	dc	dc	dc	m,f
Food consumption[1]						d	dc	dc	m,f
Ophthalmoscopy	no treatment related findings								
Urinalysis	no treatment related findings								
Clinical chemistry	no treatment related findings								
Haematology									
- RBC[2]					d	dc	dc	dc	m,f
- Hb [2]					d	dc	dc	dc	m,f
- Ht [2]						d		dc	f
Organ weights									
- liver					d^a	i^a, ic^r	dc^a	i^a, ic^r	m,f
- kidneys					i^r	ic^r	ic^r	ic^r	m,f
- spleen					i^r	ic^r	ic^r	ic^r	m,f
- adrenals						i^r		ic^r	f
Pathology									
macroscopy	no treatment related findings								
microscopy									
liver:									
- hypertrophy, centrolobular					+	++	+++	++	m,f
- haematopoiesis, extramedullary							++		
- pigment, Kupffer cell							++		
- necrosis, individual cell					++	+	++		m
spleen:									
- pigment increased in red pulp	+	+++	+	++	++	+++	+++	+++	m
- haematopoiesis, extramedullary	+		+		++	++	+++	+	m
adrenals:									
- vacuolisation in cortex	++		++		+++		+++		m

1 The extent of the reduced body weight gain and food consumption observed tended to decrease with time on study
2 The reductions in RBC, Hb and Ht were 10% or less in all dose groups and both sexes.

dr	dose related
dc/ic	statistically significantly decreased/increased compared to the controls
d/i	decreased/increased, but not statistically significantly compared to the controls
a/r	absolute/relative organ weight
+	present in one/a few animals

++ present in more than a few animals
+++ present in most/all animals

At food concentrations of 200 and 400 mg/kg increases in relative organ weights of liver, kidneys, spleen and adrenals were observed, as well as decreases, 10% or less, in RBC, haemoglobin and haematocryt. The changes in the liver were accompanied by histopathological changes, like centrilobular hypertrophy and necrosis in the mid and high dose group (m/f), and extramedullary haematopoiesis and pigment in Kupfer cells in high dose males. The changes in the spleen were accompanied with an increase in pigmentation in the red pulp and extramedullary haematopoiesis. Furthermore, food consumption and body weight (gain) were reduced. These latter reductions seemed to be reversible as their extent decreased with time during the study. This indicates that there might be a palatability problem with the test substance. The incidence of the histopathological changes observed in the spleen and adrenals of the 40 mg/kg food group was similar to the control group. Based on the effects above, the NOAEL is set at 40 mg/kg food, equal to 2.7 mg/kg bw/day. The LOAEL was 200 mg/kg food, equal to 13.8 mg/kg bw per day. No treatment related findings were observed for the FOBs.

In a dietary study in mice, the animals were exposed to bifenazate (purity 91%) at concentration levels in the food 0, 50, 100, or 150 mg/kg food during 90 days. The dose levels were equal to 0, 8.0, 16.2 and 24.0 mg/kg bw/d for males and 0, 10.3, 21.7, 32.9 mg/kg bw/d for females. The study was performed in accordance with OECD 408, however, the dose level range chosen in this study differ only a factor 1.5-2 from each other which make profound conclusions difficult. The only effects observed were on liver and spleen. Liver weights were increased in males of the mid- and high dose group. The microscopic observation revealed only an effect in the spleen in which an increased incidence of pigment was observed in males of the 100 and 150 mg/kg dose group. The severity grade of this effect increased with dose in males as well in females of the mid and high dose groups. There were no effects on haematological parameters. The NOAEL was 50 mg/kg food, equal to 8 mg/kg bw/day. The LOAEL was 100 mg/kg food, equal to 16.2 mg/kg bw per day.

In a dietary study in dogs, the animals were exposed to bifenazate (purity 92.4%) at concentration levels in the food 0, 40, 400, or 1000 mg/kg food during 90 days. The dose levels were equal to 0, 0.9, 10.4 and 25.0 mg/kg bw/d for males and 0, 1.3, 10.7 and 28.2 mg/kg bw/d for females. The study was performed in accordance with OECD 409. The results are shown in Table 4.7-6.

Table 4.7.-6. Summary of results from a 90 day diet study in dogs with bifenazate

Dose (mg/kg food)	**0**		**40**		**400**		**1000**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality	none								
Clinical signs	no treatment related findings								
Body weight	no treatment related findings								
Body weight gain							d[1]	d[1]	
Food consumption	no treatment related findings								
Ophthalmoscopy	no treatment related findings								

Dose (mg/kg food)	0		40		400		1000		dr
	m	f	m	f	m	f	m	f	
Haematology									
- RBC					dc	dc	dc	dc	m
- Hb					d	dc	dc	dc	m,f
- Ht					d	dc	dc	dc	m
- platelets					ic	ic	ic	ic	m
- MCV					ic	ic	ic	ic	
- MCH					ic	i	ic	i	m
- reticulocytes					i	i	i	ic	m,f
Clinical chemistry									
- Alkaline phosphatase							ic		
- cholesterol							ic		
- protein peak 4					dc	d	dc	d	m
Urinalysis									
- brown coloration					+		++		m
- bilirubin[2]					i		i		m
Organ weights									
- liver					i^{a},i^{r}	$ic^{a,r}$	i^{a}, ic^{r}	$ic^{a,r}$	m,f
Pathology									
macroscopy	no treatment related findings								
microscopy									
liver:									
- hypertrophy, hepatocellular						+	+	++	f
- pigment, brown, trace					+	++	++	++	m,f
- vacuolation, hepatocyte					++	++	++		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
1 Controls had gained 1.6-1.1 kg (m-f) by the end of the study, while the high dose groups had gained 0.5-0.4 kg.
2 Semi-quantitative determination, no statistical analysis performed

At a dose of 1000 mg/kg food, body weight gain in both sexes was reduced in comparison to control animals. At 400 and 1000 mg/kg food, changes in haematological parameters like decreases in RBC, haemoglobin and haematocrit and decreases in platelet count, MCV and MCH were observed in males, but Hb was also decreased in females (no information on the level of change available). Increased liver weights and histopathological changes in the liver (hypertrophy, brown pigment and vacuolation) were observed in both sexes, and changes in urine parameters were observed in males. These observed changes are indicative for the occurrence of haemolysis and for effects on the liver. The toxicological relevance of the observed changes in clinical chemistry parameters is not clear. No effects were observed at 40 mg/kg food equal to 0.9 mg/kg bw per day, hence the NOAEL was set at this level. The LOAEL was 400 mg/kg food, equal to 10.4 mg/kg bw per day.

In a one year dietary dog study, the animals were exposed to bifenazate (purity 92.4%) at concentration levels in the food 0, 40, 400, or 1000 mg/kg food. The dose levels were equal to 0,

1.0, 8.9 and 23.9 mg/kg bw/d for males and 0, 1.1, 10.4 and 29.2 mg/kg bw/d for females. The study was performed in accordance with OECD 452. The results are shown in Table 4.7-7.

Table 4.7-7 Summary of results from a 1 year diet study in dogs with bifenazate

Dose (mg/kg food)	0		40		400		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	none								
Clinical signs	no treatment related findings								
Body weight	no treatment related findings								
Body weight gain[1]					d	d	d	d	
Food consumption[2]					d		d		
Ophthalmoscopy	no treatment related findings								
Haematology									
- WBC					i		ic	i	m
- RBC					d	d	dc	dc	m,f
- Hb (a)					d	d	dc	dc	m,f
- Ht					d	d	dc	dc	m,f
- MCV					ic	ic	i	ic	
- segmented neutrophils					i		ic	i	m
- platelets					ic	ic	ic	ic	m,f
Clinical chemistry									
- total bilirubin					i	ic	ic	ic	m,f
- protein peak 3							ic	i	
- protein peak 4					dc	dc	dc	dc	m,f
Urinalysis									
- brown coloration					+	+	++	++	m,f
- bilirubin[3]					i	i	i	i	
Organ weights									
- liver							$i^{a,r}$	i^{a},ic^{r}	
Pathology									
macroscopy	no treatment related findings								
microscopy									
bone marrow:									
- hyperplasia, myeloid					++	++	++	++	
kidney:									
- pigment in epithelium of convoluted tubules					++	++	++	++	
- pyelitis					+		+	+	
liver:									
- pigment, brown, trace					++	++	++	++	

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
1 body weight gain at the end of the study, from 0-1000 mg/kg food: 4.1, 5.1, 3.6 and 3.6 kg (m), respectively, 3.1, 3.7, 2.5 and 2.1 kg (f)
2 17% and 12%, respectively at 400 and 1000 mg/kg food
3 Semi-quantitative determination, no statistical analysis performed
(a) compared to the control group, the decrease in haemoglobin was less than 10% in the middose group at

3, 6 and twelve months for males and females, was 20, 16 and 21% respectively in the high dose group males and was 16, 14 and 20% in the high dose group females.

The test substance caused haemolysis at mid dose and high dose, based on the observed changes in haematological parameters (decrease in RBC, Hb and haematocrit) on increased bilirubin in plasma as well as in urine, on presence of brown pigment in kidney, liver and urine and on the presence of myeloid bone marrow hyperplasia. A decrease in plasma protein peak 4 was observed in the clinical chemistry data, which (according to the authors) may also be secondary to the observed haemolysis. At the mid and high dose level, there was also some reduction in body weight gain and food consumption, although differences with the control groups did not reach statistical significance. At the high dose, liver weights were increased as well. The toxicological significance of the increase in plasma protein peak 3 and of the pyelitis in the kidney, both observed at 400 and 1000 mg/kg food, is not clear. The NOAEL was 40 mg/kg food, equal to 1.0 mg/kg bw/d, based on the observed effects at the next higher dose level. The LOAEL was 400 mg/kg food, equal to 8.9 mg/kg bw per day.

In a 104 week combined toxicity and carcinogenicity study rats were exposed to bifenazate (purity 90.2%) at dietary levels of 0, 20, 80, or 200 (m) / 160 (f) mg/kg food. The dose levels were equal to 0, 1.0, 3.9, and 9.7 mg/kg bw/d for males and 0, 1.2, 4.8, and 9.7 mg/kg bw/d for females. The study was performed according to OECD 453. The results are shown in Table 4.7-8.

Table 4.7-8. Summary of results from a 104 week combined toxicity and carcinogenicity study in rats with bifenazate

Dose (mg/kg food)	**0**		**20**		**80**		**200(m)/160(f)**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Mortality (n=50)	25	31	30	32	15	28	11	36	
Clinical signs	no treatment related findings								
Body weight (gain)						dc[1]	dc	dc	f
Food consumption						dc[2]	dc	dc	f
Ophthalmoscopy	no treatment related findings								
Haematology - RBC - Hb - Ht						dc[4]		dc[3] dc[3] dc[3]	
Urinalysis	no treatment related findings								
Clinical chemistry - cholesterol							dc[5]		
Organ weights	no treatment related findings								
Pathology									
macroscopy	no treatment related findings								
microscopy *neoplastic lesions*	no treatment related findings								
microscopy *non-neoplastic lesions* *spleen*									

Dose (mg/kg food)	0		20		80		200(m)/160(f)		dr
	m	f	m	f	m	f	m	f	
- increased severity of haemosiderin pigment *pancreas*					+[6]	+[6]	+[6]	+[6]	
- chronic inflammation	13/60	5/60	5/30	2/31	1/16	1/27	22/60	9/60	
- basophilic foci							+		

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
\+ present in one/a few animals
[1] cumulative body weight gain in week 1-13 and body weight in week 3-18
[2] cumulative food consumption in week 1-13
[3] in week 13, 26 and 52
[4] in week 26
[5] in week 26, 52 and 78
[6] at interim sacrifice (week 53)

Toxicologically relevant effects were noted in rats treated with bifenazate at 80 and 160/200 mg/kg food for 104 weeks. The effects included decreased body weight and body weight gains, decreased mean total food consumption in male and female rats of the high dose group and in females of the middose group. Erythrocyte counts were decreased in mid and high dose females. Haemoglobin and haematocryt were decreased in high dose females. The decreases were less than 10% at all time points. In both males and females an increased severity of haemosiderin pigment in the spleen was observed. The test substance is not oncogenic to rats when fed in the diet at concentrations up to 200 mg/kg food for 104 weeks. The NOAEL was 20 mg/kg food, equal to 1.0 mg/kg bw/day. The LOAEL was 80 mg/kg food, equal to 3.9 mg/kg bw per day.

In a 78 week carcinogenicity study mice were exposed to bifenazate (purity 90.2%) at dietary levels of 0, 10, 100 or 225 (m) / 175 (f) mg/kg food. The dose levels were equal to 0, 1.5, 15.4, and 35.1 mg/kg bw/d for males and 0, 1.9, 19.7, and 35.7 mg/kg bw/d for females. The study was performed according to OECD 451. Toxicologically relevant effects were noted in mice treated with bifenazate at 100 and 225/175 mg/kg food for 78 weeks (see table 4.10-2). The effects included decreased body weight and body weight gains, decreased mean total food consumption in male rats of the high dose group. Erythrocyte counts were decreased in high dose males and white blood cell and lymphocyte counts were decreased in mid and high dose males. Liver weights were increased in the high dose group and kidney weights were decreased in males of the mid and high dose group. The test substance is not oncogenic to mice when fed in the diet at concentrations up to 175/225 mg/kg food for 78 weeks. The NOAEL was 10 mg/kg food, equal to 1.5 mg/kg bw/day. The LOAEL was 100 mg/kg food, equal to 15.4 mg/kg bw per day.

Table 4.7-9 Summary of results from a 78 week carcinogenicity study in mice with bifenazate

Dose (mg/kg food)	0		10		100		225 (m) / 175 (f)		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	10	9	8	13	3	5	5	11	
Clinical signs	no treatment related findings								

Dose (mg/kg food)	0		10		100		225 (m) / 175 (f)		dr
	m	f	m	f	m	f	m	f	
Body weight (gain)							d[1]	dc	
Food consumption							dc		
Haematology									
- RBC							dc[2]		
- WBC					dc[2]		dc[2]		
- lymphocytes					dc[2]		dc[2]		
Organ weights									
- liver							ic[a,r]	ic[r]	
- kidneys					dc[a,r]		dc[a,r]		m
Pathology									
macroscopy	no treatment related findings								
microscopy *neoplastic lesions*	no treatment related findings								
microscopy *non-neoplastic lesions*	no treatment related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

a/r absolute organ weight/relative organ weight

1 first 26 weeks only

2 at 52 weeks, no statistically significant effects present at 79 weeks

4.7.1.2 Repeated dose toxicity: inhalation

No studies were submitted.

4.7.1.3 Repeated dose toxicity: dermal

A dermal toxicity study was performed in which rats were exposed to the bifenazate (purity 92.5%), under a semi-occlusive wrap for 6 hours per day during 21 days. The dose levels in the study were 0, 80, 400, and 1000 mg/kg bw per day. The study was performed in accordance with OECD 410.

Table 4.7-10 Summary of results from a 21 day dermal study in rats with bifenazate

Dose (mg/kg bw/d)	0		80		400		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	none								
Clinical signs	no treatment related findings								
Body weight gain					d	dc	dc	dc	
Food consumption					d	d	dc	dc	m, f

Dose (mg/kg bw/d)	0		80		400		1000		dr
	m	f	m	f	m	f	m	f	
Haematology									
- RBC								dc	
- Hb							dc	dc	m
- Ht								dc	
- hypochromasia						2/10		2/10	
- anisocytosis						2/10		2/10	
- polychromasia						1/10		3/10	
- platelet count							ic	i	
Clinical chemistry									
- total bilirubin								ic	
Urinalysis									
- increased ketone level					+		+		
- increased protein level					+	+	+	+	
- specific gravity					i	i	ic	i	m
- volume					dc		dc		m
Ophthalmoscopy	no treatment related findings								
Organ weights									
- spleen					$i^{a,r}$	i^{r}	$ic^{a,r}$	i^{a}, ic^{r}	m, f
- adrenal					i^{r}		i^{a} ,ic^{r}		m
Pathology									
Macroscopy	no treatment related findings								
Microscopy									
spleen									
- extramedullary haematopoiesis[1]			+		++	++	++	++	m, f

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

\+ present in one/a few animals

++ present in most/all animals

[1] 1, 0, 5, and 1 (males) and 0, 1, 1, and 0 (females) of the number of incidences were graded as 'trace' increases; 1, 4, 2, and 8 (males) and 2, 0, 4, and 4 (females) as 'mild' and 0, 0, 0, and 0 (males) and 0, 0, 2, and 6 (females) as 'moderate' for the 0, 80, 400, and 1000 mg/kg bw/d dose groups, respectively.

Dermal exposure of rats to bifenazate at dose levels of 400 and 1000 mg/kg bw for 21 days resulted in decreased body weights and food consumption, haematological changes (level of changes unknown), effects on urinary parameters, increased spleen weights, and extramedullary haematopoiesis in the spleen in both males and females. At 80 mg/kg bw/day the incidence of extramedullary haematopoiesis was slightly increased in males. A similar increased incidence did not occur in females at 80 mg/kg bw/d, although females showed more haematological changes at higher dose levels than males. Furthermore, the spleen weight was not substantially different from controls at 80 mg/kg bw/day in males and females, and there were no haematological changes in peripheral blood at this dose level. After considering these facts, it was concluded that the slightly increased incidence of splenic extramedullary haematopoiesis in males at 80 mg/kg bw/day was incidental, having no clear treatment-relation. In conclusion, the NOAEL is set at 80 mg/kg bw/day.

4.7.1.4 Repeated dose toxicity: other routes

No studies were submitted.

4.7.1.5 Human information

No human data available.

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

After repeated oral administration, bifenazate caused decreases in RBC, Hb and Ht in the three species investigated (dog, mouse and rat). In most studies these haematological changes were part of the critical effects, sometimes in association with histopathological changes related with haemolysis in one or more organs (liver, spleen, bone marrow). The haemolytic symptoms were accompanied by reduced body weight (gain) and food consumption. In most studies bifenazate also caused increased liver weights, although this effect was not always critical and sometimes not very distinct. Based on the (semi)chronic studies in dogs and rats, the overall NOAEL was 1.0 mg/kg bw/day.

After 21 days of dermal exposure of rats to bifenazate, the critical effects observed at a dose level of 400 mg/kg bw per day included histopathological changes in the spleen indicative of haemolysis (and reduced RBC, Hb and Ht at the next higher dose of 1000 mg/kg bw per day), as well as reduced body weight (gain) and food consumption, increased spleen weights and changes in urinary parameters. The NOAEL in this study was 80 mg/kg bw/day, which was higher than the one found in the corresponding oral rat study (<33.3 mg/kg bw/day). Based on these findings, no clear route-specific toxicity of bifenazate was demonstrated in rats.

The type of effects at the dose levels relevant for classification for R48 and STOT RE are included in the table below.

Table 4.7-11 Overview of the effects at the guidance value dose levels.

Study	Guidance value STOT RE 1 / 2	Guidance value R48/25 / R48/22	Dose level	Observed effects	Conclusion CLP	Conclusion DSD
Rat 28 day diet	30 / 300 mg/kg bw/day	15 / 150 mg/kg bw/day	319 (males) and 397 (females) mg/kg bw/day	Mortality (females), Pale appearance, reduced Hb, Ht and RBC and many others	STOT RE 2 is required based on the severe effects at a dose level just above the guidance value	Likely as at twice the guidance value already mortality is observed.
			66 (males) and 82 (females) mg/kg bw/day	reduced Hb, Ht and RBC and spleen congestion, pigment and lymphoid depletion	Unknown for STOT RE 2 as the percentage of reduction in Hb is unknown.	Unknown as the percentage of reduction in Hb is unknown.
Mice 28	30 / 300 mg/kg	15 / 150 mg/kg	155 (males)	Mortality (20% in males, 100% in	STOT RE 2 is required based on	R48/22 is required based on

day diet	bw/day	bw/day	~ 200 (females) mg/kg bw/day	females)and many other effects	the severe effects at a dose level below the guidance value	the severe effects at a dose level just above the guidance value
			34 (males) and 47 (females) mg/kg bw/day lowest dose tested	Decreased body weight gain, effects on WBC in haematology (females), increased pigment in the spleen (females) and lymphoid necrosis in the thymus	The effects observed do not indicate a need for STOT RE	The effects observed do not indicate a need for R48/25
Dogs 28-day diet	30 / 300 mg/kg bw/day	15 / 150 mg/kg bw/day	58 (males) and 67 (females) mg/kg bw/day highest dose tested	Non-significant effect as only 2 dogs per sex were tested. Decreased body weight gain and food consumption, haematological effects on RBC, liver pigmentation and erythrophagocytosis in the lymph nodes	The observed effects do not justify classification. However, the highest dose was clearly below the guidance value for STOT RE 2	The observed effects do not justify classification. However, the highest dose was clearly below the guidance value for R48/22
Rats 90 day diet	10 / 100 mg/kg bw/day	5 / 50 mg/kg bw/day	28 (males) and 33 (females) mg/kg bw/day highest dose tested	Decreased body weight gain, body weight and food consumption. Decreases in RBC, Hb and Ht. changes in liver, kidney, spleen and adrenal weight. centrilobular hypertrophy, Kupfer cell pigmentation and individual cell necrosis in the liver , spleen pigmentation and extramedullary hematopoiesis and increased vacuolisation of the adrenal cortex	The observed effects are limited and do not indicate a need for classification. However, the tested dose levels are clearly below the guidance values showing that classification cannot be excluded based on these data.	The observed effects are limited and do not indicate a need for classification. The tested dose levels are just below the guidance values
Mice 90-day diet	10 / 100 mg/kg bw/day	5 / 50 mg/kg bw/day	24 (males) and 33 (females) mg/kg bw/day highest dose tested	The main effects were limited to an increase in incidence and severity of the pigmentation in the spleen.	The observed effects are limited and do not indicate a need for classification. However, the tested dose levels are clearly below	The observed effects are limited and do not indicate a need for classification. The tested dose levels are just below the

					the guidance values showing that classification cannot be excluded based on these data.	guidance values
Dogs, 90-day diet	10 / 100 mg/kg bw/day	5 / 50 mg/kg bw/day	25 (males) and 28 (females) mg/kg bw/day highest dose tested	Decrease in RBC, Hb and Ht with increases in platelets, MCV, MCH and reticulocytes, brown coloration and bilirubin in the urine and liver hypertrophy, vacuolation and pigmentation.	The observed effects are limited and do not indicate a need for classification. However, the tested dose levels are clearly below the guidance values showing that classification cannot be excluded based on these data.	The observed effects are limited and do not indicate a need for classification. The tested dose levels are just below the guidance values
Dogs, 1 year, diet	2.5 / 25 mg/kg bw/day	1.25 / 12.5 mg/kg bw/day	24 (males) and 29 (females) mg/kg bw/day highest dose tested	Decrease in RBC, Ht and Hb (above 20%), bilirubin increases in plasma and urine, myeloid hyperplasia in the bone marrow, liver pigmentation and kidney pigmentation and pyelitis	As the reduction of Hb is above 20% the criteria for classification with STOT RE 2 are fulfilled.	
			8.9 (males) and 10.4 (females) mg/kg bw/day	Decrease in RBC, Ht and Hb (below 10%), bilirubin increases in plasma and urine, myeloid hyperplasia in the bone marrow, liver pigmentation and kidney pigmentation and pyelitis	The effects at the guidance value for STOT RE 1 are most likely limited and do not fulfil the criteria based on interpolation between the LOAEL and the NOAEL.	The effects at the guidance value for R48/22 are most likely limited and do not fulfil the criteria based on interpolation between the mid and the high dose.
			1.0 (males) and 1.1 (females) mg/kg bw/day	NOAEL		
Rats chronic diet	1.25 / 12.5 mg/kg bw/day	0.625/6.25 mg/kg bw/day	9.7 (males) and 9.7 (females) mg/kg bw/day	Decreased body weight gain and food consumption, decreased RBC, Ht and HB (less than 10%) and increase in spleen pigmentation	The effects at the guidance value for STOT RE 2 are limited and do not fulfil the criteria	The effects at the guidance value for R48/22 are limited and do not fulfil the criteria
Mice 78-	1.7 / 17	0.8 / 8.0	35.1 (males)	Decreased body	The effects above	The effects above

weeks diet	mg/kg bw/day	mg/kg bw/day	and 35.7 (females) mg/kg bw/day	weight gain and food consumption, decreased RBC, WBC and lymphocytes and increase in spleen pigmentation	the guidance value for STOT RE 2 are limited and do not fulfil the criteria	the guidance value for R48/22 are limited and do not fulfil the criteria
Rat, 21-days dermal	86 / 857 mg/kg bw/day	43 / 429 mg/kg bw/day	1000 mg/kg bw/day	Haematological changes, increased spleen and adrenal weights and spleen extramedullary haematopoiesis	Interpolation of the effects between 400 and 1000 mg/kg bw/day to the guidance value indicate that the effects are limited and not warrant classification with STOT RE 2.	
			400 mg/kg bw/day	Limited haematological changes, increased spleen and adrenal weights and spleen extramedullary haematopoiesis		The effects at the guidance value for R48/21 are limited and do not fulfil the criteria

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

See paragraph 4.7.1.7.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The relevant dose levels are indicated in the table above. Haematological symptoms were a common effect in the repeated dose studies. The DSD criteria state that R48 should be applied for consistent changes in haematology which indicates severe organ dysfunction. Haematological disturbances are considered to be particularly important if the evidence suggests that they are due to decreased bone marrow production of blood cells. The increases in extramedullary haematopoiesis and the increase reticulocytes clearly show that the haematological effects are not due to bone marrow suppression but due to a haemolytic anaemia. More specific criteria for classification with R48 based on anaemic effects are described in Muller et al, 2006. The main criteria are pallor, Hb reduction below 20% and histological changes such as fibrosis of the spleen, liver or kidney. The effects at the relevant guidance levels are compared to these criteria in the table above.

The results indicate that there are differences in effects at the relevant dose levels depending on species and exposure duration. The DSD criteria state that when studies of more than one duration are available, then those of the longest duration should normally be used. Chronic studies should be evaluated on a case by case basis.

Oral

Rat

It is unclear from the 28-day study whether the criteria are fulfilled because the level of Hb reduction is unknown. However, the occurrence of mortalities (> 50% in females) and pallor at a dose level twice of the guidance value indicate that the effects at the guidance value are probably severe enough for classification. The dose levels tested in the 90-day rat study were below the guidance value. The effects at the highest dose tested were limited and do not fulfil the criteria. However, it cannot be excluded that more severe effects could occur at the guidance value. In the chronic study in rats the highest dose tested was above the guidance value but the effects were limited. The Hb reduction was also limited at the other time points. Overall, the rats show clear anaemic effects. The dose levels at which these appear are probably only relevant for classification when exposed for a short period (28-days) but not for the longer study durations. Given the preference in the criteria for longer studies, the effects in the rat are not considered to fulfil the DSD classification criteria.

Dog

The highest dose level in the 28-day study in dogs was clearly below the guidance value. Although the limited effects at the highest dose do not warrant classification, it cannot be excluded that more severe effects occur at the guidance level. The same applies to the 90-day study. In the one year dog study, the effects at the guidance value as estimated by interpolation are not sufficient for classification as the Hb reduction is probably below 20%. Overall, also in dogs clear anaemia is observed however, at dose levels not warranting classification.

Mice

The increased mortality observed at 155 (males) or ~200 (females) mg/kg bw/day in the 28-day study clearly warrants classification with R48/22. However, there is no indication for a requirement for R48/25. The effects in the 90-day study are limited around the guidance value level and do not indicate a need for R48/22. The same applies to the results in the chronic mice study. Overall, mice show clear anaemic effects. The dose levels at which these appear are relevant for classification when exposed for a short period (28-days) but not for the longer study durations. Given the preference in the criteria for longer studies, the effects in mice are not considered to fulfil the DSD classification criteria.

Overall, the three tested species show anaemia that fulfils the criteria for R48 when exposed for a short duration (28-days) but not for longer durations. Several weeks are required before the compensatory effects of haemolytic anaemia such as additional erythropoiesis become fully effective (Muller et al, 2006). With study duration longer than 90-days the haemolytic effects are probably partly compensated by the increased production of RBC. Given the preference in the criteria for longer studies, the effects in the rat, dog and mouse are not considered to fulfil the DSD classification criteria.

Dermal

The effects around the guidance value in the 21-day dermal study in rats are limited and do not fulfil the criteria for R48/21.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

No classification for repeated dose toxicity through the oral and dermal route is required.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See paragraph 4.7.1.7

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The relevant dose levels are indicated in the table above. Haematological symptoms were a common effect in the repeated dose studies. The CLP criteria state that STOT RE should be applied for consistent and significant adverse changes in haematology. More specific criteria for classification with STOT RE based on anaemic effects are described in the CLP guidance and are based on Muller et al, 2006. The main criteria are pallor, Hb reduction below 20% and histological changes such as fibrosis of the spleen, liver or kidney. The effects at the relevant guidance levels are compared to these criteria in the table above.

The results indicate that there are differences in effects at the relevant dose levels depending on species and exposure duration. The CLP criteria do not state a preference for studies with a certain duration. The guidance only states that for very short studies the guidance values become unrealistic and should be adapted. From a scientific perspective it could be argued that chronic studies may not be the best studies to determine repeated dose toxicity because of an increase in variability of the test parameters due to old age diseases. Therefore, sensitivity of chronic studies may be reduced and may be less relevant for classification.

Oral

Rat

The occurrence of mortalities and pallor at the guidance value in the 28-day study show that the criteria are fulfilled. The dose levels tested in the 90-day rat study were clearly below the guidance value. The effects at the highest dose tested were limited and do not fulfil the criteria. However, it cannot be excluded that more severe effects could occur at the guidance value. In the chronic study in rats the highest dose tested was just below the guidance value but the effects were limited. The Hb reduction was also limited at the other time points. Overall, the rats show clear anaemic effects. The dose levels at which these appear are relevant for classification when exposed for a short period (28-days), possibly relevant at 90-days but not for the chronic study. As there is no preference in the criteria for classification the result in rats are considered to indicate a requirement for classification.

Dog

The highest dose level in the 28-day study in dogs was clearly below the guidance value. Although the limited effects at the highest dose do not warrant classification, it cannot be excluded that more

severe effects occur at the guidance level. The same applies to the 90-day study. In the one year dog study, the effects at the guidance value are sufficient for classification as the Hb reduction is above 20%. Overall, also in dogs clear anaemia is observed however, at dose levels warranting classification with STOT RE 2.

Mice

The increased mortality observed at 155 mg/kg bw/day in the 28-day study clearly fulfil the criteria for STOT RE 2. However, there is no indication for a requirement for STOT RE 1. The effects in the 90-day study are limited at dose levels below the guidance value level but do not indicate a need for STOT RE 2. However, more severe effects at the guidance value cannot be excluded. The effects in the chronic study which was tested at a dose level above the guidance value do not indicate a need for classification. Overall, mice show clear anaemic effects. The dose levels at which these appear are relevant for classification when exposed for a short period (28-days), unknown for the 90-day study but not for the chronic study. As there is no preference in the criteria for classification the result in rats are considered to indicate a requirement for classification.

Conclusion

Overall, the three tested species show anaemia that fulfils the criteria for STOT RE 2 when exposed for a short duration (28-days) in most species. In most 90-day studies the used dose levels were below the guidance value indicating that classification cannot be excluded. The chronic and 1-year studies differ between the species as a requirement for classification is determined in the dog but not in rats and mice. Several weeks are required before the compensatory effects of haemolytic anaemia such as additional erythropoiesis become fully effective (Muller et al, 2006). With study duration longer than 90-days the haemolytic effects are probably partly compensated by the increased production of RBC. As there is no preference for certain study duration in the CLP criteria and it is unknown which species is more relevant to humans the results of the combination of exposure duration and species that fulfil the criteria are considered relevant and classification as STOT RE 2 is required

Dermal

The effects around the guidance value in the 21-day dermal study in rats as estimated by interpolation are probably limited and do not fulfil the criteria for STOT RE 2.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification with STOT RE 2 is required. As there is no information regarding repeated dose toxicity after inhalation exposure, route specificity cannot be indicated in the labelling. The blood is determined as the target organ.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 4.9: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Ames test (OECD 471)	negative	Indicator cells: S. typh.TA 98, TA 100, TA 1535, TA 1537 E.coli WP2uvrA	Wagner and Coffman, 1996 [a]
gene mutation (OECD 476)	negative	mouse lymphoma cells L5178Y (TK)	San and Clarke, 1996 [a]
chromosome aberration (OECD 473)	negative	Chinese hamster ovary (CHO) cells	Gudi and Schadly, 1996 [a]
Micronucleus test *in vivo* (OECD 482)	negative	mouse	Gudi, 1996 [a]

[a] As summarised in DAR_2003_vol3 B6

4.9.1 Non-human information

4.9.1.1 In vitro data

An Ames test was performed with *S. typhymurium* and *E. coil*, in accordance with OECD 471. In this test no cytotoxicity was observed, but precipitation was observed at dose levels of 1000 ug/plate and higher. In this well performed test at dose levels up to and including 5000 ug/plate, the substance bifenazate (purity 90.2%) did not induce point mutations either in presence or absence of metabolic activation.

A gene mutation test was performed with mouse lymphoma L5178Y cells in accordance with OECD 476. Dose levels ranged from 0 to 50 ug/mL when tested without activation and from 0 to 500 ug/ml with activation. In this well performed test, the substance bifenazate (purity 90.2%) did not induce gene mutations either in presence or absence of metabolic activation.

A chromosome aberration test was performed with Chinese Hamster Ovary (CHO) cells in accordance with OECD 473. Dose levels ranged from 12 to375 ug/mL when tested without activation, and from 20 to 1250 ug/mL with activation. In this well performed test, the substance bifenazate (purity 90.2%) did not induce chromosome aberrations either in presence or absence of metabolic activation.

4.9.1.2 In vivo data

In a micronucleus test, mice (15-20 animals per sex per dose level) were exposed to bifenazate (purity 90.2%) by intraperitoneal (ip) administration and were sacrificed at 24, 48, or 72 h after dosing. The vehicle was corn oil. Dose levels were 0, 96, 193 and 384 mg/kg bw for males and 0, 50, 100, and 200 mg/kg bw for females. Signs of toxicity (lethargy) were observed at dose levels from 100 mg/kg bw and there was mortality at 384 mg/kg bw. The test was in accordance with OECD 482. The test substance did not induce micronuclei in mouse bone marrow cells.

4.9.2 Human information

No human data available.

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

Bifenazate was negative in the following *in vitro* genotoxicity tests, both without and with metabolic activation: Ames tests with S. typhimurium strains TA98, TA100, TA1535 and TA1537 and with E. coli strain WP2uvrA, a gene mutation test with mouse lymphoma cells L5178Y and a chromosome aberration test with CHO cells. Bifenazate was also negative in the *in vivo* mouse micronucleus test with bone marrow.

4.9.5 Comparison with criteria

Bifenazate was found negative in well performed genotoxicity studies both *in vitro* and *in vivo*, and does not meet the criteria for classification.

4.9.6 Conclusions on classification and labelling

No classification is needed.

4.10 Carcinogenicity

Table 4.10: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Long term toxicity and carcinogenicity study in rats OECD 453	NOAEL: 20 mg/kg food, equal to 1.0 mg/kg bw per day LOAEL: 80 mg/kg food, equal 3.9 mg/kg bw per day	No carcinogenicity at doses up to the higest dose level tested (200 mg/kg food, equal to 9.7 mg/kg bw/day)	Ivett, 1999a [a]
Carcinogenicity study in mice OECD 451	NOAEL: 10 mg/kg food, equal to 1.5 mg/kg bw per day	No carcinogenicity at doses up to the higest dose level tested (225 mg/kg food, equal to 9.7 mg/kg bw/day)	Ivett, 1999b [a]

[a] As summarised in DAR_2003_vol3 B6

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In a 104 week combined toxicity and carcinogenicity study rats were exposed to bifenazate (purity 90.2%) at dietary levels of 0, 20, 80, or 200 (m) / 160 (f) mg/kg food. The dose levels were equal to 0, 1.0, 3.9, and 9.7 mg/kg bw/d for males and 0, 1.2, 4.8, and 9.7 mg/kg bw/d for females. The study was performed according to OECD 453. Toxicologically relevant effects were noted in rats treated with bifenazate at 80 and 160/200 mg/kg food for 104 weeks (see table 4.10-1). The effects included decreased body weight and body weight gains, decreased mean total food consumption in male and female rats of the high dose group and in females of the mid dose group. Erythrocyte counts were decreased in mid and high dose females. Haemoglobin and haematocryt were decreased in high dose females. In both males and females an increased severity of haemosiderin pigment in the spleen was observed. The test substance is not oncogenic to rats when fed in the diet at concentrations up to 200 mg/kg food for 104 weeks. The NOAEL was 20 mg/kg food, equal to 1.0 mg/kg bw/day. The LOAEL was 80 mg/kg food, equal to 3.9 mg/kg bw per day.

Table 4.10-1 Carcinogenicity study in rats

Dose (mg/kg food)	0		20		80		200(m)/160(f)		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	25	31	30	32	15	28	11	36	
Clinical signs	no treatment related findings								
Body weight (gain)						dc[1]	dc	dc	f

Dose (mg/kg food)	0		20		80		200(m)/160(f)		dr
	m	f	m	f	m	f	m	f	
Food consumption						dc[2]	dc	dc	f
Ophthalmoscopy	no treatment related findings								
Haematology									
- RBC						dc[4]		dc[3]	
- Hb								dc[3]	
- Ht								dc[3]	
Urinalysis	no treatment related findings								
Clinical chemistry									
- cholesterol							dc[5]		
Organ weights	no treatment related findings								
Pathology									
macroscopy	no treatment related findings								
microscopy *neoplastic lesions*	no treatment related findings								
microscopy *non-neoplastic lesions spleen*									
- increased severity of haemosiderin pigment					+[6]	+[6]	+[6]	+[6]	
pancreas									
- chronic inflammation	13/60	5/60	5/30	2/31	1/16	1/27	22/60	9/60	
- basophilic foci							+		

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

\+ present in one/a few animals

[1] cumulative body weight gain in week 1-13 and body weight in week 3-18

[2] cumulative food consumption in week 1-13

[3] in week 13, 26 and 52

[4] in week 26

[5] in week 26, 52 and 78

[6] at interim sacrifice (week 53)

In a 78 week carcinogenicity study mice were exposed to bifenazate (purity 90.2%) at dietary levels of 0, 10, 100 or 225 (m) / 175 (f) mg/kg food. The dose levels were equal to 0, 1.5, 15.4, and 35.1 mg/kg bw/d for males and 0, 1.9, 19.7, and 35.7 mg/kg bw/d for females. The study was performed according to OECD 451. Toxicologically relevant effects were noted in mice treated with bifenazate at 100 and 225/175 mg/kg food for 78 weeks (see table 4.10-2). The effects included decreased body weight and body weight gains, decreased mean total food consumption in male rats of the high dose group. Erythrocyte counts were decreased in high dose males and white blood cell and lymphocyte counts were decreased in mid and high dose males. Liver weights were increased in the high dose group and kidney weights were decreased in males of the mid and high dose group. The test substance is not oncogenic to mice when fed in the diet at concentrations up to 175/225 mg/kg food for 78 weeks. The NOAEL was 10 mg/kg food, equal to 1.5 mg/kg bw/day. The LOAEL was 100 mg/kg food, equal to 15.4 mg/kg bw per day.

Table 4.10-2 Carcinogenicity study in mice

Dose (mg/kg food)	0		10		100		225 (m) / 175 (f)		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	10	9	8	13	3	5	5	11	
Clinical signs	no treatment related findings								
Body weight (gain)							d^{1}	dc	
Food consumption							dc		
Haematology									
- RBC							dc^{2}		
- WBC					dc^{2}		dc^{2}		
- lymphocytes					dc^{2}		dc^{2}		
Organ weights									
- liver							$ic^{a,r}$	ic^{r}	
- kidneys					$dc^{a,r}$		$dc^{a,r}$		m
Pathology									
macroscopy	no treatment related findings								
microscopy *neoplastic lesions*	no treatment related findings								
microscopy *non-neoplastic lesions*	no treatment related findings								

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

a/r absolute organ weight/relative organ weight

1 first 26 weeks only

2 at 52 weeks, no statistically significant effects present at 79 weeks

4.10.1.2 Carcinogenicity: inhalation

No data.

4.10.1.3 Carcinogenicity: dermal

No data.

4.10.2 Human information

No data.

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

Bifenazate has not demonstrated an oncogenic potential in the studies of carcinogenicity in mice and rats.

4.10.5 Comparison with criteria

Bifenazate does not does not meet the criteria for classification.

4.10.6 Conclusions on classification and labelling

No classification is needed.

4.11 Toxicity for reproduction

Table 4.11-1: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
2-generation reproduction study	NOAEL parental: 20 mg/kg food or 1.4 mg/kg bw per day NOAEL developmental: ≥ 15.0 mg/kg bw/d	At LOAEL of 200 mg/kg food or 5.8 mg/kg bw per day decreased bw (gain) No effects	Schardein, 1999 [a]
Teratogenicity study rat	Maternal NOAEL: 10 mg/kg bw/day Developmental NOAEL: ≥ 500 mg/kg bw/day Teratogenicity NOAEL ≥ 500 mg/kg bw/day	At the LOAEL of 100 mg/kg bw decreased bw and food consumption; red material around nose, dorsal head and forelimbs No effects No effects	Schardein, 1997[a] a
Teratogenicity study rabbit	Maternal ≥ 200 mg/kg bw/day Developmental ≥ 200 mg/kg bw/day Teratogenicity ≥ 200 mg/kg bw/day	No effects No effects No effects	Schardein, 1997b [a]

[a] As summarised in DAR_2003_vol3 B6

4.11.1 Effects on fertility

4.11.1.1 Non-human information

A two generation reproduction study was performed in accordance with OECD 416. The animals were exposed to bifenazate (purity 92.5%) at dietary levels of 0, 20, 80 and 200 mg/kg food[1]. Next to this study a subsequent two generation study was performed, which was attached as an appendix to the study report. In this latter study the dose levels were 0, 7.5, 15, and 20 mg/kg food.The results are summarised in table 4.11-2.

In the parental F0 and F1 animals of the 80 and 200 mg/kg food dose groups, decreased body weight and body weight gain were observed. The effect on parental body weight were also noted at 20 mg/kg food in the F1 animals, but these effects were not reproduced in a subsequent 2-generation reproduction study wich also included the same dose level[2]. Therefore, this effect is not attributed to test substance administration. Parental animals did not show any clinical signs and there were no effects on food consumption. There were no effects on mating-, fertility-, and gestation parameters, except for the observation of an abnormal oestrus cycle in one or a few F0 females of the high dose group. This effect was not observed in the F1 generation. Sperm was evaluated in the F0 generation and there were no abnormalities. Some effects on organ weights were observed. The absolute increased spleen weight in females of the 200 mg/kg food group of the F0-generation and absolute increased liver weight observed in the F1-generation were slight and no microscopic findings were observed in the organs. Therefore, the increases were not considered to be test substance related. The increase of relative organ weights of kidneys, ovaries and adrenal glands are considered to be attributed to the decreased body weight. There were no treatment related findings at at the macro- and microscopic observations.

Spleen weight of the female pups of the F1-generation and male and female pups of the F2-generation of the 20 mg/kg group was increased. However, as no increase was observed in neither of the higher dose groups (80 and 200 mg/kg groups) in this study nor in the subsequent study, these findings are considered not to be related to treatment.

Physical development of the F1-pups was considered as normal by the authors since in all male F1-pups preputial separation and in nearly all female F1-pups vaginal opening was observed. However, there was a minimal delay in sexual maturation for the males in the 80 and 200 mg/kg groups and for the females in the 200 mg/kg group.

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[1] Dose levels were equal to the following values (copied from DAR bifenazate):
F0-generation: 0, 1.5, 6.1, and 15.3 mg/kg bw/d for males and 0, 1.7, 6.9, and 17.2 mg/kg bw/d for females prior to breeding and for females during gestation: 0, 1.4, 5.8, and 15.6 mg/kg bw/d and during lactation 0, 3.1, 12.1, and 32.5 mg/kg bw/d
F1-generation: 0, 1.7, 6.9, and 17.4 mg/kg bw/d for males and 0, 1.9, 7.8, and 19.4 mg/kg bw/d for females prior to breeding and for females during gestation 0, 1.4, 5.8, and 15.0. mg/kg bw/d and during lactation 0, 3.2, 13.1, and 33.6 mg/kg bw/d

[2] A subsequent 2-generation study was performed at concentrations of 7.5, 15, and 20 mg/kg food in order to further assess the equivocal parental body weight effects noted at 20 mg/kg food. The effects were not reproduced in the subsequent study. In addition the effect on spleen weight in the pups of the 20 mg/kg group was not reproduced. Therefore, the reduction in mean body weight at 20 mg/kg food was not attributed to test substance administration. (copied from DAR bifenazate)

In male pups, preputional separation was checked from day 40 onwards. In the control group 100% separation was reached within 51 days. In the low, mid and high dose group it was reached within 48, 53 and 51 days respectively. This delay is not considered an adverse effect.

In female pups the process of vaginal opening was completed in the control group on day 36 and in the low dose group somewhat earlier on day 34. However, in the mid dose group the process was completed on day 40 and in the high dose group on day 47. This is considered to be induced by exposure to bifenazate and to be an adverse effect. However, it is unclear whether it is due to the in utero exposure or due to the post-natal exposure and related to the decreased body weight gain.

No other toxicological relevant effects were observed.

Table 4.11-2 Summary of results from a two generation study in rats with bifenazate

Dose (mg/kg food)	**0**		**20**		**80**		**200**		**dr**
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
F0 animals									
Mortality	no treatment related mortality								
Clinical signs	no treatment related findings								
Body weight					d	d	dc	dc	m, f
Body weight gain							dc[1]	dc[2]	
Food consumption	no treatment related findings								
Mating/fertility/gestation	no treatment related findings								
Oestrus cycle -abnormal oestrus cycle								+	
Sperm evaluation	no treatment related findings								
Organ weight - spleen - kidneys - ovaries - adrenal glands								ic[a, r] ic[r] ic[r] ic[r]	
Pathology									
macroscopy	no treatment related findings								
microscopy	no treatment related findings								
F1 pups									
Litter size	no treatment related findings								
Survival index	no treatment related findings								
Sex ratio	no treatment related findings								
Body weight	no treatment related findings								
Physical development - vaginal opening - preputial separation					d		d	d	

Dose (mg/kg food)	0		20		80		200		dr
	m	f	m	f	m	f	m	f	
Organ weight -spleen				ic[a,r,4]					
Pathology									
macroscopy	no treatment related findings								
F1 animals									
Mortality	no treatment related mortality								
Clinical signs	no treatment related findings								
Body weight (gain)				d[3, 4]	dc	dc	dc	dc	m, f
Organ weight									
- liver					ic[a]		ic[a]	Ic[a]	
- kidneys								Ic[r]	
- ovaries								ic[r]	
- adrenal glands				ic[r]				ic[r]	
- pituitary								dc[a]	
Food consumption	no treatment related findings								
Mating/fertility/gestation	no treatment related findings								
Pathology									
macroscopy	no treatment related findings								
microscopy	no treatment related findings								
F2 pups									
Litter size	no treatment related findings								
Survival index	no treatment related findings								
Sex ratio	no treatment related findings								
Body weight	no treatment related findings								
Organ weight - spleen			i[a] ic[r,4]	ic[a,r,4]					
Pathology									
macroscopy	no treatment related findings								

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
[1] in week 3 – 5
[2] in week 1- 4
[3] throughout the pre-breeding period
[4] A subsequent 2-generation study was performed at concentrations of 7.5, 15, and 20 mg/kg food in order to further assess the equivocal parental body weight effects noted at 20 mg/kg food. The effect was not reproduced in the subsequent study. In addition the effect on spleen weight in the pups of the 20 mg/kg group were not reproduced. Therefore, the reduction in mean body weight at 20 mg/kg food were not attributed to test substance administration.

Based on these findings, the NOAEL for parental toxicity is set at 20 mg/kg food (equal to 1.4 mg/kg bw per day); the NOAELs for reproduction and developmental toxicity are set at 200 mg/kg food (equal to 15.0 mg/kg bw/day).

4.11.1.2 Human information

No data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

A developmental toxicity study was performed in rats according to OECD 414. Dams were exposed by gavage to bifenazate (purity 92.5%) at dose levels of 10, 100 and 500 mg/kg bw per day during days 6-15 of gestation. Maternal toxicity was observed in the mid and high dose group: clinical signs (red material around the nose and on head and/or forelimbs), decreased defaecation, brown vaginal discharge and decreases were found in body weight and food consumption. No adverse effects were observed in the foetuses. The results are summarised in table 4.11-3. The NOAEL for maternal toxicity is 10 mg/kg bw per day and the NOAEL for developmental and teratogenic effects 500 mg/kg bw per day.

Table 4.11-3 Summary of results from a developmental toxicity study in rats with bifenazate

Dose (mg/kg bw/day)	**0**	**10**	**100**	**500**	**dr**
Maternal effects					
Mortality	none				
Clinical signs - red material around the nose - pale extremities - red material on the dorsal head or forelimbs - decreased defecation - brown vaginal discharge			++ +	++ ++ ++ ++ +	
Pregnant animals	25	22	25	24	
Body weight (gain)			dc	dc	
Gravid uterus weight	no treatment related findings				
Food consumption			dc	dc	
Pathology					
macroscopy	no treatment related findings				
Litter response					
Live foetuses	no treatment related findings				
Foetal weight	no treatment related findings				
Post implantation loss	no treatment related findings				
Sex ratio	no treatment related findings				
Examination of the foetuses					
External observations	no treatment related findings				
Skeletal findings	no treatment related findings				
Visceral findings	no treatment related findings				

A developmental toxicity study was performed in rabbits according to OECD 414. Does were exposed by gavage to bifenazate (purity 92.5%) at dose levels of 0, 10, 50, and 200 mg/kg bw per day during days 7-19 of gestation. The number of pregnant does was 17, 20, 15 and 17, respectively, and in each dose group there was one abortion. Maternal toxicity was not observed in this study and no adverse effects were observed in the foetuses. The dose levels in this study were based on the results of a range finding study in which dose levels of 0, 125, 250, 500, 750 and 1000 mg/kg bw/d were used. Clear toxicity (maternal body weight changes, abortions and deaths) was observed at ≥250 mg/kg bw/day. Since there is clearly a steep dose-effect relationship for bifenazate exposure in rabbits (no effects whatsoever at a dose of 200 mg/kg bw/day and deaths at a dose of 250 mg/kg bw/day), the highest dose used would be quite close to the LOAEL. Therefore, the lack of maternal toxicity at the highest dose level is, in this case, considered to be acceptable.

4.11.2.2 Human information

No data.

4.11.3 Other relevant information

No data.

4.11.4 Summary and discussion of reproductive toxicity

In an oral 2-generation reproduction study in rats, the NOAELs for developmental and reproduction toxicity were set at 200 mg/kg food, equal to 15.0 mg/kg bw/day At the dose level of 80 and 200 mg/kg food, a parental effect noted was decreased body weight (gain). Therefore, the NOAEL for parental toxicity was set at 20 mg/kg food, equal to 1.4 mg/kg bw/day. No effect on sexual function and fertility was observed.

In a teratogenicity study in rats, a NOAEL-maternal of 10 mg/kg bw/day was derived, based on decreased body weight and food consumption and clinical signs observed at the next higher dose level. No effects were observed in the foetuses. The NOAEL for developmental effects was 500 mg/kg bw/day, the highest dose tested.

In a teratogenicity study in rabbits, maternal toxicity was not observed at the top dose level of 200 mg/kg bw. However, since in a preliminary study it was found that there was a steep dose response relation ship for maternal toxicity in rabbits it was considered acceptable that the substance was not tested at higher dose levels. A NOAEL-maternal and NOAEL-developmental was set at the highest dose level, being 200 mg/kg bw/day.

No irreversible structural effects were observed in either the rat or the rabbit in the respective studies.

A delay in vaginal opening was observed in the females of the 2-generation study. This could be considered as a post-natal developmental effect.

4.11.5 Comparison with criteria

As no effects on sexual function and fertility were observed, comparison to the criteria is not relevant.

The only possible developmental effect was a decrease in post-natal vaginal opening in the presence of a decrease in body weight gain. This is considered as a limited effect as it is a delay in development in the presence of reduced body weight gain. Further, it is unclear whether this effect is due to the in utero exposure or through the postnatal exposure. Food uptake after weaning can be clearly above the average uptake over the whole exposure period. The estimated average exposure of approximately 20 mg/kg bw/day is a dose level inducing anaemia in mature rats. therefore, it is likely that such effects also occurred in the post-natal period. As this effect is considered to be only a delay and therefore of limited adversity and probably related to the same toxicity as observed in mature rats, no classification for developmental toxicity is required.

4.11.6 Conclusions on classification and labelling

No classification is needed.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

In a 2 week oral study on cholinergic toxicity rats were exposed to bifenazate (purity 90.9%) at feeding levels of 0, 20, 200 and 400 mg/kg food. The animals were monitored twice daily for overt signs of cholinergic toxicity (e.g. changes in general behaviour, gait, and excretory functions) and mortality and morbidity. Physical observations, body weight, and feed weight assessments were performed on all animals once a week. There were no overt signs of cholinergic toxicity and no effects on plasma cholinesterase, erythrocyte acetylcholinesterase and brain acetylcholinesterase activities seen in animals fed diets containing up to 400 mg/kg food, the highest dose tested. The NOAEL for cholinergic toxicity was set at ≥ 400 mg/kg food (equal to ≥ 34.6 mg/kg bw/day).

In a 90-day oral toxicity study in rats, a battery of behavioural tests and observations (FOB) was performed after 7 weeks feeding and at the end of the study. No treatment related findings were observed for these FOBs.

4.12.1.2 Immunotoxicity

No studies were submitted.

4.12.1.3 Specific investigations: other studies

No data.

4.12.1.4 Human information

No data.

4.12.2 Summary and discussion

In a 2-week feeding study in rats (see paragraph 4.7.1.1), no overt signs of cholinergic toxicity and no effects on plasma cholinesterase, erythrocyte acetylcholinesterase and brain acetylcholinesterase activities were seen in the animals fed diets containing bifenazate up to 400 mg/kg food.

No sign of any neurotoxic effects were observed in a 90-day oral toxicity study in rats in which a battery of behavioural tests and observations (FOB) was performed (see paragraph 4.7.1.1).

4.12.3 Comparison with criteria

The substance does not meet the criteria for classification for STOT-Repeated Dose.

4.12.4 Conclusions on classification and labelling

No classification is needed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties and hazard assessment for bifenazate are based on the Draft Assessment Report, the Addendum to the Draft Assessment Report prepared in the context of the possible inclusion of bifenazate in Annex I of Council Directive 91/414/EEC (DAR 2003 + subsequent addenda, RMS The Netherlands).

5.1 Degradation

Table 5.1-1: Summary of relevant information on degradation

Method	Results	Remarks	Reference[a]
Hydrolysis test	DT_{50} pH 4 = 8.2 days DT_{50} pH 5 = 4.8 days DT_{50} pH 7 = 12.3 hours DT_{50} pH 9 = 0.97 hours		Shah, JF (1997a)
Photolysis test, artificial sunlight	DT_{50} = 20.3 hours	25°C, pH5	Shah, JF (1997b)
Photolysis test, natural water	DT_{50} = 0.83 hours	25°C, pH7	Shah, JF (1998)
Photolysis test, artificial sunlight	DT_{50} = 21.1 hours	25°C, pH5	Lewis, CJ (2001)
Quantum yield	1.22% (0.0122 moles/einstein)		Nag, JK (2000)
Ready biodegradability (OECD 301B)	11.7% degradation after 28 days		Armstrong, K (2000)
Water/sediment system aerobic	DT_{50} water = < 0.25 days DT_{50} sediment = n.d. DT_{50} system = < 0.25 days	Test performed with sandy loam and clay loam type sediment	Mamouni,A (2001)
Water/sediment system anaerobic	DT_{50} system = 77.7 days		Lentz, NR (1998)

[a]As summarized in the DAR for Bifenazate Volume 3, B.8 Environmental fate and behavior (annex point IIA).

5.1.1 Stability

Hydrolysis

Bifenazate was shown to be susceptible to oxidation and hydrolysis at pH 4, 5, 7 and 9 (25°C) with DT_{50} values of 8.2 days, 4.8 days, 12.3 hours, and 0.97 hours, respectively.

Table 5.1-2: Summary of results of the hydrolysis study for bifenazate

Substance	Water type	Duration [d]	T [°C]	pH	Transformation at end [%]	DT_{50} hydrolysis [d, h]
^{14}C-bifenazate	sodium acetate	30	25±1	4	100	8.2 d
^{14}C-bifenazate	sodium acetate	30	25±1	5	100	4.8 d
^{14}C-bifenazate	sodium phosphate	32	25±1	7	100	12.3 h
^{14}C-bifenazate	sodium borate	7	25±1	9	100	0.97 h
D3598	sodium phosphate	32	25±1	7	100	29.0 h
D3598	sodium borate	7	25±1	9	100	0.57 h

Two primary degradation products D3598 and D9472, and several minor degradation products were detected. The DT_{50} of D3598 was 29.0 hours at pH 7 and 0.57 hours at pH 9. DT_{50}s at other pHs and for other degradation products could not be calculated because too few data points were available.

Photolysis in water

Bifenazate was demonstrated to be susceptible to photolysis. In the three submitted tests a, b and c, an unknown amount of acetonitrile was used to prepare the fortification solutions and it is uncertain how this may have influenced photolysis. When exposed to artificial sunlight (>290 nm, 25°C) at a 12:12 hours light:dark cycle, the DT_{50} in a pH 5 sodium acetate buffer was 20.3 hours after correction for other ways of degradation. Degradation in natural water was considerably faster with a DT_{50} of 0.83 hours. It is suggested that natural water contains sensitisers that increase the photolytic degradation.

Test d resulted in a DT_{50} of 21.1 hours, which is comparable to the previous test. Five known degradation products were detected in the studies with buffered solution: D3598 (maximum 58.6% of AR), D1989 (max. 13.1% of AR), D9472 (max. 18.6% of AR), D9963 (max. 30.4% of AR), A1530 (max. 1.0% of AR). In study d, an unidentified peak reached 18.0% of AR. This peak was shown not to consist of D9963.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

The ready biodegradability of bifenazate technical (purity 97.9%) was assessed in a Modified Sturm test according to OECD guideline 301B. The CO_2 production, expressed as a percentage of theoretical after 28 days, was 11.7% for bifenazate. Based on these results, bifenazate is considered as *not readily biodegradable.*

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems

Aerobic water/sediment system

In an aerobic water/sediment study with a sandy loam and a clay loam system, ^{14}C-bifenazate dissipated from the system with a DT_{50} of <0.25 days (6 hours) and a DT_{50} for water of < 0.25 days as well. No DT_{50} value was determined in sediment. Non-extractable residues in the sediment increased to 46.9 and 65.2% of AR after 100 days in the sandy loam and clay loam system, respectively. Mineralisation after 100 days amounted to 33.7 and 18.9% of AR, respectively.

Table 5.1-3: Summary of results of the aerobic water/sediment study for bifenazate and degradation products

Substance	Type of system	Water phase [% AR]	Sediment phase [% AR]	DT_{50} water [d]	DT_{50} sediment [d]	DT_{50} system [d]
Bifenazate	Sandy loam			< 0.25		< 0.25
D3598	Sandy loam	30.9	< 10	< 1		3.4
D9472	Sandy loam	20.4	< 10	10.5		14.4
Bifenazate	Clay loam			< 0.25		< 0.25
D3598	Clay loam	32.8	10.5		10.1	3.4
D9472	Clay loam	13.7	< 10	3.2		5.3

Major degradation products in the water phase were D3598 and D9472. Other degradation products were formed to a lesser extent.

Anaerobic water/sediment system

In an anaerobic water/sediment study with a loam system, ^{14}C-bifenazate dissipated from the system with a DT_{50} of 77.7 days at 25 °C, equivalent to 116 days at 20 °C. Non-extractable residues in the sediment increased to 28.4% of AR after 119 days and then further increased to 51.5% of AR after 12 months. Mineralisation after 119 days amounted to 0.07% of AR and was 0.17% after 12 months. Distribution of radioactivity was given for the system only. Major degradation products were A1530 with a maximum of 24.8% of AR in the system after 10 months, and desmethyl-D3598 with a maximum of 14.7% of AR after 8 months. DT_{50}s could not be estimated for these degradation products on the basis of the data. D3598 was detected, but the maximum level reached in the system was 3.7% of AR at test initiation.

5.1.3 Summary and discussion of degradation

Bifenazate is susceptible to both hydrolysis and photolysis in water. In water sediment tests, primary degradation of the substance happens fast, mineralization however is minimal. Bifenazate is shown to disappear very rapidly from the water phase. Biodegradation was determined in an OECD 301B test, showing that bifenazate is not readily biodegradable.

Based on the results of the aquatic toxicity tests, the primary degradation products of bifenazate are considered classifiable for the environment.

In Section 4.1.2.9 of Annex I of CLP it is stated that rapid degradation can be demonstrated by ready biodegradability or other evidence of rapid degradation in the environment (≥ 70% abiotic or biotic degradation in the environment in 28 days). Further, it is stated that primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that

the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Therefore:

Bifenazate is considered not readily biodegradable according to the result of the OECD 301B test.

Bifenazate is considered not rapidly degradable based on the results of the simulation tests.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Information not applicable for classification and labelling

5.2.2 Volatilisation

Bifenazate has vapour pressure of $<1 \cdot 10^{-7}$ torr at 25 °C, equivalent to $<1.33 \cdot 10^{-5}$ Pa, and a Henry's law constant of $< 1.01 \cdot 10^{-3}$ $Pa \cdot m^3 \cdot mol^{-1}$ (at 20 °C). Based on the information submitted it is considered that significant volatilisation of bifenazate is unlikely to occur.

5.3 Aquatic Bioaccumulation

No bioaccumulation study is available.

Bifenazate has a logKow of 3.4. Bifenazate therefore fulfils the criteria for bioaccumulation potential according to Directive 67/548/EEC but not the criteria for bioaccumulation potential according to the criteria of CLP.

5.4 Aquatic toxicity

Table 5.4-1 lists the results from the relevant aquatic acute toxicity studies that were performed with bifenazate and its major degradation products D3598, D9472 and D1989. The key acute and chronic studies carried out with bifenazate at each tropic level are described in more detail below.

Table 5.4-1: Summary of relevant information on acute aquatic toxicity

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [mg/L]	Remarks	Reference[b]
Acute toxicity to fish								
EPA/ASTM	Bifenazate	92.4	*Lepomis macrochirus*	Flow through	96h LC_{50}	0.58	Based on mean measured bifenazate concentrations	Graves and Swigert (1997a)
EPA/ASTM	Bifenazate	92.4	*Oncorhynchus mykiss*	Flow through	96h LC_{50}	0.76	Based on mean measured bifenazate concentrations	Graves and Swigert (1997b)
Screening test	D3598		*Oncorhynchus mykiss*	Static	96h LC_{50}	0.32	Based on nominal concentrations	Anon (1992a)

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [mg/L]	Remarks	Reference[b]
Screening test	D3598		*Lepomis macrochirus*	Static	96h LC_{50}	0.32	Based on nominal concentrations	Anon (1992b)
OECD/EPA/ASTM	D3598		*Oncorhynchus mykiss*	Flow through	96h LC_{50}	0.044	Based on mean measured concentrations	Palmer et al (2001a)
OECD/EEC	D9472		*Oncorhynchus mykiss*	Flow through	96h LC_{50}	0.21	Based on mean measured concentrations	Seyfried, B (2001a)
Acute toxicity to invertebrates								
EPA/ASTM	Bifenazate	92.4	*Daphnia magna*	Flow through	48h LC_{50}	0.5	Based on mean measured bifenazate concentrations	Graves and Swigert (1997c)
EPA/ASTM	Bifenazate	92.2	*Crassostrea virginica*	Flow through	96h EC_{50}	0.417	Based on mean measured bifenazate + D3598 concentrations	Graves and Krueger (1999b)
Screening test	D3598		*Daphnia magna*	Static	48h LC_{50}	0.25	Based on nominal concentrations	Anon (1992c)
EPA/ASTM	D1989		*Daphnia magna*	Flow through	48h EC_{50}	0.24	Based on measured bifenazate concentrations at test initiation	Drottar and Kreuger (2000)
OECD/EPA/ASTM	D3598		*Daphnia magna*	Flow through	48h EC_{50}	0.051	Based on mean measured concentrations	Palmer et al (2001b)
OECD/EEC	D9472		*Daphnia magna*	Static	48h EC_{50}	0.78	Based on mean measured concentrations	Seyfried, B (2001b)
Acute toxicity to algae and aquatic plants								
EPA/OECD	Bifenazate	92.6	*Pseudokirchneriella subcapitata*	Static	96h ErC_{50}	> 2.02	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999c)
EPA/OECD	Bifenazate	92.6	*Navicula pelliculosa*	Static	96h ErC_{50}	1.4	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999d)
EPA/OECD	Bifenazate	92.6	*Anabaena flos-aquae*	Static	96h ErC_{50}	> 4.48	Based on measured bifenazate concentrations	Drottar and Krueger (1999e)

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [mg/L]	Remarks	Reference[b]
							at test initiation	
EPA/OECD	Bifenazate	92.6	*Skeletonema costatum*	Static	96h ErC_{50}	0.36	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999)
EPA	Bifenazate	92.6	*Lemna gibba*	Renewal	7d IC50	>3.82	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999f)
OECD/EC/EPA	D3598		*Pseudokirchneriella subcapitata*	Static	96h ErC_{50}	> 1.8	Based on measured D3598 concentrations at test initiation	Palmer et al (2001c)
OECD/EEC	D9472		*Scenedesmus subspicatus*	Static	96h ErC_{50}	2.75	Based on mean measured concentrations	Seyfried, B (2001c)

[b] As summarized in the DAR for Bifenazate Volume 3, B.9 Ecotoxicology (annex point IIA).

Table 5.4-2: Summary of relevant information on chronic aquatic toxicity

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [mg/L]	Remarks	Reference[b]
Chronic toxicity to fish								
ELS acc. to EPA	Bifenazate	92.6	*Oncorhynchus mykiss*	Flow through	87d NOEC growth	0.017	Based on mean measured concentrations	Drottar and Krueger (1999a)
Chronic toxicity to invertebrates								
EPA/ASTM	Bifenazate	92.6	*Daphnia magna*	Flow through	21d NOEC reproduction	0.15	Based on mean measured concentrations	Drottar and Krueger (1999b)
Chronic toxicity to algae and aquatic plants								
EPA/OECD	Bifenazate	92.6	*Pseudokirchneriella subcapitata*	Static	96h NOEC	0.252	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999c)
EPA/OECD	Bifenazate	92.6	*Navicula pelliculosa*	Static	96h NOEC	0.517	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999d)
EPA/OECD	Bifenazate	92.6	*Anabaena flos-aquae*	Static	96h NOEC	1.13	Based on measured bifenazate	Drottar and Krueger (1999e)

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [mg/L]	Remarks	Reference[b]
							concentrations at test initiation	
EPA/OECD	Bifenazate	92.6	*Skeletonema costatum*	Static	96h NOEC	0.2	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999)
EPA	Bifeanzate	92.6	*Lemna gibba*	Renewal	7d NOEC	≥3.82	Based on measured bifenazate concentrations at test initiation	Drottar and Krueger (1999f)
OECD/EC/EPA	D3598		*Pseudokirchneriella subcapitata*	Static	96h NOEC	0.56	Based on measured D3598 concentrations at test initiation	Palmer et al (2001c)
OECD/EEC	D9472		*Scenedesmus subspicatus*	Static	96h NOEC	0.11	Based on mean measured concentrations	Seyfried B (2001c)

[b] As summarized in the DAR for Bifenazate Volume 3, B.9 Ecotoxicology (annex point IIA).

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of bifenazate technical (purity 92.4%) for the bluegill sunfish (*Lepomis macrochirus*) was tested in a flow-through study in accordance with EPA and ASTM guidelines. Juvenile bluegill sunfish (length 28-40 mm, weight 0.40-1.6 g) were exposed to nominal concentrations of 0.19, 0.32, 0.54, 0.90, and 1.5 mg/L in two replicates of ten fish each (mean loading 0.61 g/L). The test water had a temperature of 22°C and a pH of 8.0-8.2.

Tan precipitates were observed on the sides of the mixing chambers at concentrations 0.32 mg/L and higher, a tan/white precipitate was observed on the bottom of the test chamber of the 1.5 mg/L treatment. Actual concentrations, corrected for purity of the test compound and analytical recovery, were on average 87-95% of nominal and amounted to 0.18, 0.30, 0.51, 0.85, and 1.3 mg/L. Metabolite D3598 was detected in the lowest and highest test concentration, average concentrations were 0.049 and 0.13 mg/L after correction for recovery. The metabolite D1989 was not detected. An actual 96-hours LC_{50} of 0.58 mg bifenazate/L was calculated using Probit analysis.

5.4.1.2 Long-term toxicity to fish

The toxicity of bifenazate technical (purity 92.6%) on early life stages of the rainbow trout (*Oncorhynchus mykiss*) was tested in a flow-through study in accordance with EPA guidelines (87 days). Rainbow trout embryos were exposed to nominal concentrations of 0.025, 0.050, 0.10, 0.20,

and 0.40 mg/L, a negative control and a solvent control (acetone, 0.1 mL/L) were included in the test. Four replicates were tested. The test water had a temperature of 21°C and a pH of 8.0-8.4.

Average measured concentrations over the whole test period, corrected for purity of the test compound and procedural recovery, were 0.017, 0.037, 0.079, 0.14 and 0.28 mg/L, which represents 68 to 79% of nominal. The metabolite D3598 was detected in samples of all bifenazate treatments, concentrations expressed as bifenazate equivalents were 11 to 78% of the nominal bifenazate concentrations. Concentrations of the metabolite D1989 were always below the LOQ. The mean summed concentrations of bifenazate and D3598, expressed as total bifenazate equivalents, were 0.0192, 0.044, 0.091, 0.163, and 0.31 mg/L. This represents 77 - 91% of nominal.

The NOEC for hatching and larval and fry survival until thinning was 0.14 mg bifenazate/L, the NOEC for swimming up was 0.079 mg bifenazate/L, the NOEC for larval survival from 14 to 60 days post-hatch was 0.037 mg bifenazate/L, the NOEC for growth at 32 days post-hatch was 0.037 mg bifenazate/L, and the NOEC for growth at 60 days post-hatch was 0.017 mg bifenazate/L.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The effect of bifenazate technical (purity 92.2%) on shell deposition of the eastern oyster (*Crassostrea virginica*) was determined in a flow-through study in accordance with EPA and ASTM guidelines. Oysters were exposed to nominal concentrations of 0.075, 0.15, 0.30, 0.60, and 1.2 mg/L, one replicate per concentration with 20 oysters each. The test water had a temperature of 22°C and a pH of 7.8-8.2.

A tan/white precipitate was observed on the sides of the mixing chambers of the three highest test concentrations and on the bottom of the test chamber of the 1.2 mg/L treatment. Actual concentrations of bifenazate, corrected for purity of the test compound and analytical recovery, were on average 44-60% of nominal and amounted to 0.040, 0.079, 0.17, 0.36, and 0.53 mg/L. Metabolite D3598 was detected in all test concentrations, average concentrations were 0.0377, 0.0661, 0.107, 0.154, and 0.227 mg/L expressed as bifenazate equivalents (not corrected for procedural recovery). This represents 19 to 50% of the nominal bifenazate concentrations. Test concentrations, expressed as total equivalents of bifenazate, were 0.0624, 0.145, 0.280, 0.516, and 0.761 mg/L. This represents 83.2-96.7% of the nominal bifenazate concentration for the lower four concentrations and 63.4% for the highest concentration. The metabolite D1989 was identified in most chromatograms, but concentrations were below the LOQ.

The 96-h EC_{50}, based on total bifenazate equivalents, was calculated as 0.417 mg/L using a linear interpolation method based on bifenazate + D3598.

5.4.2.2 Long-term toxicity to aquatic invertebrates

The chronic toxicity of bifenazate technical (purity 92.6%) on neonate daphnids was tested in a flow through study in accordance with EPA and ASTM guidelines. Daphnids were exposed to nominal concentrations of 0.05, 0.10, 0.20, 0.40 and 0.80 mg/L, two replicates per concentration. The test water had a temperature of 20°C and a pH of 8.0-8.1.

A small brown precipitate was observed during the test in the mixing chamber of the highest test concentration and at 0.40 mg/L on day 21, solutions in all other mixing chambers and test chambers were clear. The average measured concentrations of bifenazate over 21 days, corrected for procedural recovery and purity of the test compound, were 64 to 76% of nominal and amounted to

0.036, 0.076, 0.15, 0.27 and 0.51 mg/L. The metabolite D3598 was detected in the 0.40 and 0.80 mg/L samples on days 0 and 7 only. Average concentrations were 0.061 mg/L in the 0.40 mg/L-treatment and 0.095 mg/L in the 0.80 mg/L-treatment. Recalculated into bifenazate equivalents, this is 13-17% of nominal on average. Test concentrations, expressed as total equivalents of bifenazate, were 0.0359, 0.0764, 0.153, 0.298, and 0.58 mg/L. This represents 72-77% of the nominal bifenazate concentration. Concentrations of the metabolite D1989 were below the LOQ. The 21-days NOEC was determined to be 0.15 mg/L for reproduction and growth, based on mean measured concentrations of bifenazate.

5.4.3 Algae and aquatic plants

A growth inhibition test was performed with bifenazate technical (purity 92.6%) on marine diatoms in accordance with EPA and OECD guidelines on the saltwater algae *Skeletonema costatum*. Diatoms were exposed to five nominal concentrations of 0.063, 0.13, 0.25, 0.50, and 1.0 mg/L, three replicates per concentration. Initial cell density was ca. $7.7 \cdot 10^4$ cells/mL. Tests were performed under 14 hours lighting of 4300 lux, temperature 20°C, pH 8.0-9.0.

Actual concentrations of bifenazate in t=0 samples, corrected for purity of the test compound, were 71.7 to 84.1% of nominal and amounted to 0.0452, 0.101, 0.200, 0.420, and 0.815 mg/L. Concentrations at t=96 h were below the LOQ for the lowest and the two highest test concentrations, and were 43 and 46% of nominal at test concentrations of 0.13 and 0.25 mg/L (0.055 and 0.114 mg/L). Concentrations of the metabolites D3598 and D1989 were below the LOQ in all samples. The E_rC_{50} was estimated as 0.36 mg/L (95% CI 0.24-0.71 mg/L), the NOE_rC was reported as 0.200 mg/L based on measured bifenazate concentrations at test initiation.

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Bifenazate produces acute $L(E)C_{50}$ values in concentrations < 1 mg/L for fish, crustaceans and algae, and produces chronic NOEC values in concentrations > 0.1 < 1 mg/L for fish, crustaceans and algae. The substance disappears very rapidly from the water phase, but is not rapidly degradable: two metabolites (D3598, D9472) are formed in concentrations > 10% AR. Both metabolites show toxicity comparable to the toxicity of bifenazate.

<u>CLP- Acute aquatic hazards</u>

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of ≤ 1 mg/l is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

The lowest $L(E)C_{50}$ obtained for bifenazate are 0.36, 0.42 and 0.76 mg/l in algae, invertebrates and fish, respectively. Bifenazate therefore fulfils the criteria for classification as Aquatic Acute Cat. 1. An M-factor of 1 for acute toxicity is proposed based on $L(E)C_{50}$ values of 0.36, 0.42 and 0.76 mg/l in algae, invertebrates and fish, respectively.

<u>CLP - Aquatic chronic hazards</u>

According to the criteria of the 2nd ATP to the CLP Regulation, when NOEC values are available for all trophic levels, a substance is classified for aquatic chronic hazards if a NOEC or EC_{10} of ≤ 1 mg/l is obtained in a long-term aquatic toxicity study. The assignment of a hazard category depends on the NOEC value and whether the substance is rapidly degradable or not.

Bifenazate is considered not rapidly degradable (see section 5.1.3). NOEC values for bifenazate are available for all trophic levels. The lowest NOEC is 0.017 mg/l obtained for fish. Bifenazate therefore fulfils criteria for classification as Aquatic Chronic Cat.1. An M-factor of 1 for chronic toxicity is proposed based on the NOEC value of 0.017 mg/l in fish.

Directive 67/548/EEC

According to the criteria of Directive 67/548/EEC, a substance can be classified for acute or chronic hazards to the environment. If a substance has acute aquatic toxicity of <100 mg/l and is not readily biodegradable or has a log Kow of ≥3, it is classified for long-term hazards to the environment..

The lowest acute aquatic toxicity values for bifenazate are 0.36, 0.42 and 0.76 mg/l in algae, invertebrates and fish, respectively. Bifenazate is not readily biodegradable (see section 5.1.3). Furthermore, the log Kow value of bifenazate is 3.4. Bifenazate therefore fulfils the criteria for classification with N;R50/53. The specific concentration limits (SCL) of Cn ≥ 25% N; R50-53, 2.5% ≤ Cn <25% N; R51-53 and 0.25% ≤ Cn <2.5%; R52-53 where Cn is the concentration of bifenazate in a mixture are proposed.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Conclusion on environmental classification according to CLP

Bifenazate fulfils the criteria for classification as Aquatic Acute 1 with an M-factor of 1

Bifenazate fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 1

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

Bifenazate fulfils the criteria for classification as

N;R50/53 with SCL of
Cn ≥ 25% N; R50/53,
2.5% ≤ Cn <25% N; R51/53
0.25% ≤ Cn <2.5%; R52/53
where Cn is the concentration of bifenazate in a preparation

6 OTHER INFORMATION

None

7 REFERENCES

DAR bifenazate 2003 volume 3 annex B, Part B1-5

DAR bifenazate 2003 volume 3 annex B, Part B6

DAR bifenazate 2003 volume 3 annex B, Part B8

DAR bifenazate 2003 volume 3 annex B, Part B9

Muller A. et al., 2006. Hazard classification of chemicals inducing haemolytic anaemia: An EU regulatory perspective. Regul Toxicol Pharmacol. 45(3):229-41.

8 ANNEXES