Committee for Risk Assessment

RAC

Annex 1

Background document

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

Cycloxydim

EC number: 405-230-9

CAS number: 101 205-02-1

ECHA/RAC/CLH-O-0000003157-76-01/A1

Adopted

28 November 2012

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name (Iso common name):	***Cycloxydim***
EC number:	***405-230-9***
CAS number:	***101 205-02-1****
Annex VI Index number:	**-**
Degree of purity:	***TC** (technical compound): min. 940 g/kg*** ***ratio E : Z 99.2 : 0.8*** ***ratio R : S 1:1 (racemic mixture)*** ***TK (technical concentrate) :*** ***min. 400 g/kg max. 450 g/kg***
Impurities:	***No relevant impurities***

***The ratio of the E/Z isomers is proven to be: **99.2 : 0.8**. Therefore Cycloxydim is a racemic mixture to which the CAS number refers.

**** Due to its limited stability cycloxydim technical is not isolated as TC, but handled, transported and processed solely as TK which is a (liquid) mixture containing cycloxydim and a solvent.
The classification for mixtures is not at issue at the moment.

1.2 Dossier submitter's proposal for harmonised classification and labelling

Table 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	No current entry	No current entry
Current proposal for consideration by RAC	TC: No classification TK: No classification	TC: R 11 Highly flammable TK: No classification
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	TC: No classification TK: No classification	TC: R 11 Highly flammable TK: No classification

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria by the dossier submitter

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	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.2.	Flammable gases	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.3.	Flammable aerosols	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.4.	Oxidising gases	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.5.	Gases under pressure	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.6.	Flammable liquids	TK** No classification	-	-	Conclusive, but not sufficient for classification TK**
2.7.	Flammable solids	TC* No classification	-	-	Conclusive, but not sufficient for classification TC*
2.8.	Self-reactive substances and mixtures	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.9.	Pyrophoric liquids	TK** No classification	-	-	Conclusive, but not sufficient for classification TK**
2.10.	Pyrophoric solids	TC* No classification	-	-	Conclusive, but not sufficient for classification TC*
2.11.	Self-heating substances and mixtures	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.12.	Substances and mixtures which in	TC* and TK** No	-	-	Conclusive, but not sufficient for

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
	contact with water emit flammable gases	classification			classification TC* and TK**
2.13.	Oxidising liquids	TK** No classification	-	-	Conclusive, but not sufficient for classification TK**
2.14.	Oxidising solids	TC* No classification	-	-	Conclusive, but not sufficient for classification TC*
2.15.	Organic peroxides	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
2.16.	Substance and mixtures corrosive to metals	TC* and TK** No classification	-	-	Conclusive, but not sufficient for classification TC* and TK**
3.1.	Acute toxicity - oral	No classification	-	-	Conclusive, but not sufficient for classification
	Acute toxicity - dermal	No classification	-	-	Conclusive, but not sufficient for classification
	Acute toxicity - inhalation	No classification	-	-	Conclusive, but not sufficient for classification
3.2.	Skin corrosion / irritation	No classification	-	-	Conclusive, but not sufficient for classification
3.3.	Serious eye damage / eye irritation	No classification	-	-	Conclusive, but not sufficient for classification
3.4.	Respiratory sensitisation	No classification	-	-	Conclusive, but not sufficient for classification
3.4.	Skin sensitisation	No classification	-	-	Conclusive, but not sufficient for classification
3.5.	Germ cell mutagenicity	No classification	-	-	Conclusive, but not sufficient for classification
3.6.	Carcinogenicity	No classification	-	-	Conclusive, but not sufficient for classification
3.7.	Reproductive toxicity	No classification	-	-	Conclusive, but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.8.	Specific target organ toxicity –single exposure	No classification	-	-	Conclusive, but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	No classification	-	-	Conclusive, but not sufficient for classification
3.10.	Aspiration hazard	No classification	-	-	Conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	No classification	-	-	Conclusive, but not sufficient for classification
5.1.	Hazardous to the ozone layer	No classification	-	-	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
* TC (technical compound) will never be isolated. Cycloxydim is always manufactured, transported and processed as TK
** TK (technical concentrate) mixture of Cycloxydim and solvent

Labelling: Signal word: -
Hazard statements: -
Precautionary statements: -

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD by the dossier submitter

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	No classification	-	-	Conclusive, but not sufficient for classification
Oxidising properties	No classification	-	-	Conclusive, but not sufficient for classification
Flammability	TC*: R11 highly flammable TK**: no classification	-	-	-
Other physico-chemical properties *[Add rows when relevant]*	No classification	-	-	Conclusive, but not sufficient for classification
Thermal stability	No classification	-	-	Conclusive, but not sufficient for classification
Acute toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	No classification	-	-	Conclusive, but not sufficient for classification
Repeated dose toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Irritation / Corrosion	No classification	-	-	Conclusive, but not sufficient for classification
Sensitisation	No classification	-	-	Conclusive, but not sufficient for classification
Carcinogenicity	No classification	-	-	Conclusive, but not sufficient for classification
Mutagenicity – Genetic toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Toxicity to reproduction – fertility	No classification	-	-	Conclusive, but not sufficient for classification
Toxicity to reproduction –	No classification	-	-	Conclusive, but not sufficient for

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
development				classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	No classification	-	-	Conclusive, but not sufficient for classification
Environment	No classification	-	-	Conclusive, but not sufficient for classification

1) Including SCLs
2) Data lacking, inconclusive, or conclusive but not sufficient for classification
* TC (technical compound) will never be isolated. Cycloxydim is always manufactured, transported and processed as TK
** TK (technical concentrate) mixture of Cycloxydim and solvent

Labelling: Indication of danger:
F Highly flammable

R-phrases:
R11 Highly flammable (only TC)

S-phrases:
S16 Keep away from sources of ignition - No smoking.
S20/21 When using do not eat, drink or smoke.

2 BACKGROUND TO THE DOSSIER SUBMITTER'S CLH PROPOSAL

2.1 History of the previous classification and labelling

Cycloxydim is a herbicide used for outdoor foliar spraying against perennial grasses. At the timepoint of submission of CLH report cycloxydim has not been yet approved for Annex I listing as a third stage Part A Review compound under Council Directive 91/414/EEC (the Inclusion in Annex I is forthcoming), with Austria as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, cycloxydim should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physical and chemical properties, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of cycloxydim under Directive 91/414/EEC. This assessment was based on one full data package submitted by one company.

Cycloxydim is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). Following evaluation of the data this proposal seeks to propose classification for the physical and chemical properties (only according to DSD; no C&L necessary according to CLP regulation). No classification for human health and environment is proposed. No disagreement on classification and labeling proposal were given between Austria as Rapporteur Member State and other Member States during the peer review procedure for Annex I inclusion. At the time of resubmission of the CLH report no other registration dossier is available.

2.2 Dossier submitter's short summary of the scientific justification for their CLH proposal

Regarding physical and chemical properties classification as R11 highly flammable (according to DSD) was proposed for the technical compound (TC i.e the dried technical active substance [min. purity 940 g/kg]). Due to its limited stability cycloxydim technical is not isolated as TC, but handled, transported and processed solely as TK which is a mixture containing Cycloxydim and a solvent (purity min. 400 g/kg max. 450 g/kg). Based on the studies provided, TC has to be classified with R11 highly flammable. For TK no classification is justified.

According to CLP a new study was required referring to the "Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, 2nd revised edition, Part III, Test N.1, Section 33.2.1.4" resulting in NO CLASSIFICATION for the technical compound (TC) (see below in table 9 point B.2.1.20 Flammability). The new study provided has been considered relevant with respect to CLP.

Classification for self heating (CLP) requires a different testing method (N.4) to DSD classification. However, according to ECHA guidance document (ECHA-09-G-02-EN) no testing is applicable if the substance is completely molten below 160 °C. Data on corrosiveness to metals are not considered under DSD as well. According to the ECHA guidance document (ECHA-09-G-02-EN) no reclassification is applicable if the substance is not "skin corrosive".
Therefore no classification and labeling (according to CLP) is proposed regarding physical and chemical properties for cycloxydim TC and TK.

For cycloxydim, no classification and labelling is proposed regarding human health.

Regarding environment for cycloxydim no classification is proposed with regard to DSD and CLP.

2.3 Current harmonised classification and labelling

Cycloxydim has not been previously discussed or agreed at TC C&L (Dir. 67/548/EEC); no harmonised classification and labelling exists.

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

No entry in Annex VI, Table 3.1.

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

No entry in Annex VI, Table 3.2.

2.4 Current self-classification and labelling

In the initial submitted dossier the Notifier stated that "based on the results of physical/chemical, toxicological, e-fate and ecotoxicological studies, classification and labelling of technical grade cycloxydim (TC, TGAI) is not required".

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification (cycloxydim is a pesticide)

Part B.

SCIENTIFIC EVALUATION OF THE DATA

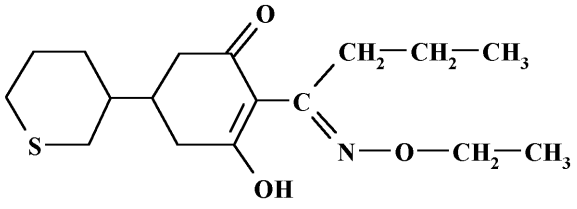
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

ISO name:	Cycloxydim
EC number:	405-230-9
CAS number:	101 205-02-1
CAS name:	2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(tetrahydro-2H-thiopyran-3-yl)-2-cyclohexen-1-one
IUPAC name:	(5*RS*)-2-[(*EZ*)-1-(ethoxyimino)butyl]-3-hydroxy-5-[(3*RS*)-thian-3-yl]cyclohex-2-en-1-one
CLP Annex VI Index number:	-
Molecular formula:	$C_{17}H_{27}NO_3S$
Molecular weight range:	325.5 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cycloxydim	TC* (technical compound) 940 g/kg TK (technical concentrate) min. 400 g/kg max. 450 g/kg	TC*: no range minimum purity is stated TK: min. 400 g/kg max. 450 g/kg	

***** Due to its limited stability cycloxydim technical is not isolated as TC but handled, transported and processed solely as TK which is a mixture containing Cycloxydim and a solvent.

Current Annex VI entry: -

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No relevant impurities	-	-	-

For further information see DAR confidential and DAR confidential resubmission

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives				

Current Annex VI entry: -

1.2.1 Composition of test material

Physico-chemical properties: purity of tested technical material in the range from 99.6% [TC pure] to 42.0%[TK technical])

Human health hazard assessment: purity of tested technical material in the range from 90.14% to 94.8%

Environmental hazard assessment: purity of tested technical material in the range from > 90% to 98.7%
Remark: The specification of min. 94 % (TC) as given in IUCLID dossier is based on the most recent 5-batch profile dated 2005 and may differ to the purity used in older studies.

1.3 Physico-chemical properties

Please note that physical and chemical properties reported should be interpreted with caution as the E/Z isomer ratio is dependent on the sample environment (pH and polarity of solvents). Therefore the values reported relate only to the conditions of the specific tests and may vary with varying conditions.

Due to its limited stability cycloxydim technical is not isolated as TC but handled, transported and processed solely as TK which is a mixture containing Cycloxydim and a solvent.

Table 9: Summary of physico - chemical properties

Property (Annex point as reference to the DAR)	Method	Results	Conclusion/Comment	Reference (Study)
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	EEC/A1 Differential scanning calorimetric method (DSC) OECD 102 GLP	Purified product (purity: 99.6% w/w) The melting point range was observed to be 37.1 - 41.2 °C (demonstrated by a DSC graph)	Acceptable	Tuerk W., (1996c) (BASF RegDoc# 1996/10961) Daum A., (2006) (BASF RegDoc# 2006/1019582)
B.2.1.2 Boiling point (IIA 2.1.2)	EEC/A2 Differential scanning calorimetric method (DSC) OECD 103 GLP	Purified product (purity: 99.6% w/w) There is no endothermic effect other than the melting point. Therefore, boiling or sublimation of the test substance can be excluded. (demonstrated by a DSC graph)	Acceptable	Tuerk W., (1996c) (BASF RegDoc# 1996/10961) Daum A., (2006) (BASF RegDoc# 2006/1019582)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	EEC/A2 Differential scanning calorimetric method (DSC) OECD 103 GLP	Purified product (purity: 99.6% w/w) Decomposition is observed at approx. 200 °C (demonstrated by a DSC graph)	Acceptable	Tuerk W., (1996c) (BASF RegDoc# 1996/10961) Daum A., (2006) (BASF RegDoc# 2006/1019582)
B.2.1.4 Relative density (IIA 2.2)	EEC/A3 Hydrometer OECD 109 GLP	Purified product (purity: 99.6% w/w) D_4^{20} = 1.165 20 °C	Acceptable	Kaestel R., (1997a) (BASF RegDoc# 1997/10238)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
	EEC/A3 Gas comparison pycnometer OECD 109 GLP	TK: content of cycloxydim: 42.0 % w/w D_4^{20} = 0.99 at 20 °C	Acceptable	Kaestel R., (1997c) (BASF RegDoc# 1997/10654)
B.2.1.5 Vapour pressure (IIA 2.3.1)	EEC/A4 GLP	Purified product (purity: 99.6% w/w) 1.0 x 10^{-5} Pa at 20 °C 2.2 x 10^{-5} Pa at 25 °C	Acceptable	Kaestel R., (1997a) (BASF RegDoc# 1997/10238)
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	Calculations based on vapour pressure, molecular weight and water solubility	6.141 x 10^{-5} Pa.m³.mol^{-1} (20 °C) values used for calculation: water solubility: 53 mg/L at pH 4.3 and 20 °C vapour pressure: 1.0 x 10^{-5} Pa at 20 °C	Acceptable	Ohnsorge U., (2000) (BASF RegDoc# 2000/1013164)
B.2.1.7 Appearance: physical state and colour (IIA 2.4.1)	Visual examination; subjective evaluation by independent persons GLP	Purified product (purity: 99.6% w/w) colourless solid consisting of white crystals	Acceptable	Tuerk W., (1996c) (BASF RegDoc# 1996/10961)
		Technical product (purity: 92.3% w/w) yellow liquid, a viscous paste	Acceptable	Kaestel R., (1997b) (BASF RegDoc# 1997/10385),
		TK: content of cycloxydim: 42.0 % w/w yellow liquid	Acceptable	Kaestel R. (1997c) (BASF RegDoc# 1997/10654)
B.2.1.9 Appearance: odour (IIA 2.4.2)	Organoleptic determination; subjective evaluation by independent persons GLP	Purified product (purity: 99.6% w/w) odourless	Acceptable	Tuerk W., (1996c) (BASF RegDoc# 1996/10961)
		Technical product (purity: 92.3% w/w) moderate aromatic odour	Acceptable	Kaestel R., (1997b) (BASF RegDoc# 1997/10385)
		TK: content of cycloxydim: 42.0 % w/w moderate aromatic odour	Acceptable	Kaestel R. (1997c) (BASF RegDoc# 1997/10654)

<table>
<tr><th>Property (Annex point as reference to the DAR)</th><th>Method</th><th>Results</th><th>Conclusion/Comment</th><th>Reference (Study)</th></tr>
<tr><td>B.2.1.10
Spectra of the active substance
(IIA 2.5.1)</td><td>UV/VIS - Spectroscopy
GLP</td><td>Purified product (purity: 99.6% w/w)
c = 10.48 mg/L
<table><tr><th>Solvent</th><th>$\lambda_{max\ [nm]}$</th><th>ε_{max} [$L \cdot mol^{-1} \cdot cm^{-1}$]</th></tr><tr><td>MeOH</td><td>210
227
259
278
290
300</td><td>7.2×10^3
4.1×10^3
1.0×10^4
9.3×10^3
7.9×10^3
4.4×10^3</td></tr></table>**ε** above 290 nm > 10; absorbance until approx. 320 nm</td><td>The spectrum measured in neutral medium is acceptable
In addition a spectrum in acidic medium is required since the pKa of cycloxydim is 4.17
➔ the spectra are submitted and reported in the resubmission report and given below</td><td>Tuerk W., (1996b)
(BASF RegDoc# 1996/10960)</td></tr>
<tr><td></td><td>OECD 101 UV/VIS – Absorption spectra
GLP</td><td>Purified product (purity: 99.6% w/w) at 25 °C
concentration = 19.9 mg/L
<table><tr><th>Solvent</th><th>$\lambda_{max\ [nm]}$</th><th>ε_{max} [$L \cdot mol^{-1} \cdot cm^{-1}$]</th></tr><tr><td>MeOH/HCL
pH = 1.4</td><td>228
260
290
310</td><td>6753
13788
4199
2930</td></tr></table>**ε** above 290 nm > 10; absorbance until approx. 330 nm</td><td>Acceptable</td><td>Kroehl T. (2007)
BASF Reg Doc# 2007/1017028</td></tr>
</table>

Property (Annex point as reference to the DAR)	Method	Results	Conclusion/Comment	Reference (Study)
	OECD 101 UV/VIS – Absorption spectra GLP	Purified product (purity: 99.6% w/w) at 25 °C concentration = 19.92 mg/L Solvent: MeOH/NaOH pH = 12.1 $\lambda_{max\ [nm]}$: 215; 234; 283; 290 ε_{max} [L·mol^{-1}·cm^{-1}]: 4983; 2140; 22105; 18079 **ε** above 290 nm > 10; absorbance until approx. 320 nm	Acceptable	Kroehl T. (2008) BASF Reg Doc# 2008/1009861
	FTIR - Spectroscopy KBr disk, 600 – 4000 cm^{-1} GLP	Purified product (purity: 99.6% w/w)	Acceptable The IR spectrum of cycloxydim is in agreement with the chemical structure	Tuerk W., (1996b) (BASF RegDoc# 1996/10960)
	^{1}H - NMR-Spectroscopy GLP	Purified product (purity: 99.6% w/w)	Acceptable The NMR spectrum of cycloxydim is in agreement with the chemical structure	
	MS - Spectroscopy EI Direct Inlet GLP	Purified product (purity: 99.6% w/w	Acceptable The MS-spectrum is consistent with the chemical structure	
B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)			No impurities of toxicological or environmental significance	

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.12 Solubility in water (IIA 2.6)	EEC/A6 flask method OECD 105 HPLC GLP	Purified product (purity: 99.6% w/w) at 20 °C Solubility in purified water (Millipore quality): 53 mg/L The saturated solution is acidic (pH 4.3)	Acceptable	Tuerk W., (1996a) (BASF RegDoc# 1996/10935)
	EEC/A.6 flask method OECD 105 HPLC GLP	Purified product (purity: 99.6% w/w) all at 20 °C pH 4 (phthalate buffer) 0.05 g/l pH 7 (phosphate buffer) 0.9 g/l pH 9 (borate buffer) 8 g/l	Acceptable	Class T. (2008) BASF Reg Doc# 2008/1021518
	CIPAC MT 157.3 flask method HPLC according to GLP*)	Purified product (purity: 99.4% w/w) at 20 °C 38 mg/L in bi-distilled water 30 mg/L in water adjusted to pH 3 with HCl Cycloxydim forms salts in the alkaline range (pH 9-11) (> 50 % w/w of pure a.i. is soluble in bi-distilled water (pH 10.7) in form of its sodium salt)	Acceptable CIPAC MT 157.3 flask method is comparable to EEC/A6 flask method	1. Pawliczek J.B., (1988) (BASF RegDoc# 11669)

Property (Annex point as reference to the DAR)	**Method**	**Results**		**Conclusion/Comment**	**Reference (Study)**
B.2.1.13 Solubility in organic solvents (IIA 2.7)	Visual classification. CIPAC MT 181 [formerly CIPAC BASF RegDoc# No. 3869/M] GLP	Purified product (purity: 99.6% w/w)		Acceptable	Daum A., (1998) (BASF RegDoc# 1998/10949)
		solvent	solubility at 20 °C [g/100mL]		
		n-heptane toluene dichloromethane methanol acetone ethyl acetate	> 25 > 25 > 25 > 25 > 25 > 25		
B.2.1.14 Partition coefficient n-octanol/water (IIA 2.8)	OECD 107 flask method HPLC according to GLP*)	Purified product (purity: 99.4% w/w) at 25 °C log P_{OW} = 3.09 at pH 5 (mean value of 3.13, 3.22 and 2.92) = 1.36 at pH 7 (mean value of 1.55, 1.46 and 1.08) = - 0.42 at pH 9 (mean value of -0.42, -0.40 and -0.45) The different values are determined with concentrations of 10^{-2}, 10^{-3} and 10^{-4} mol/L. Phase separation is achieved by centrifugation at 25 °C and 12 000 g.		Acceptable The method is comparable to the EEC/A8 shake flask method. Although the substance is surface active, phase separation is described.	Redeker J., (1988a) (BASF RegDoc# 1988/10545)
	OECD 107 HPLC GLP	BH 517-T1SO (BH 625-7) (purity: 98.5% w/w) at 20 °C metabolite in rats, goats, hens, and plants The test substance shows two signals for the cis/trans isomere at the S=O group. Concentration: 10.01 mg/50mL. log P_{OW} (isomere 1) = 1.35 log P_{OW} (isomere 2) = 1.42 Since the substance does not dissociate, the log P_{OW} does not depend on pH.		Acceptable The method is comparable to the EEC/A8	Daum. A., (1997) (BASF RegDoc# 1997/11453)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
	OECD 107 flask method HPLC GLP	BH 517-TSO (purity: 97.1% w/w) at 20 °C metabolite in rats, goats, hens, and plants log P_{OW} = 1.22 deionized water; conc. = 9.208 mMol/L = 0.96 deionized water; conc. = 0.921 mMol/L = 1.15 at pH 4; conc. = 9.208 mMol/L = 1.16 at pH 4; conc. = 0.921 mMol/L = -0.81 at pH 7; conc. = 9.208 mMol/L = -1.00 at pH 7; conc. = 0.921 mMol/L = -1.77 at pH 9; conc. = 9.208 mMol/L = -1.97 at pH 9; conc. = 0.921 mMol/L	Acceptable The method is comparable to the EEC/A8 shake flask method.	Daum. A., (2000) (BASF RegDoc# 2000/1013186)
B.2.1.15 Hydrolysis rate (IIA 2.9.1)	US-EPA, Subdivision N, § 161-1, OECD Guideline 111, 2004, SETAC, March 1995 GLP	[^{14}C]-cycloxydim radio-chemical purity > 95 % <u>Half lives of a.i. at 25°C</u> pH 4: 2.1 days pH 5: 12.2 days pH 7: 264 days* pH 9: 958 days* * extrapolated	Acceptable. The claim to address the formation of the Z-isomer [see reporting table 1(17)] is not possible since E/Z isomerization takes place in solution leading to an equilibrium of both isomers which depends on the polarity and the pH value of the solvent. For further explanation, please refer to Volume 4 C.1.2.2 Identity of isomers.	Hassink J., (2009) BASF Reg Doc# 2008/1090871

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.16 Direct phototrans-formation (IIA 2.9.2)	FAO, Rev. 3; US-EPA, Subdivision N, § 161-2 GLP	[^{14}C]-cycloxydim radio-chemical purity: 95.2% (pH 5), 92.6% (pH 7), 96.2% (pH 9) Unlabelled reference substance: Purity: 99.0 % w/w) <u>Half lives at 22 °C:</u> pH 5: 5.8 h pH 7: 17.6 h pH 9: 22.3 h <u>Metabolites:</u> At pH 5: TSO: 32%, T2S: 12% and T1SO ($C_{15}H_{23}NO_3S$): 69% T2SO ($C_{15}H_{21}NO_3S$): 18% At pH 7: TSO: 53% and T1SO: 64% T2SO: 16% At pH 9: TSO: 18%, T1S ($C_{15}H_{23}NO_2S$): 28%, T1SO: 68%	Acceptable For details see B.8.4 Fate and behaviour	Goetz N., (2000) (BASF RegDoc# 2000/1000143)
B.2.1.17 Quantum yield (IIA 2.9.3)	Calculation	Quantum yield in moles degraded per Einstein absorbed pH 5: $\Phi = 5.68 \times 10^{-3}$ pH 7: $\Phi = 1.87 \times 10^{-4}$ pH 9: $\Phi = 2.02 \times 10^{-4}$	Acceptable For details see B.8.4 Fate and behaviour	Goetz N., (2000) (BASF RegDoc# 2000/1000143)
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.4)	OECD 112 according to GLP*)	Purified product (purity: 99.4% w/w) $pK_A = 4.17$ at 20 °C $pK_A = 4.04$ at 25 °C	Acceptable	Redeker J., (1988b) (BASF RegDoc# 1988/10557)
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Calculation according to Atkinson's method	Calculated atmospheric degradation half life: T ½ = 6.3 h	Acceptable For details see B.8.7.1 Fate and behaviour in air	Sarafin R. (1991a) (BASF RegDoc# 1991/10319)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.20 Flammability (IIA 2.11.1)	EEC/A10 GLP	Technical product (purity: 92.3% w/w) Preliminary test: A burning rate of 145 s was measured Main test : 31 s	Acceptable Technical cycloxydim is considered as "highly flammable" Purity is mentioned in Tier 2	Loeffler U., (1997a) (BASF RegDoc# 1997/10446)
		TK: content of cycloxydim: 42.0 % w/w Not applicable for liquids	Acceptable	Loeffler U., (1997b) (BASF RegDoc# 1997/10655)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.20 Flammability	N.1 Section 33.2.1.4 no GLP required	Technical product (Batch COD-001412) TC (solid) Preliminary test is negative (brief burning followed by rapid extinction) Not considered a flammable solid (TC) of division 4.1.	Acceptable No classification according to Regulation 1272/2008 According to CLP a new study was required referring to the "Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, 2nd revised edition, Part III, Test N.1, Section 33.2.1.4" resulting in NO CLASSIFICATION for the technical compound (TC). The new study provided has been considered relevant with respect to CLP.	Löhr, (2010) (BASF RegDoc# 2010/1155866)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.21 Auto-flammability (IIA 2.11.2)	EEC/A15 GLP	Technical product (purity: 92.9% w/w) Auto-ignition temperature is 295 °C	Acceptable according to Directive 67/548/EEC. Compound is not considered as auto-flammable under test condition Purity is mentioned in Tier 2 According to the to an ECHA guidance document (ECHA-09-G-02-EN) no test procedure (N.4) for self heating is applicable if the substance is completely molten at 160 °C. This is demonstrated by DSC plots for determination of melting point and boiling point (refer to B.2.1.1 and B.2.1.2 No classification.	Loeffler U., (2000) (BASF RegDoc# 2000/1013215)
		TK: content of cycloxydim: 42.0 % w/w Auto-ignition of the TK occurs at 360 °C	Acceptable TK is not considered as auto-flammable under test condition	Loeffler U., (1997b) (BASF RegDoc# 1997/10655)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.22 Flash point (IIA 2.12)	EEC/A9 Non-equilibrium method GLP	Technical product (purity: 92.9% w/w) Ignition was observed at the flash point 89.5 °C	Acceptable Purity is mentioned in Tier 2	Loeffler U., (2000) (BASF RegDoc# 2000/1013215)
		TK: content of cycloxydim: 42.0 % w/w The TK has a flash point of 62 °C	Acceptable	Kaestel R. (1997c) (BASF RegDoc# 1997/10654)
B.2.1.23 Explosive properties (IIA 2.13)	EEC/A14 GLP	Technical product (purity: 92.9% w/w) No explosive properties were observed, neither thermal nor by impact	For pasty substances also mechanical sensitivity (friction) has to be tested. However, due to it's limited stability, cycloxydim technical is not isolated as TC but handled, transported and processed as TK (technical concentrate). Therefore, no data are required.	Loeffler U., (2000) (BASF RegDoc# 2000/1013215) Petersen-Thiery M. (2006). (BASF RegDoc# 2006/1019535)
		TK: content of cycloxydim: 42.0 % w/w Not applicable because the test substance is a liquid	Acceptable	Loeffler U., (1997b) (BASF RegDoc# 1997/10655)
B.2.1.24 Surface tension (IIA 2.14)	EEC/A5 1.6.4. OECD harmonized ring method GLP	Purified product (purity: 99.6% w/w) at 20 °C σ = 58.0 mN/m at 0.5 % (w/w) σ = 57.0 mN/m at 2.0 % (w/w) in pure water. The solutions/suspensions were filtered before determination i.e. a saturated solution	Due to a low water solubility the test is acceptable, although according to EEC/A5 90% of the saturation solubility is required.	Kaestel R., (1997a) (BASF RegDoc# 1997/10238)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
		Technical product (purity: 92.3% w/w) at 20 °C The surface tension of Cycloxydim TC at 1.0 % in pure water is 56.2 mN/m The solution/suspension was centrifuged before determination	Acceptable	Kaestel R., (1997b) (BASF RegDoc# 1997/10385)
		TK: content of cycloxydim: 42.0 % w/w at 20 °C At 1 % concentration the TK causes a surface tension of 52.4 mN/m	Acceptable	Kaestel R. (1997c) (BASF RegDoc# 1997/10654)

Property (Annex point as reference to the DAR)	**Method**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.25 Oxidising properties (IIA 2.15)	Statement according to EEC/A17	Technical product (purity: 92.9% w/w) Not applicable because of the chemical structure of the test substance	According to the UN manual: Recommendations on the transport of dangerous goods, a test has to be provided as the compound contains oxygen that is not only bonded to carbon or hydrogen, but to nitrogen. The performance of the test is only required, if the TC is transported or marketed. However, a statement was provided by the notifier that due to it's limited stability, cycloxydim technical is not isolated as TC but only handled, transported and processed as TK (technical concentrate). Therefore, no further data are required.	Loeffler U., (2000) (BASF RegDoc# 2000/1013215) Petersen-Thiery M. (2006). (BASF RegDoc# 2006/1019535)

Property (Annex point as reference to the DAR)	Method	Results	Conclusion/Comment	Reference (Study)
	EEC/A.21 GLP	TK: Batch 7073 (purity: 41.55%) Cycloxydim TK is not an oxidizing agent. The mean pressure rise time of the test mixture is greater than the mean pressure rise time of the reference mixture.	Acceptable	Bitterlich S. (2007) BASF Reg Doc# 2007/1013309

Parts marked in grey: Additional studies or statements to the DAR submitted for (non)classification according to Regulation 1272/2008.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Cycloxydim is used as herbicide for outdoor foliar spraying against perennial grasses.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies on flammability

Method	Results	Remarks	Reference
EEC/A10	TC (purity: 92.3% w/w) Preliminary test: Burning rate of 145 s was measured Main test : 31 s Cycloxydim is considered as "highly flammable" R11	-	Loeffler U., (1997a) (BASF RegDoc# 1997/10446)
N.1 Section 33.2.1.4 no GLP required	Technical product (Batch COD-001412) TC (solid) Preliminary test is negative (brief burning followed by rapid extinction) Not considered a flammable solid (TC) of division 4.1.	Acceptable No classification according to Regulation 1272/2008 According to CLP a new study was required referring to the "Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, 2nd revised edition, Part III, Test N.1, Section 33.2.1.4" resulting in NO CLASSIFICATION for the technical compound (TC). The new study provided has been considered relevant with respect to CLP.	Löhr, (2010) (BASF RegDoc# 2010/1155866)

Parts marked in grey: Additional studies or statements to the DAR submitted for (non)classification according to Regulation 1272/2008

3.1 *Summary of dossier submitter's proposal*

The dossier submitter proposes to classify with F; R11 under DSD, due to the results observed in the EEC/A10 study (burning time of less than 45 seconds). However, classification in accordance with CLP is not proposed based on the result of a study performed according to the

UN Recommendations on the Transport of Dangerous Goods (TDG), Manual of Tests and Criteria, 2nd revised edition, Part III, Test N.1, Section 33.2.1.4 in which only brief burning followed by rapid extinction was observed in a preliminary study.

3.2 *Comments received during public consultation*

One comment regarding flammability was received from the UK pointing out that substances that are classified as flammable (F; R11) under DSD are in general classified as flammable also under CLP. However, it was recognised that there are differences between the two test systems, which means that it is not always possible to make a direct translation and that in some cases a different classification could be justified. In spite of the fact that the UN study gave a negative result in the preliminary study, the UK does not think it appropriate to ignore the results obtained in the main EEC/A10 study, also when classifying according to CLP.

3.3 *Any key elements not included in the original CLH report but used to formulate the RAC opinion*

The full study reports for the EEC/A10 and UN tests as well as the characterisation of the test substance were requested to the dossier submitter (Austria) in order to check the validity of these tests and if the presence of some solvents could be responsible of the different results.

In response to this request, the dossier submitter has supplied an analytical characterization of the test substance used in the UN test. However, unfortunately the submitter was not able to trace back the exact composition of the test substance used in the EEC/A10 test, although indicating that the residues of toluol could be responsible of the flammability results.

3.4 *The RAC assessment and comparison with criteria*

Classification as F; R11, highly flammable according to DSD was proposed for the technical compound (TC, i.e. the dried technical active substance; min. purity 940 g/kg) due to the results observed in the EEC/A10 study (i.e. a burning time of less than 45 seconds).

However, according to the Dossier submitter, the flammability test EEC/A.10 was conducted with the TC isolated from cycloxydim technical concentrate (TK i.e. the mixture containing cycloxydim and a solvent [purity 400 - 450 g/kg]) dissolved in toluene. Since toluene is classified as R11, the test result "highly flammable" may be explained by toluene residues in the test substance, as suggested by the Dossier submitter.

According to CLP, a new study was required referring to the UN "Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria, 2nd revised edition, Part III, Test N.1, Section 33.2.1.4". The result of the flammability test was negative, which leads to no classification for the TC under CLP.

The UN test can be considered valid, in spite of the fact that several repetitions should have been conducted. According to the dossier submitter, this test was conducted with cycloxydim TC, isolated through a thin film-evaporator using cycloxydim TK dissolved in Solvesso 150 (test report DocID 2010/1155866), which is not classified as R11.

The only difference between the tests EEC/A10 and the UN method is that, when applying the flame, the decision whether the combustion propagates along a 200 mm train of the substance is taken over 2 minutes in the UN method and over 4 minutes in EEC/A10 method. The different time considered in the decision could be responsible of the different results under the two methods.

Another possible reason for the different results could be the presence of toluene solvent residues in the test substance used in the EEC/A10 test.

However, since the exact composition of the test substance used in the EEC/A10 test is not known, the RAC was not in a position to challenge the outcome of that test.

In conclusion, the RAC supports the dossier submitter's proposal to classify as flammable (F; R11) under DSD and no classification under CLP.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption/Excretion: From urine and bile studies, *bioavailability (enteral absorption) of Cycloxydim* was identified to be > 95 % and occurred rapidly, of both the free acid and the sodium salt. The majority (74 – 86 %) of the single oral dose of 10 mg/kg bw of the sodium salt of Cycloxydim is eliminated renally, most of which was excreted within 24 hours. Only 14 – 26 % of the administered radioactivity was excreted in faeces. After a 14 day pre-treatment of 10 mg/kg bw, the excretion balance was very similar to that of a single dose of 10 mg/kg bw. Biliary excretion plays an important role in the elimination of the substance (55 – 65 % of the dose being excreted via this route). A significant part of the material excreted via bile was re-absorbed and subsequently eliminated via urine.

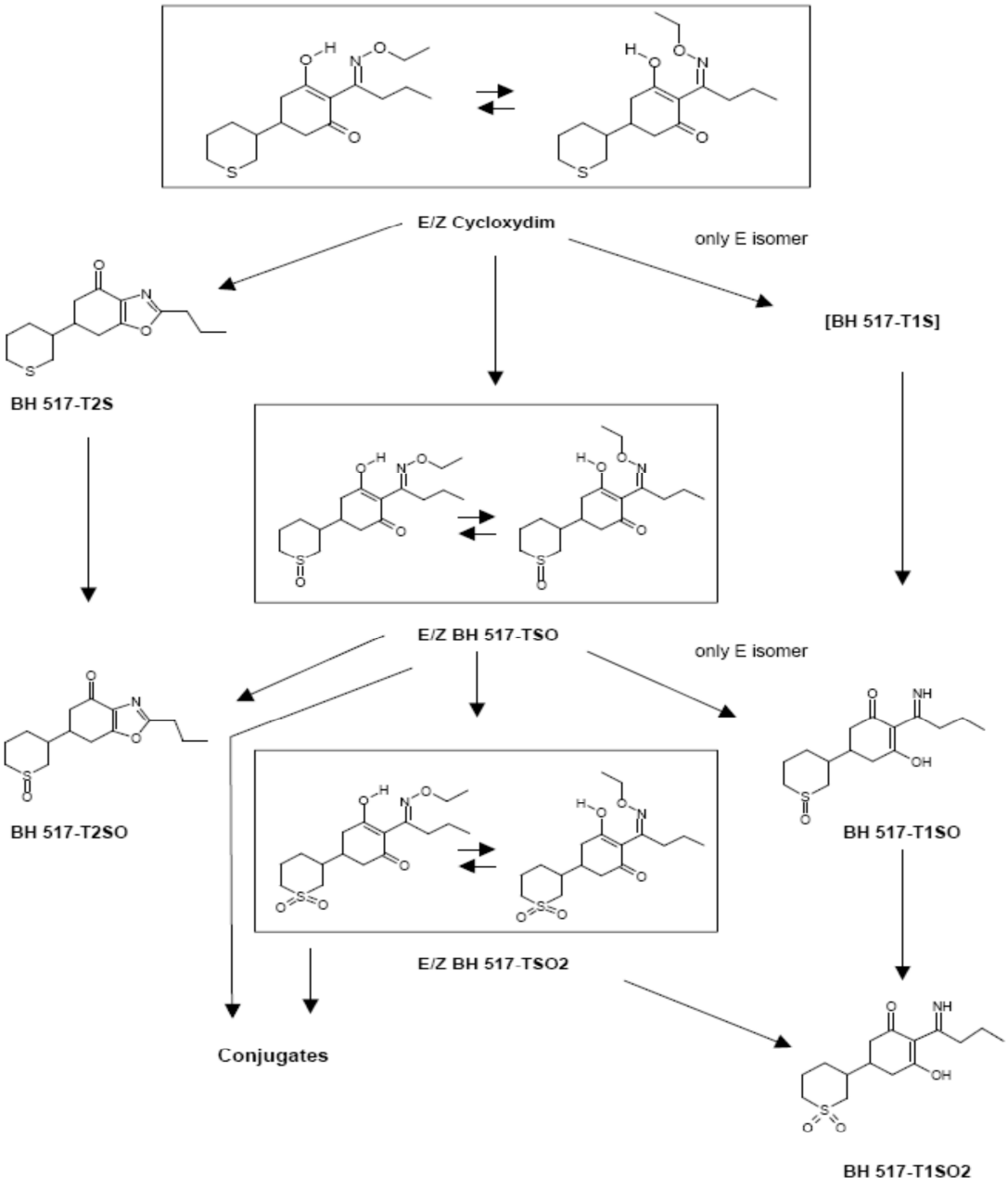
The pharmacokinetic parameters of the free acid and the sodium salt of Cycloxydim at a dose of 10 mg/kg bw were comparable, with the exception that T_{max} of the free acid was reached 5 - 7 hours after oral administration with a C_{max} of 5 and 6.8 µg/mL for males and females, respectively. In case of oral administration of the sodium salt T_{max} was 0.5 - 1 hour with a C_{max} of 8.4 and 9.3 µg/mL for male and female rats, respectively. C_{max} was similar after single or seven consecutive repeated oral doses of the corresponding concentrations of the free acid. Intravenously applied sodium salt reached a C_{max} that was 4-fold (33.5 and 39.5 µg/mL for males and females, respectively) of that reached by oral administration. The AUC data indicate a more than proportional increase of AUC with dose.

Tissue distribution studies indicated that after the last of seven daily oral administrations, radioactivity in the organs rapidly declined over time. At 72 h after the last dose radioactivity was < 1 µg/g tissue for all organs. The highest amounts of radioactivity were found in the biotransformation active organs: liver (0.07 and 0.16 µg/g in males and females, respectively) and kidneys (0.07 and 0.12 µg/g in males and females, respectively), but also drastically in thyroid glands (0.13 and 0.15 µg/g in males and females, respectively). Compared to plasma levels (0.03 and 0.06 µg/mL in males and females, respectively), concentrations in the other organs were equal or lower with the exception of whole blood (0.05 and 0.08 µg/g in males and females, respectively) and gut (0.06 and 0.10 µg/g in males and females, respectively) There was evidence of some retention in the thyroid gland and blood cells, also nasal mucosa was found to be a feature of disposition. No potential of bioaccumulation can be assumed.

The analysis of urine, bile, faeces and organs indicated that several metabolites were formed. Eight compounds were isolated and five (including parent compound) could be identified spectrometrically. As far as possible the proposed structures for the metabolites were corroborated by comparison with synthetic standards. Patterns of metabolites in the urine were qualitatively and quantitatively similar after administration of 10 mg/kg bw of either the free acid or the sodium salt. At a dose of 300 mg/kg bw (free acid) the qualitative distribution of urinary metabolites was similar, but relatively less metabolised. The major metabolite in urine, liver and kidneys was in all cases the sulphoxide of Cycloxydim (*TSO*), accounting for about 21 - 47 %, depending on dose and sex. It has also to be mentioned that in most cases TSO co-chromtographed with $T1SO_2$. The second quantitatively important metabolite (< 15 % of administered radioactivity) was T1SO, resulting from N-de-ethylation of TSO. Minor components in urine were $T1SO_2$ (sulphone of T1SO), T2SO (Beckmann rearrangement of T1SO) and unchanged Cycloxydim. Metabolites of hydroxylation at the 5-position of the

cyclohexene ring of the parent were identified to be of little quantitative relevance (2.3 % activity in urine). Following enzymatic hydrolysis of bile secretion the major metabolites corresponded to TSO (including $T1SO_2$), unchanged parent and T2S (N-de-ethoxylated and rearranged parent product).

Proposed metabolic pathway of Cycloxydim in mammals



Dermal absorption:

In vivo rat study (Beimborn & Leibold, 2003)

The dermal absorption considering material detected in urine, feces, cage wash, blood cells, plasma, kidneys, liver and the carcass for the aqueous solution and for the formulation concentrate were found to be very similar, after 8-hour exposure the total amount of radiolabel absorbed ranged from approx. 22.3 to 29.3 % irrespective of the high or low dose concentration tests. At the end of the 8-h period, a certain amount of substance located in the skin was available for further absorption, as indicated by a decline of radioactivity at the application site and an increase in the material absorbed with time. At the high dose level, results indicate that about 90 % of the radioactive material remaining in the skin after end of exposure penetrated subsequently through the skin during the post-observation period (after 96h), whereas at the low dose level, about 65 % of the radioactivity remaining in the skin after the end of exposure penetrated through the skin during the post-observation period. Therefore the potentially (additionally includes the residing material in the skin of the application site) absorbable amount of material ranges in the aqueous dilution between 31.3 – 38.1 % and in the concentrate from 24.9 – 30.7 %.

For hazard assessment the "worst-case" assumption needs to be anticipated, therefore 31% dermal absorption is suggested for the undiluted formulation (mixing loading) and 38% for the aqueous diluted formulation (spray application) from in vivo rat study.

In vitro human and rat skin study (Gamer et al., 2007)

For the aqueous dilutions of the representative formulation BAS 517 24, the dermal penetration of ^{14}C-labelled cycloxydim through human skin was 17.96 % (radioactivity found in receptor fluid, receptor samples including wash out, receptor chamber including washing, skin preparation); for rat skin, the dermal penetration was higher (47.44%). Comparing the dermal penetration through rat skin and human skin, the ratio rat : human for the dilution can be calculated to be 2.6.

For the concentrate of the representative formulation BAS 517 24, the dermal penetration of ^{14}C-labelled cycloxydim through human skin was 7.87 % (radioactivity found in receptor fluid, receptor samples including wash out, receptor chamber including washing, skin preparation); for rat skin, the dermal penetration was higher (63.12%). Comparing the dermal penetration through rat skin and human skin, the ratio rat : human for the dilution can be calculated to be 8.0.

Combining the results of the *in vivo* study (31% dermal absorption was suggested for the undiluted formulation (mixing loading) and 38% for the aqueous diluted formulation (spray application)) with the results of the *in vitro* study (concentrate: 8 times more permeable through rat skin; dilution: 2.6 times more permeable through rat skin), 3.9% dermal absorption for undiluted formulation and 14.6% for dilution can be calculated.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption	Rapid and extensive (>95%), based on urinary and bilary excretion within 48 h
Distribution	Widely distributed; highest levels found in liver, kidneys, thyroid gland and blood cells
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	95% excreted within 120 h, mainly via urine (72 - 86%), 14 -26% via faeces
Metabolism in animals	Extensively metabolized: main metabolites TSO (oxidation of sulphur); and T1SO (oxidation of sulphur and N-de-ethoxylation)

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity (OECD 401)	♀ LD_{50} = 3830 mg/kg bw ♂ LD_{50} = 4420 mg/kg bw Combined LD_{50} = 3940 mg/kg bw	Wistar rat, Purity 92.2%	1; Kieczka H., Kirsch P. 1984(a)
Acute oral toxicity (OECD 401)	♂/♀ $LD_{50} \geq$ 5000 mg/kg bw	NMRI mouse Purity 94.8%	2; Kieczka H., Kirsch P. 1985(b)
Acute dermal toxicity (OECD 402)	♂/♀ LD_{50} > 2000 mg/kg bw	Wistar rat, Purity 92.2%	3; Kieczka H., Kirsch P. 1984(b)
Acute inhalative toxicity (OECD 403)	♂/♀ LC_{50} > 5.28 mg/L	Wistar rat, Purity 94.8%	Klimisch H.J. et al. 1985

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Rat:

Mortalities occurred between day 3 and 7 in males and females at dose levels of 3830 and 5000 mg/kg body weight, with the exception of one high-dose group female that died within 24 h after dosing. No mortality was noted at lower dose levels. Clinical symptoms recorded were dyspnea, apathia, abnormal position, staggering, atonia, paresis, tremors, twitching, spastic gait, piloerection, exsiccosis, salivation, lacrimation and poor general state. The onset of symptoms was observed between 30 minutes and 5 hours after dosing. In animals that survived to the end of the study, symptoms were observed up to day 7 of the study and occurred in a dose-dependent fashion starting at 2150 mg/kg body weight. No symptoms were noted at the lowest dose level of 1210 mg/kg body weight. At study termination the surviving animals were free of symptoms. Macroscopic examination in animals that died revealed bloody ulceration of the glandular stomach and hematinized contents of the intestine in several cases. No substance-related necropsy findings were noted in animals that survived.

The LD_{50} for Wistar rats was calculated at 3830 mg/kg bw for females and 4420 mg/kg bw for males (combined 3940 mg/kg bw).

Mouse:

At the dose level of 5000 mg/kg bw, mortality was restricted to one male mouse, which died within one day after test substance administration. Clinical symptoms recorded were dyspnea, apathia, abnormal position, staggering, paresis, absent pain reflex, narcotic-like state, twitching, piloerection, imbalance and poor general state. The onset of symptoms was

observed between 30 minutes and 1 day after dosing. These symptoms were observed in animals that survived up to one day of the study, and were dose dependent starting at 1470 mg/kg body weight. At study termination the surviving animals were free of symptoms. Macroscopic examination in the mouse that died revealed a yellow-brown discoloration of the liver. No substance-related findings were noted in animals that survived.

The oral LD_{50} in NMRI mice was calculated > 5000 mg/kg body weight.

4.2.1.2 Acute toxicity: inhalation

There were no mortalities in the study. During exposure to cycloxydim, weak attempts to escape were noted as well as eyelid closure, and shallow, jerky respiration. After exposure slight piloerection and jerky respiration were noted. No abnormalities apart from slight alopecia on the head were noted on day 6 after exposure.

The body weight gain in males and females revealed no substance related effects. No macroscopic findings were noted at study termination (day 14).

Cycloxydim has low acute inhalation toxicity as liquid aerosol. The 4-hour LC_{50} by head-nose exposure was calculated to be: > 5.28 mg/L (male and female Wistar rats).

4.2.1.3 Acute toxicity: dermal

Body weight gain increased consistently up to nine days of observation, no later time point were measured. No deaths occurred. Clinical signs, local findings as well as macroscopic examination revealed no abnormalities at a concentration of 2000 mg/kg bw.

Cycloxydim is of low acute toxicity in rats after dermal administration. The LD_{50} is higher than 2000 mg/kg bw in Wistar rats.

4.2.1.4 Acute toxicity: other routes

No information on other routes.

4.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.2.3 Summary and discussion of acute toxicity

Cycloxydim has low oral acute toxicity in rats and mouse, and low dermal and inhalative toxicity in rats (rat oral LD_{50} = 3940 mg/kg bw, mouse oral LD_{50} > 5000 mg/kg bw, dermal LD_{50} > 2000 mg/kg bw, LC_{50} > 5.28 mg/L air).

4.2.4 Comparison with criteria

All estimated LD_{50} values are above the guidance values in the criteria (both DSD and CLP).

4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for cycloxydim regarding acute toxicity.

4.2.6 Comments received during public consultation

No specific comments were received on this endpoint.

4.2.7 The RAC assessment and comparison with criteria

Acute toxicity

Cycloxydim has a low acute toxicity following application via oral, inhalation and dermal route. For the oral route, the LD_{50} in Wistar rats (3830 mg/kg for females and 4420 mg/kg for males, combined 3940 mg/kg bw) and in NMRI mice (>5000 mg/kg bw) was above the guidance value of 2000 mg/kg bw (CLP and DSD).

Dermal application to Wistar rats revealed an LD_{50}>2000 mg/kg which is above the guidance value for classification for CLP and DSD.

The 4-hour LD_{50} by head-nose inhalation exposure for cycloxydim as a liquid aerosol was >5.28 mg/l which was above guidance concentration of >5 mg/l and no classification is adequate.

The Dossier submitter proposed no classification for acute toxicity for the oral, inhalation and dermal route. The RAC agreed that no classification on acute toxicity is justified.

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

No specific, non lethal, target organ toxicity after single exposure was observed in acute toxicity studies. The observed effects in acute toxicity studies covered mostly clinical signs like dyspnoe, apathia, abnormal position, staggering, paresis, twitching and piloerection. No acute neurotoxicity studies were provided. In addition, no human data are available that would support classification for this endpoint.

No classification as STOT-SE under the CLP Regulation is proposed.

4.3.1 Dossier submitter's summary and discussion of Specific target organ toxicity – single exposure

No specific target organ toxicity after single exposure was observed in acute toxicity studies. No acute neurotoxicity studies are provided.

4.3.2 Dossier submitter's comparison with criteria

No effects observed in acute toxicity studies would trigger criteria for classification and labelling STOT SE.

4.3.3 Dossier submitter's conclusions on classification and labelling

No classification and labelling is proposed forc regarding specific target organ toxicity after single exposure.

4.3.4 Comments received during public consultation

No specific comments were received on this these hazard classes.

4.3.5 The RAC assessment and comparison with criteria

Specific target organ toxicity – single exposure (STOT SE)

As no specific target organ toxicity was observed after single exposure and no evidence from other studies was available, the RAC agreed that no classification for STOT-SE was appropriate.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation study (OECD 404)	Slightly irritating	White Vienna rabbits, Purity 92.2%	2. Kieczka H., Kirsch P. 1984(c)

4.4.1.1 Non-human information

After 4 hours there were slight erythemas observed in 2 of the 6 animals, which were subsided within 48 h (see Table below*.)* At 24 h only two animals showed slight skin redness. The overall mean value for erythema and edema for all animals was 0.1 and 0.0, respectively. No other findings were noted. Thus the study was terminated 72 hours after test substance administration..

Table 13: Skin irritation results of Cycloxydim in White Vienna rabbits

Animal	1	2	3	4	5	6
	R/ED*	R/ED	R/ED	R/ED	R/ED	R/ED
24 h	0/0	1/0	1/0	0/0	0/0	0/0
48 h	0/0	0/0	0/0	0/0	0/0	0/0
72 h	0/0	0/0	0/0	0/0	0/0	0/0
Mean value	0/0	0.3/0	0.3/0	0/0	0/0	0/0

*R/ED = redness/edema

In a skin irritation study cycloxydim showed only minimal irritating properties with an overall mean score of 0.1 for erythema (barely perceptible), considered as very slightly irritating to the rabbit skin**.** According to classification criteria, classification and labelling is not warranted.

4.4.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.1.3 Dossier submitter's summary and discussion of skin irritation

According to the results of the rabbit skin irritation study, cycloxydim is not irritant to the intact shaved rabbit skin.

4.4.1.4 Dossier submitter's comparison with criteria

Estimated skin irritation scores (0.1 for erythema) are below the criteria for classification and labelling (according to both DSD and CLP).

4.4.1.5 Dossier submitter's conclusions on classification and labelling

No classification and labelling is proposed for cycloxydim regarding skin irritation.

4.4.1.6 Comments received during public consultation

No specific comments were received on this endpoint.

4.4.1.7 The RAC assessment and comparison with criteria

Cycloxydim showed only minimal irritating properties. The overall mean score of 0.1 for erythema is well below the guidance values (≥2.3 for CLP, ≥2 for DSD) for classification.

The RAC concluded that no classification as a skin irritant was appropriate.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation study (OECD 405)	Slightly irritating	White Vienna rabbits, Purity 92.2%	Kieczka H., Kirsch P. 1984(d)

4.4.2.1 Non-human information

Eye effects observed in the rabbits were confined to conjunctival redness and discharge. No reactions could be found on the cornea and iris in any rabbit, furthermore, no conjunctival swelling was noted throughout the study (grade 0 at all time-points). Conjunctival redness (grade 1 in one rabbit and grade 2 in 5 rabbits) and discharge (grade 2) was noted 1 hour after application. Ocular findings at the 24 h reading were confined to slight conjunctival redness (grade 1 in five rabbits and grade 2 in one rabbit). Recovery continued to progress and by day 8 of the study (study termination); no findings were noted in any of the animals tested. The mean (24h–48h–72h) score for conjunctival redness was 0.8 (Table below).

Table 15: Eye irritation study in White Vienna rabbits: Scoring for conjunctival redness

	Time point of scoring					Score	Overall mean score
Rabbit	1 h	24 h	48 h	72 h	8 d	24–48–72 h	
1	2**	1	0	0	0	0.3	0.8

	Time point of scoring					Score	Overall mean score
Rabbit	**1 h**	**24 h**	**48 h**	**72 h**	**8 d**	**24–48–72 h**	
2	2**	1	1	1	0	1.0	
3	2**	1	0	0	0	0.3	
4	1**	2	1	1	0	1.3	
5	2**	1	1	0	0	0.6	
6	2**	1	1	1	0	1.0	

** = Discharge (Grade 2 = with moistening of the lids and hairs just adjacent to lids)

According to the results of the study with the 24h-48h-72h score values (none of 6 rabbits had conjuctival erythema ≥ 2), cycloxydim is considered as very slightly irritant to the rabbit eye. Therefore, classification and labelling is not warranted.

4.4.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.2.3 Dossier submitter's summary and discussion of eye irritation

According to the results of the eye irritation study, cycloxydim is slight irritant to the rabbit eye; according to classification criteria, classification and labelling is not warranted.

4.4.2.4 Dossier submitter's comparison with criteria

The estimated eye irritation scores (24 – 72 hours) are below the guidance values of the criteria for classification (according to both DSD and CLP).

4.4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for cycloxydim regarding eye irritation.

4.4.2.6 Comments received during public consultation

No specific comments were received on this endpoint.

4.4.2.7 The RAC assessment and comparison with criteria

Indications of slight eye irritation were observed in rabbits. Individual mean scores for conjunctival redness at 24, 48 and 72 hours after instillation were 0.3 (n=2), 0.6, 1.0 (n=2) or 1.3. The condition that 2 of 3 tested animals should show conjunctival redness ≥2 (CLP) or ≥2.5 (DSD) was never fulfilled at any time point and redness resolved from 48 h until (latest) 8 days after treatment.

The RAC agreed with the proposal of the Dossier submitter that classification for eye irritation is not warranted.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

There is no specific information regarding the ability of cycloxydim to cause irritation to the respiratory tract during the acute inhalation toxicity study.

4.4.3.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.4.3.3 Dossier submitter's conclusions on classification and labelling

No classification is proposed for respiratory tract irritation.

4.4.3.4 Comments received during public consultation

No specific comments were received on this endpoint.

4.4.3.5 The RAC assessment and comparison with criteria

There is no human information and no information from animal studies indicating irritating effects on the respiratory tract.

The RAC followed Dossier submitter's proposal and agreed with no classification for this endpoint.

4.5 Corrosivity

Based on the data from the skin and eye irritation studies it can be concluded that cycloxydim is not corrosive.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Skin sensitization, Maximisation test (OECD 406)	Not sensitising	Guinea pig, Purity: 94.8%	3. Kieczka H., Kirsch P. 1985(a)

4.6.1.1 Non-human information

The intradermal induction caused distinct erythema and edema formation. The injections of Freud's adjuvant/aqua dest. (1:1) caused distinctive erythema and edema in the control animals, whereas the injected olive oil only caused distinct erythema. After dermal induction distinct erythema and incrustation were observed in addition to distinct edema in the test group. After challenge with a 75% test substance formulation as well as an olive oil solution,

no skin changes could be observed either in the control groups or in the test group. The challenge reactions can be derived from table below.

Table 17: Results – Guinea Pig Maximization Test (GPMT) with Cycloxydim

Challenge	1st challenge		2nd challenge	
Test substance concentration	75% in olive oil	Olive oil	75% in olive oil	Olive oil
Control group 1	0/10	0/10	0/10	0/10
Control group 2	no application of test substance	0/10	0/10	
Test group	0/20	0/20	0/20	0/20

X/Y: number of positive reactions/number of animals tested (reading 48 h after beginning of test substance application

Cycloxydim is negative in the Guinea Pig Maximization Test under the test conditions chosen, and thus considered as not sensitizing to the skin.

4.6.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.6.1.3 Dossier submitter's summary and discussion of skin sensitisation

According to the results of the skin sensitisation study in guinea pig (Maximisation test), cycloxydim is not sensitising to guinea pig skin. According to classification criteria, classification and labelling is not warranted.

4.6.1.4 Dossier submitter's comparison with criteria

Effects observed in the skin sensitisation study on guinea pig are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.6.1.5 Dossier submitter's conclusions on classification and labelling

No classification and labelling is proposed for cycloxydim regarding skin sensitisation.

4.6.1.6 Comments received during public consultation

No specific comments were received on this endpoint.

4.6.1.7 The RAC assessment and comparison with criteria and justification

No positive responders were observed in a Guinea Pig Maximization Test with cycloxydim. The RAC agreed on the dossier submitter's proposal that no classification is proposed.

4.6.2 Respiratory sensitisation

No data on respiratory sensitisation available.

4.6.3 Comments received during public consultation

No specific comments were received on this endpoint.

4.6.4 The RAC assessment and comparison with criteria

The RAC considered that a conclusion on the classification is not possible due to the lack of data.

4.7 Repeated dose toxicity

Table 18: Summary table of relevant repeated dose toxicity studies

Method	NOAEL	Remarks	Reference
28 days oral drinking water study in rats (OECD 407), range finding study	1000 ppm (106 mg/kg bw/d in females) and 3000 ppm (272 mg/kg bw/d in males) <u>Main effects at 9000 ppm in males and females:</u>: - food consumption, bw ↓ - urea ↑ - triglycerides↓ - liver (rel.,abs.) ↑ - kidney (rel., abs.) ↑ <u>Main effects at 3000 ppm Females</u>: - food, consumption↓, bw ↓ - rel. liver weight ↑ Males - kidney (rel.) ↑ Main effects at 300 ppm and above: <u>Males and females: dose-related reduction in water consumption</u>	Wistar rat, Purity: 92.8% Dose range: 0, 300, 1000, 3000, 9000 ppm (drinking water) equivalent to: 0, 32, 102, 272, 683 mg/kg bw/d (males) 0, 35, 106, 252, 678 mg/kg bw/d (females)	Kuehborth B. et al. 1986(c)
90 days oral drinking water study in rats with 6 months observation period (OECD 408, EPA 82-1), range finding study	300 ppm (22 mg/kg bw/d in males and 28 mg/kg bw/d in females) <u>Main effects at 2700 ppm</u>: <u>Males and females:</u> <u>Water consumption ↓</u> <u>Food consumption ↓</u> bw↓ <u>Main effects at 900 ppm</u>: <u>Males</u>: - ALT ↑ - ALP ↓ <u>Females</u>: <u>Water consumption ↓</u> - ALT ↑ - ALP ↓ - creatinine ↑ - urea ↑	Wistar rat, Purity: 94.8% Dose range: 0, 30, 100, 300, 900, 2700 ppm (drinking water) equivalent to 0, 2.2, 7.3, 22, 72, 178 mg/kg bw/d (males) 0, 3.2, 10, 28, 74, 201 mg/kg bw/d (females)	Kuehborth B. et al. 1985

	Complete recovery (900 and 27000 ppm) at the end of 6- month observation period		
28 days oral drinking water study in mice (partially OECD 407), range finding study	1000 ppm (189 mg/kg bw/d in males and 218 mg/kg bw/d in females) Main effects at 9000 ppm: Males and females: food consumption↓ urea ↑ Males (2/10):liver hydropic vacuolar parenchymal degeneration in Main effects at 3000 ppm: Males: - cholesterol ↓ - rel. liver weight (115%) ↑ Females: - cholesterol ↓ Main effects at 300 ppm and above: Males and females: Water consumption ↓	B6C3F1 mice, Purity: 94.8% Dose range: 0, 300, 1000, 3000, 9000 ppm (drinking water) equivalent to 0, 59, 189, 462, 1008 mg/kg bw/d (males) 0, 63, 218, 591, 1177 mg/kg bw/d (females)	Kuehborth B. et al. 1986(a)
28 days oral drinking water study in mice (partially OECD 407), 2nd range finding study; only supplemental information	100 ppm (22.5 mg/kg bw/d for males) and > 900 ppm (82.5 mg/kg bw/d) for females Main effects at 900 ppm: Males: Water consumption ↓ Effects from limited data set at 300 ppm in males : - LDH ↓ Main effects at 300 ppm and above: Females: Water consumption ↓	B6C3F1 mice, Purity: 94.8% Dose range: 0, 30, 100, 300, 900 ppm (drinking water) equivalent to 0, 7.3, 22.5, 58.3, 204 mg/kg bw/d (males) 0, 8.8, 28.3, 82.5, 242 mg/kg bw/d (females) no histopathology	Kuehborth B. et al. 1986(b)
28 days oral feeding study in dogs (range finding study)	40 mg/kg bw/d in males and females Main effects at 360	Beagle dogs, Purity: 94.8%	4. Hellwig J. et al. 1985(a)

	mg/kg bw/d: Hepatocellular hypertrophy Males: RBC ↓ -23.6% Hb ↓ -20.1% MCV ↑ - Heinz bodies ↑ Females: - Heinz bodies ↑ (no data on RBC, MCV and Hb) ALP ↑ Main effects at ≥120 mg/kg bw/d: Males: - liver weight ↑ - ALP ↑ Females: - liver weight ↑ - thyroid weight ↑	Dose range: 0, 300, 1200, 3600, 10800 ppm (diet) equivalent to 0, 9.9, 39.8, 118, 356 mg/kg bw/d (males) 0, 9.6, 39.4, 119, 338 mg/kg bw/d (females)	
90 days oral feeding study in dogs (OECD 409, EPA 82-1), range finding study	300 ppm (10 mg/kg bw/d) for males and 1500 ppm (50 mg/kg bw/d) for females Main effects 1500 ppm (males): - liver weight ↑ (> 110%) - ALP ↑ Females: Main effects 7500 ppm (Males and females): - liver weight ↑ - thyroid weight ↑ (only males) - ALP ↑ - enlarged hepatocytes - RBC ↓, MCV ↑ (no data for females) - Heinz bodies ↑ - Platelets ↑ PMN granulocytes ↑ (no data for females) Hepatocellular	Beagle dogs, Purity: 94.8% Dose range: 0, 60, 300, 1500, 7500 ppm (diet) equivalent to 0, 2, 10, 50, 250 mg/kg bw/d (males and females)	Hellwig et al., 1985(b)

	hypertrophy (3/4 males and 4/4 females)		
1 year oral feeding study in dogs (OECD 452, EPA 83-1)	400 ppm (12 mg/kg bw/d) for males and females Main effects at 1600 ppm: Males and females: - liver weight ↑ - ALP ↑, - Heinz bodies ↑ - Platelets ↑ Main effects at 6400 ppm: - RBC ↓, - Hb↓ (females only) MCV ↑, MCH ↑ - Heinz bodies ↑ - Platelets ↑ ALP ↑, Liver: Haemosiderosis (3/6 females)	Beagle dogs, Purity: 93.9% 0, 400, 1600, 6400 ppm (diet) equivalent to 0, 12, 49, 206 mg/kg bw/d (males and females)	Hellwig J., Hildebrand B. 1988
28 days dermal study in rats (EEC 92/69, OECD 410, EPA 870.3200)	1000 mg/kg bw/d for female and 300 mg/kg bw/d for male Males: - body weight ↓ Females: - no substance related adverse effects	Wistar rat, Purity: 92.9% Dose range: 0, 60, 300 and 1000 mg/kg bw/d	Mellert W. et al. 2001(a)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Mouse:

28 days drinking water study in mice (1st range finding study), *Kuehborth B. et al. 1986(a)*

In this 4-week range-finding study designed to determine appropriate dose levels for a long-term carcinogenicity study, ten B6C3F1 mice (source: Charles River Breeding Lab., weighing 19.5 g and 18.0 g males and females, respectively) per sex and dose were administered Cycloxydim (batch: N 64; purity: 94.8%) as sodium salt via the drinking water at concentrations of 0, 300, 1000, 3000 and 9000 ppm.
Feed consumption and body weight were determined once per week. Water consumption was determined twice per week. The health status of the animals was checked daily.

At the end of the test substance administration, blood samples were taken for clinicochemical examinations (total bilirubin, urea, sodium, potassium, chloride, triglycerides, cholesterol, alkaline phosphatase).
All animals were sacrificed after four weeks. The organ weights of liver, kidneys, heart, brain, spleen, thymus, testes and adrenals were determined; selected organs (e.g. liver, colon, lung, seminal vesicles, ileum, colon, cecum, pancreas, spleen) of selected animals were assessed by gross pathology. Subsequently, a histopathological examination of the liver, kidney, stomach, jejunum, lung and of all gross lesions was carried out.

The test substance intake over the entire study was calculated based on the weekly test substance intake data provided in the report.

Table 19: Test substance intake (calculated as free acid)

Dietary dose level (ppm)	Test substance intake (mg/kg bw/d)	
	males	females
300	59	63
1000	189	218
3000	462	591
9000	1008	1177

General observations: There were no test substance related mortalities or clinical signs of toxicity in any treatment group.
Body weight gain was reduced in the 9000 ppm group. A temporary reduction of body weight was observed in most of the animals of all test groups on day 21 only.

Table 20: Body weight gain at day 28

Dietary dose level (ppm)	Male body weight		Female body weight	
	(g)	(% control)	(g)	(% control)
0	23.00	100%	21.44	100%
300	21.90	95%	20.67	96%
1000	22.50	98%	21.40	100%
3000	22.30	97%	20.67	96%
9000	19.90	87%	19.10	89%

Food consumption of animals of the 9000 ppm groups was reduced, too.
Water consumption was reduced in males and females of the 9000; 3000 and 1000 ppm groups as well as in females of the 300 ppm groups. The extent of the reduction varied over time, but in general the water consumption reduction was approximately 50% for the 9000 ppm animals, varied between 5 – 35 % for the 3000 ppm animals, 3 – 10 % for the 1000 ppm animals and was reduced by 13 % in the 300 ppm females.
Due to the small amount of blood available, it was not possible to evaluate all clinicochemical parameters in all animals. Therefore, for many clinicochemical parameters, the number of available data points per sex and dose varied (occasionally as low as 2 out of 10); hence the results of the clinicochemical investigation have to be viewed with caution. Effects on bilirubin

concentration did not change drastically with increased dosing; suggesting an improved analytical approach, or less sensitivity of mice concerning this parameter (N ≥ 6 data-points).
Clinicochemical examinations revealed an increase of plasma urea values. In addition, a steadily decrease of cholesterol was found with the exception of female animals of the 1000 ppm group. Summary of clinicochemical parameters are presented in table below (no statistical analysis provided). Nevertheless, similar results were found in subchronic studies in the rat and are most likely substance induced.

Table 21: Clinicochemical parameters

Dose level (ppm) / Parameter	0	300	1000	3000	9000
Bilirubin (µmol/l)					
♂ (N* ≥ 6)	2.0 ± 0.4	1.6 ± 0.3	1.4 ± 0.8	1.5 ± 0.3	1.0 ± 0.4
♀ (N ≥ 6)	1.5 ± 0.4	2.7 ± 2.1	1.1 ± 0.3	1.9 ± 1.0	1.5 ± 0.6
Urea (mmol/l)					
♂ (N = 4 - 8)	5.40 ± 0.5	5.16 ± 0.36	6.27 ± 0.48	6.34 ± 0.33	9.55 ± 1.93
♀ (N = 3 - 7)	4.25 ± 0.11	10.12 ± 4.41	5.52 ± 0.60	5.98 ± 0.44	11.39 ± 2.73
Cholesterol (mmol/l)					
♂ (N = 2 - 5)	1.78 ± 0.12	1.63 ± 0.62	1.47 ± 0.32	0.85 ± 0.21	0.80 ± 0.12
♀ (N = 2 - 4)	1.97 ± 0.37	1.23 ± 0.44	1.68 ± 0.14	0.45 ± 0.08	0.53 ± 0.11

* N = number of blood samples evaluated for assessment of parameter

Organ weight determinations revealed a test substance related decrease of absolute weight of the kidneys, spleen, testes and brain in male mice and of the heart, kidney and liver weight in female mice of the group receiving 9000 ppm test substance. Both absolute and relative heart weights were decreased in 9000 ppm male mice only. Relative liver weights were increased in male mice of all dose groups. There was, however, no statistically significant increase in absolute liver weight in males up to a dose level of 3000 ppm. In females of the high dose group (9000 ppm), absolute and relative thymus weights were significantly increased. In both sexes the high dose group relative organ weight of brain was significantly increased, in males the absolute weight was also significantly higher than in controls. Relative liver weights from male rats were found to be statistically dose-dependent.
Two male mice in the highest dose group exhibited hydropic vacuolar parenchymal degeneration in the hepatocytes, which can be regarded as a manifestation of particularly distinct cell damage. Gross pathological examination and histopathological examination could not give any further details.

Table 22: Organ weights

Dose level (ppm) / Parameter	**0**		**300**		**1000**		**3000**		**9000**	
Heart wt – males										
Absolute (g)	0.110	100 %	0.107	97%	0.106	96%	0.106	96%	0.092**	84%

Dose level (ppm) / Parameter	**0**		**300**		**1000**		**3000**		**9000**	
relative (%)	0.562	100 %	0.561	100 %	0.560	100 %	0.551	98%	0.533*	95%
Liver wt – males										
- absolute (g)	0.768	100 %	0.796	104 %	0.810	105 %	0.862	112 %	0.780	102 %
- relative (%)	3.918	100 %	4.176*	107 %	4.264*	109 %	4.491**	115 %	4.512**	115 %
Thymus wt – females										
- absolute (g)	0.0523	100 %	0.0610	117 %	0.0549	105 %	0.0576	110 %	0.0637*	122 %
- relative (%)	0.2889	100 %	0.3506	121 %	0.3006	104 %	0.3352	116 %	0.394**	136 %
Brain wt – males										
- absolute (g)	0.439	100 %	0.431	98%	0.433	99%	0.429	98%	0.416**	95%
- relative (%)	2.243	100 %	2.266	101 %	2.282	102 %	2.243	100 %	2.425**	108 %
Brain wt – females										
- absolute (g)	0.450	100 %	0.456	101 %	0.453	101 %	0.449	98%	0.441	98%
- relative (%)	2.503	100 %	2.627	105 %	2.496	100 %	2.597	104 %	2.726**	109 %

Statistical evaluation: * $p < 0.05$, ** $p < 0.01$ (Williams t-Test)

The most striking effect of the test substance was a dose-dependent significant increase in the relative liver weight of the male animals from 300 ppm upward but the increase >110% compared to control was observed only for 3000 ppm group. However, only in two animals of the highest dose group (9000 ppm) the examination by light microscopy revealed morphological changes (hepatic hydropic vacuolar parenchymal degeneration) that elucidated this effect of the test substance. Based on changes in clinicochemical parameters in males and females (primarily decreased cholesterol) and increased relative liver weights in males > 110% at 3000 ppm, the NOAEL was set as 1000 ppm (189 mg/kg bw and 218 mg/kg bw for males and females, respectively).

28 days drinking water study in mice (2nd range finding study), *Kuehborth B. et al. 1986(b)*

In this second 4-week range-finding study, ten B6C3F1/CrIBR mice (source: Charles River Breeding Lab., Wilmington, Mass.; weighing 19.1 g and 15.7 g males and females, respectively) per sex and dose (0; 30; 100; 300 and 900 ppm) were administered Cycloxydim (batch: N 64; purity: 94.8%) as sodium salt via the drinking water.

Feed consumption and body weight were determined once a week. Water consumption was determined twice per week. The health status of the animals was checked each day.
At the end of the administration period, blood samples were taken for a limited clinicochemical examination (activity of lactate dehydrogenase, alanine amino-transferase, aspartate amino-transferase, and alkaline phosphatase).
All animals were sacrificed after four weeks. The liver weights were determined and assessed by gross pathology. As there were no histopathological changes in the previous mouse study at doses up to 1000 ppm, histopathological examinations were not carried out in this study.

The test substance intake over the entire study was calculated based on the weekly test substance intake data; see table below.

Table 23: Test substance intake (calculated as free acid)

Dietary dose level (ppm)	Test substance intake males (mg/kg bw/d)	Test substance intake females (mg/kg bw/d)
30	7.3	8.8
100	22.5	28.3
300	68.3	82.5
900	204	242

General observations: There were no test substance related mortalities or clinical signs of toxicity in any treatment group.
There were no effects on body weight or food consumption at any dose level.
Water consumption was reduced in males and females of the 900 ppm group as well as in females of the 300 ppm group. The extent of the reduction varied over time, but in general the water consumption reduction was approximately 4 – 9 % for the 900 ppm females and 4 – 7 % for the 300 ppm females. In the males receiving 900 ppm test substance, water consumption was reduced by approximately 8 %, during the first week of the study only. The test substance was administered via the drinking water; the reduction of water consumption is regarded as a palatability effect rather than a specific adverse effect.
Clinical chemistry: There was a decrease of lactate dehydrogenase activity in male mice as shown in table below (no statistical analysis provided).

Table 24: LDH activity (in µkat/l) of male mice on day 30 (end of study)

Dose (ppm)	0	30	100	300	900
LDH activity	51.2 ± 5.5	41.5 ± 4.8	43.7 ± 5.5	38.9± 3.5	34.0± 2.3

No statistical analysis provided

Except from liver enzymes, no further clinical chemistry or haematological data have been assessed.
Organ weight determinations revealed an increase of absolute and relative liver weights in male mice of the 900; 300 and 100 ppm groups (table below). Relative liver weights were increased in 900 ppm females. However, gross necropsy did not show test substance related changes.

Table 25: Absolute and relative liver weights

Dose level	0 ppm		30 ppm		100 ppm		300 ppm		900 ppm	
Liver wt – males										
- absolute (g)	0.821	100%	0.871	106%	0.910**	111%	0.887**	108%	0.923**	112 %
- relative (%)	4.019	100%	4.103	102%	4.162*	104%	4.182*	104%	4.362**	109 %
Liver wt – females										
- absolute (g)	0.818	100%	0.782	96%	0.773	94%	0.788	96%	0.845	103 %
- relative (%)	4.461	100%	4.356	98%	4.378	98%	4.438	99%	4.716**	106 %

Statistical evaluation: * $p < 0.05$, ** $p < 0.01$ (Williams t-Test)

The study provides limited number of data. Biochemical data supply is scarce, thus liver weight and liver enzyme activities are the only parameters that can be used for toxicological assessment. Originally, a NOAEL in B6C3F1/CrIBR mice was established at 100 ppm (22.5 mg/kg bw/d) for males and 300 ppm (82.5 mg/kg bw/d) for females, based on increased liver weights, and a dose-dependent decrease in LDH activity in male mice. After detailed re-evaluation of the results in course of providing the CLH report, the evaluator reconsidered the NOAEL to be 100 ppm (22.5 mg/kg bw/d) for males, based on reduced LDH level at 300 ppm and > 900 ppm for females based on lack of adverse effects. However, it is questionable if reduced LDH level is an adverse effect and the approach of setting NOAEL at 100 ppm is considered very conservative based on limited data set.

Rat:

28 days drinking water study in rats, *Kuehborth B. et al. 1986(c)*

In this range-finding study Cycloxydim sodium salt (batch: N 11; purity: 92.8%) was administered to groups of 5 male and 5 female Wistar rats (source: Dr. Karl Thomae GmbH, Biberach, Germany, 42 days of age and weighing 162 g and 150 g male and female, respectively) via their drinking water at doses of 0; 300; 1000; 3000 and 9000 ppm for 4 weeks.
Feed consumption and body weight were determined once a week. Drinking water consumption was measured twice per week. The health status of the animals was checked daily.
Blood samples for clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumine, globulins, lactate dehydrogenase, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, plasma cholinesterase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) were taken at 12 and 25 days after the beginning of administration. Urinalysis (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, sediment microscopy) was performed after 22 days of test substance administration.
At the end of the 4-week administration period, all animals were sacrificed after a 16-h fasting period by decapitation after they had been anaesthetized with CO_2. Organ weights (heart,

liver, kidneys, spleen, testes, ovaries, adrenals, brain and thymus) were determined and gross as well as histopathological examinations (heart, trachea, lung, esophagus, stomach, small intestine, liver, spleen, thymus, kidneys, testes, ovaries, adrenals, thyroid and brain) were carried out.

The overall mean test substance intake for the 4-week treatment period was calculated based on the weekly test substance intake data provided in the report cited.

Table 25: Test substance intake (calculated as Cycloxydim free acid)

Concentration in drinking water (ppm)	Test substance intake males (mg/kg bw/d)	Test substance intake females (mg/kg bw/d)
300	32	35
1000	102	106
3000	272	252
9000	683	678

General observations: One female of the 9000 ppm group died on day 12 of the study. There were no further mortalities in any of the treatment groups. The general health condition of all males and females of the 9000 ppm group appeared to be temporarily impaired; in 2 high-dose group males a slight red incrustation of the nose was noted, a further male showed slight redness of the nose. Ruffled fur was observed in one female on day 8 that died four days later (see above). None of the clinical symptoms mentioned were observed at the end of the study period.
Body weight gain was reduced in males and females of the 9000 ppm group as well as in 3000 ppm group females.

Table 26: Body weight at day 28 and body weight change

	Males				Females			
Dietary dose level (ppm)	Bw (day 28)		Bw change (d1 – 28)		Bw (day 28)		Bw change (d1 – 28)	
	(g)	(% ctrl)	(g)	(% ctrl)	(g)	(% ctrl)	(g)	(% ctrl)
0	302.40	100%	136.60	100%	217.60	100%	66.40	100%
300	310.00	103%	146.60	107%	215.60	99%	65.80	99%
1000	296.80	98%	137.20	100%	216.20	99%	64.20	97%
3000	294.20	97%	130.20	95%	198.20	91%	50.40	76%
9000	216.60	72%	56.60**	41%	173.75	80%	23.50**	35%

Statistical significance * $p < 0.05$, ** $p < 0.01$ (Williams t-Test)

Food consumption of animals of the 9000 ppm group, as well as females of the 3000 ppm group was reduced throughout the entire study period. Males of the 3000 ppm group, showed a temporary reduction of food consumption during the first week.

Table 27: Mean food intake during 4-wk treatment

	♂				♀			
Dietary dose level (ppm)	Day 1 – 28		Day 1 – 7		Day 1–28		Day 1 – 7	
	(g/day)	(% ctrl)	(g/day)	(% ctrl)	(g/day)	(% ctrl)	(g/day)	(% ctrl)
0	26.07	100%	25.49	100%	20.22	100%	19.57	100%
300	26.75	103%	25.57	100%	19.65	97%	19.80	101%
1000	25.53	98%	24.20	95%	19.57	97%	19.57	100%
3000	25.44	98%	23.40	92%	17.50	87%	16.66	85%
9000	17.91	69%	13.51	53%	14.80	73%	9.83	50%

Water consumption was reduced in males and females of all dose groups. While the extent of this reduction varied during the 4-week administration period, a dose-related effect on water consumption was obvious.

Table 28: Water consumption reduction (% of controls [100 %])

Dietary dose level	Males	Females
300 ppm	4 – 12%	8 – 17%
1000 ppm	12 – 19%	11 – 24%
3000 ppm	20 – 28%	33 – 46%
9000 ppm	42 – 69%	42 – 78%

Clinicochemical examinations performed at the end of the study revealed an increase of urea values and slightly increased chloride and sodium levels at 9000 ppm in both sexes. At 3000 ppm, an increase of urea concentration was observed in females after 12 days, but not after 25 days of the study. Increased plasma-urea level, in the groups receiving either 9000 ppm or 3000 ppm, was suggested to result from renal insufficiency and increased blood cholesterol indicated hepatic dysfunction. In addition, triglyceride levels were reduced at 9000 ppm in both sexes, which was attributed to the decreased alimentary uptake.

Table 29: Clinicochemical parameters at day 25

Dose level (ppm) / Parameter	0	300	1000	3000	9000
Urea (mmol/l) ♂ ♀	 6.98 6.97	 7.06 7.25	 6.90 7.61	 7.24 7.76	 8.19** 9.23**
Sodium (mmol/l) ♂ ♀	 141.91 141.25	 141.24 139.97	 139.08* 141.29	 140.71 143.81	 143.93 145.21*
Chloride (mmol/l) ♂ ♀	 98.10 99.93	 98.15 98.60	 96.28* 98.87	 98.88 100.54	 100.55 102.11

Dose level (ppm) / Parameter	0	300	1000	3000	9000
Triglycerides (mmol/l) ♂ ♀	2.16 1.45	2.29 1.35	1.83 1.63	2.50 1.17	1.13* 1.00
Cholesterol (mmol/l) ♂	1.55	1.77	1.72	1.90	1.68
Alkaline phosphatase (µcat/l) ♂ ♀	10.69 7.56	11.71 7.32	9.93 7.26	10.99 7.68	9.74 5.34**

Statistical significance * $p < 0.05$, ** $p < 0.01$ (Williams t-Test)

It has to be mentioned that by using the diazo method for quantification, bilirubin was found to be much higher in treated rats compared to controls. It was argued that this enhancement was due to an inappropriate method, metabolites may form azo pigments from the diazonium salt. However, re-assessment of rat serum (treated, untreated) and dog serum with the HPLC method did not show an increased bilirubin content (details of the method for these re-assessments are given in the studies: "Separation of bilirubin species in serum of dogs and rats" [*Vogl W.*, 1985; Project No. 040048] and "Determination of bilirubin in the serum of rats and dogs after the administration of Reg. No. 172 999 [*Schäfer-Lüderssen U.*, no date stated in the report; Report No. 19/7-db]).

Organ weight determinations revealed a test substance related decrease of absolute weight of the heart, liver, kidneys, spleen, adrenals and brain in male rats of the 9000 ppm group and of heart and adrenal weight in females of the same dose-level. Because there was no similar decrease in relative weight, these reductions were considered to be related to the reduction in body weight at this dose level. Similarly, statistically significantly increased relative liver and kidney weights were found in males of the 9000 ppm and in females of the 3000 ppm group, respectively.

Table 30: Organ weights absolute (in g) and relative (% compared to control)

Dose level	0 ppm		300 ppm		1000 ppm		3000 ppm		9000 ppm	
Terminal bw	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**
♂	271.66	100	281.16	103	267.7	99	267.46	98	200.72**	74
♀	196.52	100	193.8	99	194.02	99	180.76	92	162.68**	83
Abs. liver wt	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**
♂	9.326	100	9.848	106	9.296	100	9.568	103	7.854*	84
♀	6.73	100	7.042	105	7.12	106	6.784	101	7.085	105

Dose level	0 ppm		300 ppm		1000 ppm		3000 ppm		9000 ppm	
Rel. liver wt	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**
♂	3.427	100	3.501	102	3.471	101	3.574	104	3.911**	114
♀	3.419	100	3.631	106	3.667	107	3.766*	110	4.347**	127
Abs. kidney wt	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**	**[g]**	**(%ctrl)**
♂	2.162	100	2.312	107	2.054	95	2.310	107	1.820**	84
♀	1.554	100	1.578	102	1.67	107	1.596	103	1.623	104
Rel. kidney wt	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**	**[%]**	**(%ctrl)**
♂	0.795	100	0.823	104	0.768	97	0.865*	109	0.907**	114
♀	0.793	100	0.815	103	0.862	109	0.892	112	1.003**	126

Statistical significance: * $p < 0.05$; ** $p < 0.01$ (Williams t-test)

Gross necropsy and histopathological investigations did not show any test substance related changes at any dose level.

The group receiving the highest concentration (9000 ppm) showed reduced body weight gain, food intake and water consumption. Also clinico-chemical parameters were changed e.g. increased urea and sodium values, increased cholesterol and chloride blood concentration in males and decreased blood triglyceride values and the alkaline phosphatase activity in both sexes. Pathology revealed increased organ weight of liver and kidneys. Reduced body weight gain and food consumption were observed also in the 3000 ppm group. 1000 ppm and 300 ppm administration of Cycloxydim resulted only in reduced water consumption. The NOAEL was assessed to be 1000 ppm (accounting for 106 mg/kg bw/d) in females, based on increased rel. liver weight (110%) and 3000 ppm in the male rats (accounting for 272 mg/kg bw/d) based on reductions in body weight gain and increased relative liver and kidney weight, increased urea and decreased trigycerides level.

90 days drinking water study in rats with 6 months observation, *Kuehborth B. et al. 1985*

Cycloxydim (batch: N 64; purity: 94.8%) was administered as sodium salt to groups of 10 male and 10 female 42 days old Wistar rats Chbb (source: Dr. Karl Thomae GmbH, Germany, weighing 192.9 g and 134.7 g, males and females respectively) for 3 months via their drinking water at dose levels of 0; 30; 100; 300; 900 and 2700 ppm Cycloxydim. In order to study the possible reversibility of effects, recovery groups each consisting of 10 males and 10 females were administered 0; 900 and 2700 ppm, respectively, for 3 months and subsequently maintained on test substance-free drinking water for an additional 6 weeks.
The animals were observed daily for clinical symptoms and mortalities. A thorough examination including palpation was performed once per week. Body weight and food intake was measured weekly. Drinking water consumption was determined twice per week.

Ophthalmoscopy was performed before the start and at the end of the treatment period and at the end of the recovery period on all animals of the control and high dose groups. Clinicochemical and haematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumine, globulins, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase,blood clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) were performed after approximately 1, 2 and 3 months of test substance administration as well as towards the end of the recovery period. Urinalyses (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, sediment microscopy) were performed after approximately 3 months of test substance administration, and toward the end of the recovery period.
Animals were sacrificed on completion of the 3-month treatment period and subjected to a full macroscopic pathological examination. Weights of selected organs (liver, kidneys, testes, adrenals) were determined and histopathological investigations (brain, pituitary, thyroid, thymus, lungs, trachea, heart, sternum salivary glands, liver, spleen, kidneys, andrenals, pancreas, testes/ovaries, uterus, cervix, aorta, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, mesenteric lymph nodes, sciatic nerve) were performed. Animals of the recovery groups were also subjected to organ weight determinations as well as to gross and histopathological examinations.

Stability and homogeneity of the test substance were verified analytically. The correctness of the concentrations in the drinking water and the stability in the water were analytically confirmed.
Test substance intake is shown in table below.

Table 31: Test substance intake (free acid)

Drinking water dose level (ppm)	Test substance intake [mg/kg bw/d]	
	Males	Females
30	2.2	3.2
100	7.3	10
300	22	28
900	72	74
2700	178	201

General observations: No mortality or clinical signs of toxicity occurred in the study at any dose level.
There was a reduction of drinking water consumption, especially during the earlier phases of treatment, in the high-dose group males (-32 %) and females (-35 %) as well as in the females (-17 %) of the 900 ppm group. However, toward the end of the administration period, drinking water consumption in nearly all animals was comparable to controls. There were no effects on drinking water consumption during the recovery period.
A reduction of food consumption was observed in males of the high-dose group (maximum -11 %) throughout the administration period and in females of the high-dose group (maximum -7 %) during the initial phases of treatment. Males completely recovered during the treatment-free period.
Body weights were reduced in males of the high-dose group throughout the administration period. In females receiving 2700 ppm test-substance there was a reduction in body weight development during the first week of treatment. Thereafter, body weight development in all treated females was comparable to controls. There were no test substance related differences in body weight between test groups in either sex at the end of the recovery period.

Table 32: Body weights at the end of the administration and recovery periods

Dose level (ppm)	0	30	100	300	900	2700
Body weight at end of administration period						
♂	448.3	450.2	462.1	468.0	439.4	407.0** (90.8%)
♀	258.7	247.2	261.8	250.0	263.0	254.5
Body weight at end of recovery period						
♂	487.1	-	-	-	475.8	475.3
♀	273.5	-	-	-	275.1	271.2

Statistical evaluation: ** $p < 0.01$ (Dunnet's Test)

There were no test substance-related ophthalmological findings in the groups.
The clinicochemical investigations revealed changes which were considered to be test substance-related by the authors of the report:
• Increased alanine aminotransferase activity in 2700 and 900 ppm males and females (after 1 month of treatment).
• Decreased alkaline phosphatase activity in 2700 ppm males and females as well as in 900 ppm males (after 1 month of treatment, with exception of 2700 ppm females, which also showed a reduced activity after 3 months).
• Increased creatinine in 2700 and 900 ppm females.
• Increased urea and cholesterol in 2700 ppm females (after 1 month of treatment).
There were no clinicochemical changes in the recovery groups.
Details of clinicochemical parameters after one month of treatment can be found in tables below
.

Table 33: Clinicochemical changes (after 1 month of treatment)

Dose level (ppm) Parameter	0	30	100	300	900	2700
Alanine aminotransferase (µcat/l)						
♂	0.89	0.94	0.93	0.92	0.97**	0.98**
♀	0.92	0.92	0.99	0.98	1.02*	1.10**
Alkaline phosphatase (µcat/l)						
♂	8.71	8.80	8.71	8.34	7.82*	7.83*
♀	6.80	6.46	6.48	6.32	6.41	6.11
Creatinine (µmol/l)						
♀	50.32	50.93	50.31	51.20	53.77**	54.50**
Urea (mmol/l)						
♀	7.61	7.98	7.40	7.53	8.05	8.38*

Dose level (ppm) Parameter	0	30	100	300	900	2700
Cholesterol (mmol/l)						
♀	1.44	1.37	1.43	1.50	1.60	1.55

* p < 0.05, ** p < 0.01 (Williams t-Test)

Table 34: Clinicochemical changes (after three months of treatment)

Dose level (ppm) Parameter	0	30	100	300	900	2700
Alanine aminotransferase (µcat/l)						
♂	1.12	1.06	1.03	1.12	1.00	1.10
♀	1.10	1.36	1.05	1.27	1.07	1.22*
Alkaline phosphatase (µcat/l)						
♂	5.25	5.50	5.53	6.18	4.94	5.25
♀	4.39	4.05	4.36	4.29	4.12	3.75**
Creatinine (µmol/l)						
♀	57.83	55.12	55.63	57.85	59.07	59.38
Urea (mmol/l)						
♀	7.56	8.11	8.13	8.06	7.76	7.91
Cholesterol (mmol/l)						
♀	1.61	1.55	1.62	1.65	1.66	1.87*

Statistical evaluation: * p < 0.05, ** p < 0.01 (Williams t-Test)

Total bilirubin plasma concentrations were increased in males after one and two months of treatment at the highest concentration. In contrast, female rats showed enhanced bilirubin concentrations at all time points tested in concentrations of 2700 ppm and 900 ppm and after the second and third month in a concentration of 30 ppm. During the recovery phase, when no test substance was administered these changes subsided again. In this context female rats revealed decreased hemoglobin concentrations after 2 months of treatment until a concentration of 100 ppm (not dose dependent). The test method (diazo salt) was suggested to be responsible for the increased values in the animals receiving Cycloxydim. Subsequent co-investigation, comparing this method with HPLC revealed no correlation between these two methods, suggesting false positive values. See also studies above.
Organ weight determinations, as well as gross and histopathological examinations did not reveal any test substance related changes at any dose level.

The high dose level of 2700 ppm resulted in a clear reduction of food consumption and body weight development. At this dose level, and also in 900 ppm females, there was also a reduction of water consumption. As the test substance was administered via the drinking water the reduction of water consumption is to be regarded as a palatability effect rather than a specific adverse effect. There were also significant changes in clinical chemical parameters at the two higher dose levels. Both sexes recovered completely with respect to reduced body

weight gain and alterations of clinicochemical changes. Reduction of food consumption abrogated during the recovery phase in male animals. Based on increases in creatinine and urea in females, and alanine aminotransferase activity in both sexes at 900 ppm, the NOAEL was assessed to be 300 ppm (22 mg/kg bw/d in males and 28 mg/kg bw/d in females).

Dog:

28 days feeding study in dogs (range finding study) *Hellwig J. et al. 1985(a)*

Cycloxydim (batch: N 64, Tox Batch II; purity: 94.8%) sodium salt was administered via the diet for 4 weeks to 2 male and 2 female Beagle dogs (BASF own breed; aged 6-8 months and weighing 8.9 kg and 8.2 kg males and females, respectively) per dose level at concentrations of 0; 300; 1200; 3600 or 10800 ppm to achieve a daily test substance intake of 0; 10; 40; 120 and 360 mg/kg bw/d (according to the free acid), respectively. The dietary preparation was made by mixing 350 g of dry dog food with 350 mL water containing the appropriate concentration of Cycloxydim sodium salt. The animals were offered the feed test substance preparation daily for 1 hour in the morning. Then the feed was removed from the kennel. For estimation of actual feed consumption, any feed left over was weighed and subtracted from the amount offered.
Feed consumption and health status were determined daily and body weights once a week. Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumine, globulins, lactate dehydrogenase, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, plasma cholinesterase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) were performed 5 days before the start of the administration period and on study days 9 and 24. Urinalyses (volume, pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, specific gravity, sediment microscopy) were performed 6 days before the start of the administration period and on study days 15 and 22.
At the end of the 4-week administration period all animals were sacrificed. Organ weights (heart, liver, kidneys, spleen, testes, ovaries, thyroids, adrenals, brain) were determined and gross as well as histopathological examinations (heart, stomach, duodenum, jejunum, liver, kidneys, spleen, testes, ovaries, thyroids, adrenals, brain, sternum) were carried out.

Dietary preparations were offered to the dogs immediately after preparation and were made available to the dogs only for one hour, therefore limited stability is considered not to have affected the intake of unchanged test substance.
The overall mean test substance intake for the 4-week treatment period was calculated based on the weekly test substance intake data provided in the report; see table below.

Table 35: Test substance intake (as free acid, in mg/kg bw/d)

Nominal test substance intake	Test substance intake males	Test substance intake females
10	9.9	9.6
40	39.8	39.4
120	118	119
360	356	338

General observations: There were no mortalities in any of the groups. A single occurrence of vomitus in a high-dose group female could be regarded as a possible treatment related sign of clinical toxicity.

Food consumption was slightly reduced in the two females of the high-dose group, which is also apparent from the reduced actual test substance intake values compared to the nominal concentration (see table below). Body weight gain was not affected in any of the treatment groups.
Hematological investigations: At the highest test dose, a substantial increase in Heinz bodies was observed, which was associated with a slight decrease in red blood cell parameters (see table below).
Clinicochemical examinations revealed an increase of alkaline phosphatase activity at 120 mg/kg in males and at 360 mg/kg bw/d in both sexes (see table below). Plasma cholinesterase values appeared to be slightly increased in dose groups compared to controls. However, no dose-response relationship could be observed; so this finding was not considered relevant.
As discussed for oral studies in rats, the increased amount of bilirubin by using the diazo method was claimed to be an effect of metabolites clotting to the dye, resulting in a product with the same absorption range as the diazotized 2,4 dichloroaniline. Nevertheless, haematological and clinicochemical data suggest effects on the hematologic system.

Table 36: Hematological and clinicochemical parameters at day 24 (mean values)

Dose level (mg/kg bw/d) / Parameter	0	10	40	120	360
Bilirubin (µmol/l) ♂	3.34	3.49	5.74	8.10	7.96
♀	2.34	3.87	6.48	8.96	5.78
Hemoglobin (mmol/l) ♂	9.43	7.99	8.41	8.32	7.53
- % of control		(-15.3)	(-10.8)	(-11.8)	(-20.1)
Red blood cell count (tera/l) ♂	7.41	6.32	6.47	6.36	5.66
- % of control		(-14.7)	(-12.7)	(-14.2)	(-23.6)
Hematocrit (l/l) ♂	0.49	0.42	0.44	0.44	0.40
- % of control		(-14.3)	(-10.2)	(-10.2)	(-18.4)
Heinz bodies (°/₀₀)					
♂	3.5	6.5	7.0	8.5	34
♀	10.00	4.5	11.0	9.5	21.5
MCV (fl) ♂	66.05	67.15	68.45	68.30	70.25
- % of control		(+1.7)	(+3.6)	(+3.4)	(+6.4)
Alkaline phosphatase (µcat/l) ♂	5.03	6.02	4.53	8.03	13.51
♀	4.47	6.32	7.03	5.34	10.05
Plasma Choline Esterase (µcat/l) ♂	31.07	40.68	35.87	45.92	53.01
♀	33.51	37.74	46.34	45.80	42.32

Organ weight determinations revealed a steadily increase of liver weight at all dose levels (both sexes) and of thyroid weights at all dose levels in females (see table below). However, these changes were minimal only at 10 and 40 mg/kg bw/d.

Table 37: Terminal body, liver and thyroid weights

Dose level (mg/kg bw/d)	0		10		40		120		360	
Terminal body weight	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl
♂	8700	100	9150	105	10250	118	9900	114	9900	114
♀	8450	100	8650	102	9600	114	9450	112	9150	108
Absolute liver weight	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl
♂	322.79	100	351.00	109	407.45	126	415.01	129	532.74	165
♀	317.62	100	341.83	108	397.74	125	410.49	129	464.98	146
Relative liver weight	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl
♂	3.713	100	3.841	103	4.040	109	4.196	113	5.371	145
♀	3.751	100	3.954	105	4.143	110	4.336	116	5.089	136
Absolute thyroid weight	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl	[g]	%ctrl
♂	1.2970	100 %	0.8920	69%	1.0055	78 %	0.8735	67%	0.7565	58%
♀	0.6945	100 %	0.7770	112 %	0.8875	128 %	1.0315	149%	1.1880	171 %
Relative thyroid weight	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl	[%]	%ctrl
♂	0.0149	100 %	0.0096	64%	0.0097	65 %	0.0090	60%	0.0077	52%
♀	0.0082	100 %	0.0090	110 %	0.0092	112 %	0.0109	133%	0.0130	159 %

Gross necropsy and did not show any test substance related changes. Histopathological investigations revealed hepatocellular hypertrophy in animals of the highest dose group. No histopathological changes were noted in the liver at 120 mg/kg bw or lower dose levels, and no changes were seen in the thyroid or other tissues examined up to the highest dose level tested.

In this range finding study in the highest dose level (ca 360 mg/kg bw/d) there were indications of an anemic process (reduced RBC and haemoglobin in males and increased Heinz bodies in males and females) with compensatory reactions (elevated MCV in males). The liver was considered as target organ, with increased weights and hepatocellular hypertrophy (in the highest dose); the bile appeared reddish. Clinicochemical examinations revealed an increase of

alkaline phosphatase activity at and above a concentration of 120 mg/kg bw/d. Liver weights were moderately increased at 120 mg/kg bw/d and above; thyroid weight increases in females and decreases in males were considered to be spurious. Beside hepatocellular hypertrophy at 360 mg/kg bw/d no further evidence of histopathological changes were observed in any tissue. The NOAEL is proposed to be 40 mg/kg bw/d considering a tendency to increased liver weight in both sexes (> 110%), increased relative thyroid weight in females and increased alkaline phosphatase in male dogs at 120 mg/kg bw/d.

90 days feeding study in dogs (range finding study) *Hellwig et al., 1985(b)*

Cycloxydim (batch: N 64; purity: 94.8%) was administered as sodium salt to groups of 4 male and 4 female Beagle dogs via their diet over a period of 3 months. The dose levels were 0; 60; 300; 1500 and 7500 ppm. The diet was prepared freshly each day by mixing 350 g of dry dog food with 350 mL drinking water containing the appropriate concentration of Cycloxydim sodium salt. The animals were offered the feed / test substance preparation daily for 1 hour in the morning. Then the feed was removed from the kennel. For estimation of actual feed consumption, any feed left over was weighed and subtracted from the amount offered. This procedure was followed in order to ensure sufficient stability of the test substance in the feed preparation.
Feed consumption of the animals was determined daily and their body weight once a week; the dogs' health was checked each day. Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumin, globulins, lactate dehydrogenase, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, plasma cholinesterase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) as well as urinalyses (volume, pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, specific gravitry, sediment microscopy) were carried out once prior to start of the administration period and after approximately 1 month and 3 months of treatment.
Ophthalmological examinations were carried out before the beginning of the study and towards the end of the administration period in the animals of the high-dose group and the controls.
All animals were assessed gross-pathologically. The liver, kidneys, testes, thyroids and adrenal glands were weighed. All gross lesions and the livers from all animals under study were histopathologically examined. Furthermore, a comprehensive set of organs (heart, lung, pituitary, esophagus, stomach, small intestine, liver, spleen, thymus, kidneys, testes, ovaries, adrenals, thyroid and brain, aorta, salivary gland, pancreas, uterus, gall bladder, duodenum, jejunum, ileum, cecum, colon, rectum, urinary bladder, mesenteric lymph node, nervus ischiadicus, sternum) from the control; 1500 and 7500 ppm dose groups were subjected to histopathological examination.

The stability of the test substance was demonstrated and the stability, homogeneity and the correctness of the concentration in the water (vehicle) was analytically verified. The dietary preparation (dog food and water containing the test substance) was offered to the dog immediately after preparation for one hour only. During this time period, the test substance was demonstrated to be sufficiently stable.

Table 38: Test substance intake (calculated as Cycloxydim free acid)

Dietary dose level (ppm)	Test substance intake (average for males and females, in mg/kg bw/d)
60	2
300	10
1500	50

Dietary dose level (ppm)	Test substance intake (average for males and females, in mg/kg bw/d)
7500	250

General observations: There were no mortalities or signs of clinical toxicity in any of the groups.
Food consumption and body weight development were not affected at any dose level.
The hematological investigations showed a reduction in red blood cell count for both sexes and increased MCH and MCV values at the high dose level. The number of Heinz bodies was increased in females and males of the high-dose groups. These data suggest a disturbance in haemoglobin metabolism and presumably also the cause of the other changes in the red blood count. Platelet counts increased significantliy at a test substance concentration of 7500 ppm. Leucocytes increased dose-dependent in male dogs although not statistically significant, but concomitant with polymorphonuclear neutrophilic granulocytes, indicating inflammatory events (see table below).
Total bilirubin was counted with two different methods, by the routine method (2,4-dichloro-aniline mehod) and by the diazotilized sulfanilic method of Jendrassik and Gróf. The routine method revealed a clear increased throughout the test groups, but the sulfanilic acid method did not show any difference.

Table 39: Hematology (3-month sampling time point)

Dose level (ppm) / Parameter	0	60	300	1500	7500
Red blood cells (tera /l) ♂	7.63	7.16	7.38	7.62	6.88*
- % of control		(-6.2)	(-3.3)	(-0.13)	(-9.8)
♀	7.66	7.39	7.81	7.65	7.02**
- % of control		(-3.5)	(+1.9)	(-0.13)	(-8.4)
MCV (fl) ♂	68.10	69.57	67.60	68.52	71.77*
- % of control		(+0.9)	(-1.9)	(-0.6)	(+4.2)
MCH (fmol) ♂	1.328	1.334	1.317	1.328	1.383*
- % of control		(+4.5)	(-0.8)	(0)	(+4.1)
Leukocytes (giga/l) ♂	8.25	8.93	10.55	10.50	12.25
- % of control		(+8.2)	(+27.9)	(+27.3)	(+48.5)
Polymorphonuclear neutrophilic granulocytes (giga/l) ♂	4.83	5.56	6.03	6.55	7.97
- % of control		(+15.1)	(+24.8)	(+35.6)	(+65)
Reticulocytes (°/₀₀) ♀	0.75	3.25	2.25	4.75	5.00
- % of control		(+333)	(+200)	(+533)	(+566)
Heinz bodies (°/₀₀) ♂	0	0	0	0	2.50 ± 2.17
♀	0	0	0	0	8.5 ± 0.64

Dose level (ppm) / Parameter		0	60	300	1500	7500
Platelets (giga/l)	♂	426	442	457	402	554*
- % of control			(+3.8)	(+7.3)	(-5.6)	(+30)
	♀	426	487	461	392	647**
- % of control			(+14.3)	(+8.2)	(-8)	(+51.9)

Statistical significance: * = $p < 0.05$; ** = $p < 0.01$ (t-test)

In clinicochemical examinations (see table below) a 2-3 fold increase in alkaline phosphatase activity was observed in high-dose group males and females, indicating a hepatic dysfunction. A statistically not significant increase of the mean alkaline phosphatase activity was seen in males at a dose level of 1500 ppm. However, no effects on alkaline phosphatase activity were seen in females receiving the same dose. The albumin concentration was decreased in both sexes at the high dose level, accompanied by an increased globulin concentration in high-dose group females; the total protein content was unaffected by treatment. Furthermore, decreased potassium concentrations were seen in males at 7500 ppm.

Table 40: Clinical chemistry (3-month sampling period)

Dose level (ppm)	0	60	300	1500	7500
Alkaline phosphatase (µcat/l)					
♂	3.03 (100%)	3.36 (111%)	3.10 (102%)	4.83 (159%)	9.46** (312%)
♀	4.64 (100%)	3.28 (71%)	3.88 (84%)	4.54 (98%)	10.04** (216%)
Albumin (g/l)					
♂	40.20 (100%)	37.30 (93%)	38.37 (95%)	40.12 (100%)	35.32* (88%)
♀	40.45 (100%)	40.73 (101%)	39.90 (99%)	38.27 (95%)	34.45** (85%)
Globulin (g/l)					
♀	19.22 (100%)	21.09 (110%)	20.74 (108%)	19.10 (99%)	23.77** (124%)
Potassium (mmol/l)					
♂	4.07 (100%)	4.10 (101%)	3.94 (97%)	3.81 (94%)	3.64* (89%)

Statistical significance: * $p < 0.05$; ** $p < 0.01$ (t-test)

Organ weight determination revealed a dose-dependent and statistically significant increase in absolute and relative liver weight in males and females of the high-dose group, as well as a statistically significant increase of absolute and relative thyroid weights in high-dose males. This increase was also found to be dose-dependent.

Table 41: Terminal body weight and organ weights

Dose level (ppm)	0		60		300		1500		7500	
Terminal bw	[kg]	(%ctrl)	[kg]	(%ctrl)	[kg]	(%ctrl)	[kg]	(%ctrl)	[kg]	(%ctrl)
♂	11.450	(100)	11.525	(101)	11.275	(98)	11.550	(101)	11.600	(101)
♀	10.275	(100)	9.900	(96)	11.025	(107)	10.925	(106)	10.475	(102)
Abs. liver wt	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)
♂	344	(100)	365	(106)	363	(106)	387	(113)	479**	(139)
♀	316	(100)	316	(100)	337	(107)	361	(114)	498**	(158)
Rel. liver wt	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)
♂	3.01	(100)	3.18	(106)	3.24	(108)	3.36	(112)	4.13**	(137)
♀	3.07	(100)	3.21	(104)	3.05	(99)	3.29	(107)	4.75**	(155)
Abs. thyroid wt	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)	[g]	(%ctrl)
♂	0.716	(100)	0.774	(108)	0.859	(120)	0.865	(121)	1.060*	(148)
♀	0.849	(100)	0.674	(79)	0.771	(91)	0.812	(96)	1.079	(127)
Rel. thyroid wt	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)	[%]	(%ctrl)
♂	0.0062	(100)	0.0068	(110)	0.0077	(124)	0.0075	(121)	0.009*	(145)
♀	0.0082	(100)	0.0069	(84)	0.0070	(85)	0.0075	(91)	0.010	(127)

Statistical significance: * $p < 0.05$; ** $p < 0.01$ (Williams t-test)

Gross pathological investigations showed a reddish tinge to the bile for high-dose males and females. Histopathology revealed an enlargement of hepatocytes in 3 out of 4 males and in all females at the high dose level. There were no other test substance related changes noted in the histopathological examinations.

3-month administration of Cycloxydim to dogs via the feed did not result in clinical findings or body weight changes up to the highest dose tested of 250 mg/kg bw/d At this dose level there were signs of an anemic process (reduced RBC and increased Heinz bodies in males and females) with compensatory reactions (elevated MCV in males and number of reticulocytes in females). The target organ was the liver, with increased weights and hepatocellular hypertrophy in the high dose group; the bile had a reddish colour. In male dogs liver weights were increased over 110% at a concentration of 1500 ppm, this effect was not statistically

significant, but coincided with increased ALP-activity (159% of control value). Furthermore, thyroid weights were significantly increased in males at 7500 ppm, and more than 120% increased at dose-levels of 300 and 1500 ppm (not statistically significant); however no associated histopathological changes of the thyroid were observed. Therefore, the NOAEL in this study is proposed to be 300 ppm (10 mg/kg bw/d) for male and 1500 ppm accounting for 50 mg/kg bw/d for female dogs. The effects on blood system were observable only at the highest dose tested (250 mg/kg bw/d).

1 year feeding study in dogs *Hellwig J., Hildebrand B. 1988*

Cycloxydim sodium salt (batch: N 88; purity: 93.9%) was administered to 6 male and 6 female Beagle dogs (from BASF breed, 6-8 months of age, weighing 9.7 kg and 9.2 kg males and females, respectively) per group at dietary dose levels (0, 400, 1600 and 6400 ppm) for 12 months. The diets were prepared each day by mixing 350 g pulverized dog food with 350 g water containing the appropriate amount of the sodium salt of Cycloxydim . Feed consumption was determined daily and body weight was determined once a week. The animals' health was checked each day.
Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumine, globulins, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count), as well as urinalyses (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, specific gravity, sediment microscopy), were carried out prior to start of treatment, and after 94 (3-months), 185 (6-months), and 367 (12-months) days of administration. Ophthalmological examinations were carried out before the start of the study and towards the end of the administration period.
All animals were assessed gross-pathologically, organ weights (brain, liver, kidneys, adrenals, thyroid, testes/ovaries) were determined and subsequently subjected to a complete histopathological examination (brain, pituitary, thyroid, thymus, lungs, trachea, heart, aorta, salivary glands, liver, spleen, kidneys, adreanals, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, mesenteric and axillary lymph nodes, pancreas, testes/ovaries, prostate, female mammary gland, skin, gallbladder, sciatic nerve, skeletal muscle, cervical, thoriacic and lumbar cord, sternum, femur, eyes, all gross lesions) according to giudeline requirements.

As the food was offered immediately after the preparation for only one hour to the dogs, the animals received the intended concentrations of the test substance as stated in table below.

Table 42: Test substance intake (free acid)

Dietary dose level (ppm)	Test substance intake (for males and females, in mg/kg bw/d)
400	12
1600	49
6400	206

General observations:No cases of death were reported. No test substance-related signs of clinical toxicity or mortalities were found in any group. Food consumption and body weight development were not adversely affected in any treatment group.
The hematological investigations demonstrated the following changes, which were confined with two exceptions to the high dose level, and generally observable at the 3- and 6-month time points (for details see table below).

6400 ppm: • decreased erythrocytes in males (6 months) and females (3 & 6 months)
• decreased hemoglobin and hematocrit in females (3 & 6 months)
• increased MCH in males (3 months), females (3 & 6 months)
• increased MCV in males (3 months), females (3 & 6 & 12 months)
• increased platelets and Heinz bodies in males and females (3 & 6 & 12 months)
1600 ppm: • increased platelets in males (3 & 6 & 12 months), and females (3 & 6 months)
• increased Heinz bodies in males (3 & 6 months) and females (6 & 12 months)

Table 43: Hematological parameters after 3, 6 and 12 months of administration

Treatment duration	3 months				6 months				12 months			
Dose level (ppm)	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
♂												
RBC (tera/l)	6.56	6.23	6.36	6.43	6.61	6.26	6.23	6.19*	7.08	7.12	6.85	6.90
Hb (mmol/l)	9.53	9.12	9.46	9.62	9.75	9.25	9.27	9.35	10.25	10.34	10.06	10.20
Ht (l/l)	0.457	0.434	0.452	0.463	0.467	0.443	0.446	0.451	0.500	0.500	0.485	0.496
MCH (fmol)	1.45	1.46	1.49	1.50*	1.48	1.48	1.49	1.51	1.45	1.45	1.47	1.48
MCV (fl)	69.55	69.52	70.88	71.95*	70.68	70.67	71.43	72.73	70.37	70.23	70.68	71.73
Platelets (giga/l)	460	516	528	583**	450	450	513*	532	471	464	521	589**
Heinz bodies (°/₀₀)	4	8	12	21**	2	3	9**	25**	3	2	5	29**
♀												
RBC (tera/l)	6.84	6.63	6.75	5.90**	6.78	6.64	6.66	5.83*	6.67	6.74	7.04	6.46
Hb (mmol/l)	10.01	9.78	10.03	8.97*	9.96	9.79	9.88	8.89*	9.62	9.68	10.14	9.56
Ht (l/l)	0.48	0.46	0.48	0.43*	0.48	0.47	0.48	0.44*	0.46	0.47	0.50	0.48
MCH (fmol)	1.46	1.48	1.48	1.52*	1.47	1.47	1.48	1.52**	1.45	1.44	1.44	1.48
MCV (fl)	69.6	69.2	70.4	72.7**	70.4	70.6	71.5	72.9*	68.6	69.6	70.7	72.2*
Platelets (giga/l)	524	452	541	712*	469	467	543	699*	576	464	517	703
Heinz bodies (°/₀₀)	3	3	3	24**	3	2	7*	27**	3	2	7*	27**

* $p < 0.05$, ** $p < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney u-test, 2-sided)

The clinicochemical investigation demonstrated the following changes, most of which were already expressed after 3 months of treatment and did not alter during the course of the study (see also table below):
- increased alkaline phosphatase: 6400 ppm in both sexes and at 1600 ppm in males,
- decreased total protein: 6400 ppm in males (6 months)
- decreased albumin: 6400 ppm both sexes and at 1600 ppm in males (6 months).

Table 44: Clinicochemical parameters after 3, 6 and 12 months of administration

Treatment duration	3 months				6 months				12 months			
Dose level (ppm)	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
♂												
ALP (µkat/l)	3.73	4.93	6.11**	10.64**	2.96	3.82	4.54*	9.38**	3.03	4.34	5.47**	11.65**
Tot. protein (g/l)	56.85	54.87	56.77	53.54	59.40	58.24	57.20	54.38*	60.12	58.96	58.10	56.03
Albumin (g/l)	35.76	34.01	33.99	30.90**	39.84	38.71	37.52**	33.77**	39.92	38.09	36.56**	33.84**
♀												
ALP (µkat/l)	4.00	4.61	4.90	9.93**	2.91	4.07	3.96	8.99**	5.83	4.70	4.55	9.53
Tot. protein (g/l)	56.29	57.95	55.95	53.63	57.13	57.33	56.81	55.72	58.57	60.30	60.07	56.83
Albumin (g/l)	34.81	35.17	33.65	30.23**	38.75	39.70	37.71	34.14**	36.73	38.29	37.42	33.98

* $p < 0.05$, ** $p < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney u-test, 2-sided)

Urinalysis and ophthalmoscopy did not demonstrate test substance-related effects.
Organ weight determinations revealed an increase in absolute and relative liver weights in males and females of the 6400 ppm group, as well as in males of the 1600 ppm dose level (see table below).

Table 44: Terminal body weight and liver weights

Dose level (ppm)	0	400	1600	6400
Terminal body wt (kg)				
♂	11.92 (100 %)	12.70 (107 %)	11.53 (97%)	11.53 (97%)

Dose level (ppm)	0	400	1600	6400
♀	10.87 (100 %)	11.32 (104 %)	12.12 (111 %)	10.63 (98%) **
Abs. liver wt (g)				
♂	349 (100 %)	385 (110 %)	407* (117 %)	449** (129 %)
♀	381 (100 %)	414 (109 %)	384 (101 %)	457* (120 %)
Rel. liver wt (%)				
♂	2.94 (100 %)	3.04 (103 %)	3.54* (120 %)	3.93** (134 %)
♀	3.51 (100 %)	3.67 (105 %)	3.17 (90%)	4.29** (122 %)

* $p < 0.05$, ** $p < 0.01$ (Dunnett's test)

Histopathology demonstrated slight or moderate hemosiderosis in the livers of three females of the 6400 ppm group. This effect is considered to have resulted from the transiently increased degradation of erythrocytes induced by the test substance. No further test substance-related changes were noted during the histopathological investigations. Particularly, there were no changes in the liver that could be associated with the increased liver weights.

In the 12 month study, dose level of 6400 ppm induced some signs of anemia in Beagle dogs. Associated with the effects on blood system, histopathology demonstrated increased hemosiderosis in the livers of females of the 6400 ppm group. Liver weights were increased at 1600 ppm and 6400 ppm dose levels, as well as some clinicochemical parameters indicating liver toxicity. The NOAEL in this 12-month dietary study in dogs can be established at 400 ppm (12 mg/kg bw/d) for males and females.

4.7.1.2 Repeated dose toxicity: inhalation

Short term inhalation toxicity studies (28-day and 90-day) were not performed/submitted based on the low volatility of cycloxydim (vapour pressure 1.0×10^{-5} Pa at 20°C) and no evidence of significant inhalation toxicity was evident from the results of the acute inhalation toxicity study.

4.7.1.3 Repeated dose toxicity: dermal

28 days dermal study in rats *Mellert W. et al. 2001(a)*

Cycloxydim (batch: WH 16884; purity: 92.9%) was applied to the skin of 10 male and 10 female Wistar rats (source: Charles River, Grmany, aged: 60 ± 1 days, weighing 196 g and 168 g male and female rat, respectively) per dose level on an area corresponding to at least 10% of the body surface for a period of six hours per day, five days per week for four consecutive weeks. The doses were 0 (vehicle control, olive oil); 60; 300 and 1000 mg/kg bw/d. The administration volume was 2 mL/kg bw/d. The skin was covered by a semi-occlusive dressing. After removal of the dressing the skin was washed with warm water.
The animals were examined at least once a day for clinical symptoms and mortalities. Additional clinical examinations, including detailed examinations of the skin, were carried out daily. Detailed clinical observations in an open field were conducted prior to the start of the

administration period and weekly thereafter. A functional observation battery (FOB) was performed towards the end of the study. A motor activity measurement was performed on the same day as the FOB. Food consumption and body weight were determined weekly. Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, magnesium, triglycerides, cholesterol, albumine, globulins, serum-gamma-glutamyltransferase, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) as well as urinalysis (volume, color, turbidity, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, specific gravity, sediment) were performed at the end of the administration period. Ophthalmological examinations were carried out prior to the start and towards the end of the administration period. Organ weights from liver, kidneys, adrenal glands, testes, epididymides, uterus, ovaries, thymus, spleen, heart, brain were recorded. All animals were subjected to a gross-pathological assessment followed by histopathological (salvary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, brain, pituitary gland, sciatic nerve, spinal cord, eyes, adrenal glands, thyroid, trachea, lungs, pharynx, larynx, nose, aorta, heart, bone marrow, lymph nodes, spleen, thymus, kidneys, urinary bladder, testes, ovaries, oviducts, uterus/vagina, epididymides, prostate gland, seminal vesicles, female mammary gland, skin, skeletal muscle, sternum with marrow, femur with knee joint, extraorbital lacrimal gland) examinations according to guideline requirements.

General observations: There were no mortalities or clinical signs of toxicity in the treatment groups. No substance-related effects on food consumption were observed in any treatment group. However, food efficiency was statistically significantly decreased in males at 1000 mg/kg bw/d on day 21. A relationship to treatment cannot be excluded with certainty. In females, food efficiency was statistically significantly decreased on day 21 at 60 and 300 mg/kg bw/d, but not at 1000 mg/kg bw/d. Due to the lack of a dose-response relationship, this was assessed as being incidental and not treatment-related.
No statistically significant deviations were seen concerning body weight. Concerning body weight change, a statistically significantly lower body weight gain was observed in high-dose group males for the 4-wk study period (-21.7 % compared to controls). The mean terminal body weight in males at 1000 mg/kg bw/d was decreased by 6 % (statistically non-significant) compared to the control group. Body weight change was also statistically significantly decreased in females at 60 and at 300 mg/kg bw/d on day 21. However, due to the lack of a dose-response relationship, this was assessed as being incidental and not treatment-related. No statistically significant changes in body weight development were noted in treatment-group females when based on the 4-week treatment period, and no effects on terminal body weight were seen that could be considered as substance-related.

Table 45: Food efficiency, body weight changes and terminal body weight (g)

Dose (ppm) / Parameter	0	60	300	1000
Males				
Food efficiency (Day 21)	10.6 (100%)	8.9 (84%)	9.3 (88%)	7.1* (67%)
Bw change (Day 0–21) [g]	65.6 (100%)	60.6 (92%)	70.2 (107%)	54.0 (82%)
Bw change (Day 0–27) [g]	66.7 (100%)	60.9 (91%)	69.9 (105%)	52.2* (78%)

Dose (ppm) / Parameter	0	60	300	1000
Terminal body weight [g]	262.4 (100%)	256.3 (98%)	266.8 (102%)	247.3 (94%)
Females				
Food efficiency (Day 21)	11.9 (100%)	5.1* (43%)	3.6** (30%)	7.7 (65%)
Bw change (Day 0–21) [g]	41.5 (100%)	31.2* (75%)	31.9* (77%)	36.9 (89%)
Bw change (Day 0–27) [g]	41.6 (100%)	37.9 (91%)	36.0 (87%)	40.5 (97%)
Terminal body weight [g]	211.8 (100%)	204.4 (97%)	203.7 (96%)	207.7 (98%)

Statistics: * = p< 0.05; ** = p< 0.01 (Dunnett's Test, 2-sided)

Open field observations, FOB and motor activity determinations did not show test substance related changes. Clinicochemical, hematological examinations and urinalysis did not reveal any test substance related changes.
Gross macroscopic observations and histopathological examinations did not show any treatment related effect. There were no signs of skin irritation in any of the animals.

The NOAEL for systemic toxicity is 1000 mg/kg bw/d for female and 300 mg/kg bw/d for male Wistar rats, based on slight effects on body weight gain and feed efficiency at 1000 mg/kg bw/d. No signs of local skin irritation were observed.

4.7.1.4 Repeated dose toxicity: other routes

No data on other routes.

4.7.1.5 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.7.1.6 Other relevant information

No other information available.

4.7.1.7 Dossier submitter's summary and discussion of repeated dose toxicity

The repeated dose toxicity of cycloxydim has been investigated after oral application in rats (28 and 90 days of exposure), mice (28 days of exposure) and dogs (28, 90 days and 12 months of exposure). In addition, one study of dermal exposure to rats (28 days) is available.

Rat studies:

In a 28 days oral (drinking water) range finding study in the rat, a NOAEL of 1000 ppm (equivalent to 106 mg/kg bw/d) in females was proposed, based on increased relative liver weight at 3000 ppm (252 mg/kg bw/d). The NOAEL of 3000 ppm (equivalent to 272 mg/kg

bw/d) was proposed for male rats based on decreased body weight, increased relative liver and kidney weight, increased urea and decreased trigycerides level at 9000 ppm (683 mg/kg bw/d).

In a 90 days oral (drinking water) toxicity study in the rat (range finding study), liver enzyme activities (ALT, ALP) were found to be significantly altered at concentrations of 2700 ppm and 900 ppm (equivalent to 178 or 201 and 72 or 74 mg/kg bw/d in male and female rats respectively). In addition creatinine was shown to be significantly increased at these dose levels in females after one month of exposure. The NOAEL was thus set as 300 ppm (equivalent to 22 mg/kg bw/d in male and 28 mg/kg bw/d in female rats, respectively). Both sexes recovered completely with respect to reduced body weight gain and clinico-chemical changes. No reduction in food consumption was noted in males during the recovery phase.

After repeated dermal application (5 times per week over 4 consecutive weeks) of cycloxydim to Wistar rats, male animals showed significantly reduced body weight gain and reduced food efficiency at a concentration of 1000 mg/kg bw, but no biochemical alterations could be observed. Therefore the NOAEL was set as 300 mg/kg bw for male and 1000 mg/kg bw for female rats.

Mouse studies:

In a 28 days oral (drinking water) toxicity study in mice (1st range finding study), the NOAEL was set as 1000 ppm (corresponding to 189 mg/kg bw/d in male and 218 mg/kg bw/d in female mice, respectively), based on a significant increase in relative liver weights over 115% (in male mice) at a concentration of 3000 ppm and 9000 ppm in combination with altered clinico-chemical parameters (enhanced plasma urea levels and deceased plasma cholesterol) in males and females, and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in two males in the highest dose group.

A second 28 days oral (drinking water) toxicity study in mice (2nd range finding study) was provided but with less data-points. Biochemical data was scarce in this study, liver weight and liver enzyme activities are the only parameters that can be used for toxicological assessment. Originally, a NOAEL in B6C3F1/CrIBR mice was established at 100 ppm (22.5 mg/kg bw/d) for males and at 300 ppm (82.5 mg/kg bw/d) for females, based on increased liver weights, and a dose-dependent decrease in LDH activity in male mice. The DS considered the NOAEL to be 100 ppm (22.5 mg/kg bw/d) for males, based on reduced LDH level at 300 ppm and >900 ppm for females based on lack of adverse effects. However, the derivation of the NOAEL upon the reduced LDH is considered to be a very conservative approach, since it is not clear if it is indeed an adverse effect.

Dog studies:

In a 28 days oral (feeding) study in dogs (range finding study with 2 dogs/sex/dose), the relative liver weights were significantly increased at $\geq$120 mg/kg bw/d in both sexes. Liver hypertrophy was noted in the highest concentration tested (360 mg/kg bw/d). In females absolute and relative thyroid weights were clearly increased at $\geq$120 mg/kg bw/d, but no histopathological changes were evident. The NOAEL was therefore determined to be 40 mg/kg bw/d in both sexes.

The results of a 90 days oral (feeding) toxicity study in dogs (range finding study) showed statistically significant changes in haematological parameters (decreased RBC, increased Heinz bodies, increased MCV and reticulocytes) at 7500 ppm (250 mg/kg bw/d) and statistically significant increase in alkaline phosphatase in male rats, with increased absolute and relative liver weights and hepatocellular hypertrophy. In addition absolute and relative thyroid weights were increased for 121% (males), but reached no statistical significance. The NOAEL in this study was set as 300 ppm (corresponding to 10 mg/kg bw/d) for male and 1500 ppm equivalent to 50 mg/kg bw/d for female dogs.

In a one year chronic toxicity study in dogs, a dose level of 6400 ppm (206 mg/kg bw/d) induced anaemic effects, associated with increased liver weights and increased hemosiderosis

in the livers of female dogs. At a dose of 1600 ppm (49 mg/kg bw/d) indications of compensatory reactions to an anaemic process (increased Heinz bodies and platelets) in both sexes were identified, as well as increased liver weight in male animals, associated with altered clinico-chemical parameters (increased alkaline phosphatase activity and reduced albumin concentration). The NOAEL in this study was 400 ppm (12 mg/kg bw/d) for males and females.

RAC comment: The RAC considered Heinz bodies not to be a compensatory reaction to anaemic processes. Moreover Heinz bodies are indicative of damage to red blood cells.

4.7.1.8 Dossier submitter's summary and discussion of repeated dose toxicity findings relevant for classification according to DSD and CLP

In the sub-acute oral rodent studies (rat and mice), the only effect observed below the cut-off values of 150 mg/kg bw/d and 300 mg/kg bw/d for DSD and CLP, respectively, was increased relative liver weight in female rats (without histological findings) and decreased LDH level in male mice (for which is not clear if it is indeed an adverse effect).

In the sub-acute dermal rat study the only effect observed below the cut-off values of 300 mg/kg bw/d and 600 mg/kg bw/d for DSD and CLP, respectively, was reduced body weight in males.

In the 90 days rat study, the effects observed below the cut-off values of 100 mg/kg bw/d for CLP (but not below the cut-off value for DSD, 50 mg/kg bw/day) were increased ALP in males and females (with no effects on liver weight or histopathological findings) and increased creatinin and urea in females.

For dogs, no cut-off criteria are available at present. In the 28 days dog study, the effects on blood system (reduced haemoglobin in males, reduced RBC in males, increased Heinz bodies in males and females) and liver (increased relative liver weight in males and females, hepatocellular hypertrophy in males and females, increased ALP in males and females) were observed primarily at the highest dose tested (360 mg/kg bw/d).

In the 90 days dog study, the effects on blood system (reduced RBC in males and females, increased MCV, leucocytes and polymorphonuclear neutrophilic granulocytes in males, increased Heinz bodies and platelets in males and females) and liver (increased relative liver weight in males and females, hepatocellular hypertrophy in males and females, increased ALP in males and females) were observed primarily at the highest dose tested (250 mg/kg bw/d).

In the 1 year dog study, the effects on blood system (reduced RBC in males and females, reduced haemoglobin in females, increased MCH, MCV, platelets and Heinz bodies in males and females) and liver (increased relative liver weight in males and females, increased ALP in males and females, but no histopathological findings) were observed primarily at the highest dose tested (206 mg/kg bw/d).

Thus effects observed in the subchronic (oral and dermal) studies in rat, mouse and dog do not meet the criteria for classification and labelling for Specific target organ toxicity - repeated exposure (CLP) or for repeated dose toxicity (DSD). Increased numbers of Heinz bodies and platelets, increased relative liver weight and elevated ALP concentration observed at 49 mg/kg bw/d (1600 ppm) were considered to be treatment-related effects.

4.7.1.9 Dossier submitter's comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Please see 4.7.1.8.

4.7.1.10 Dossier submitter's conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

Effects observed in the subchronic (oral and dermal) studies in rat, mouse and dog do not trigger the criteria for classification and labelling for repeated dose toxicity.

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

Please see 4.7.1.8.

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

Please see 4.7.1.8.

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

Effects observed in the subchronic (oral and dermal) studies in rat, mouse and dog do not meet the criteria for Specific target organ toxicity - repeated exposure (CLP).

4.8.4 Comments received during public consultation

No specific comments were received on this endpoint.

4.8.5 The RAC assessment and comparison with criteria

The target organs of interest following repeated (subacute to chronic) administration of cycloxydim are the liver (in rats, mice and dogs), the kidney (in rats) and the haematopoietic system (in dogs).

The main effects observed in the liver were:

a) increases in rat and dog liver weight (probably indicating some hypertrophic or hyperplastic liver cell response. The only microscopic finding attributable to weight increase was reported as 'enlarged (=hypertrophic) hepatocytes' in dogs at high dose of 7500 ppm (250 mg/kg bw/d, after 90 days of treatment.
b) increases in ALP in dogs which is not specific for liver cell damage, but may indicate effects on the bile duct epithelia/system.
c) reduced serum concentrations of triglycerides and/or cholesterol in rat and mouse studies are likely to be related to the observed reduction in food and water consumption.
d) increases in ALT (seen at 900 ppm (74 mg/kg bw/d)) in rats receiving cycloxydim for 90 days may be indicative for liver cell damage, however microscopic degenerative/necrotic liver cell lesions were only reported in one 28 day range-finding study in 2/10 mice (at 9000 ppm (1177 mg/kg bw/d, above limit dose).
e) ALP was elevated in some studies (a possible indication of liver dysfunction), but a reduction was found in other studies at comparably high doses.

Overall, effects on the liver of a serious nature (e.g. liver cell degeneration) were noted at doses above the normal concentration recommended for a limit test (1000 mg/kg bw/d) in the

test guidelines for repeated dose toxicity (REACH Test Method Regulation (EC) 440/2008, methods B.7, and B.26) or if observed at doses below the guidance levels for classification as STOT RE, were not considered serious enough. This is the case for liver cell hypertrophy alone, liver weight increase, increased liver enzymes.

The main effects observed on the kidneys were as follows. In rats, increased urea and creatinine concentration indicated renal dysfunction, but the effects were not associated to any other macroscopic or microscopic abnormal finding except increases in kidney weight in rats. Effects were only seen in rats at doses above guidance values for classification (at 900 ppm (201 mg/kg bw/d) after 90 days of treatment and at 9000 ppm (680 mg/kg bw/d) after 28 days of treatment).

Overall, there was no serious effect on the urinary tract that warrants classification.

The main effects observed on the haematopoietic system were:

Increased numbers of Heinz bodies, reduction of red blood cells, and (compensatory) increases in MCV, associated with haemosiderosis after chronic treatment are serious health effects indicating haemolytic anaemia. These toxic effects were only seen in dogs; no indications on anaemic effects were noted in guidance-compliant studies in rats.

a) Heinz bodies are damaged erythrocytes that are composed of denatured protein, mainly haemoglobin and are characteristic for anaemic conditions following chemical insult. Heinz bodies were seen in all three dog studies: the lowest dose was 49 mg/kg bw/d after 1 year treatment, which is above the guidance value (see calculation below).
b) Reductions of red blood cells and haemoglobin were above 20% at 360 mg/kg bw/d in the 28 day study in rats. The effect as such is adverse according to the criteria of Muller et al. (2006). However the effect dose is clearly above the guidance value for oral 28 day studies.
c) Haemosiderosis in the liver of 3/6 female dogs was observed together with reductions of red blood cells and haemoglobin reductions above 10% after 3 and 6 months. Findings are in accordance to the criteria of Muller et al., 2006 advocating classification, but the dosage of 6400 ppm (206 mg/kg bw/d) is far above the guidance value for classification when Haber's law is used (100 mg/kg bw/d divided by 4; serious effect should therefore be evident at about 25 mg/kg bw/d or below).

Overall, cycloxydim causes adverse effects on the haematopoietic system, but the doses causing these effects were high and above the guidance values as referred to in the criteria for classification (3.9.2, Annex I, CLP).

The RAC supported the Dossier submitter's proposal that no classification is warranted for STOT RE.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 46: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Dose range	Reference
In vitro studies			
Reverse mutation assay of Cycloxydim sodium salt in S. typhimurium TA 1535,TA 100, TA 1537, TA 98 (OECD 471)	Negative (+/- S-9 mix)	Study I : 0, 0.06, 0.3, 1.5, 7.5, 15.0 mg/ plate Study II : 0, 0.02, 0.1, 0.5, 2.0, 5.0 mg/plate (dissolved in DMSO) Purity: 93.9%	Engelhardt G., Gelbke H.-P. 1985(b)
Reverse mutation assay of Cycloxydim free acid in S. typhimurium TA 1535, TA 100, TA 1537, TA 98 (OECD 471)	Negative (+/- S-9 mix)	0, 0.02, 0.1, 0.5, 2.5, 5.0 mg/plate (dissolved in DMSO) Purity: 92.2%	Engelhardt G., Gelbke H.-P. 1983
Gene mutation assay of Cycloxydim sodium salt in Chinese hamster ovary (CHO) cells (HPRT locus) (OECD 476)	Negative (+/- S-9 mix)	0, 5, 10, 15, 21, 28, 35, 40 mg/mL (dissolved in DMSO) Purity: 93.9%	Boer W. C. den, Hoorn A. J. W. 1985(a)
Gene mutation assay of Cycloxydim sodium salt in Chinese hamster ovary (CHO) cells (HPRT locus) (OECD 476)	Negative (+/- S-9 mix)	Study I: 0, 0.215, 0.464, 1.0, 2.15, 4.64, 10.0, 21.5 mg/mL Study II and III: 0, 0.1, 0.215, 0.464, 0.681, 1.0, 2.15, 4.64 mg/mL (dissolved in DMSO) Purity: 93.9%	Jaeckh R., Gelbke H.-P. 1986
Gene mutation/ chromosome aberration assay of Cycloxydim sodium salt Mouse lymphoma forward mutation assay L5178Y Tk+/- cells (TK locus) (OECD 476)	Negative without S-9 mix weakly positive with S-9 mix at cytotoxic concentrations	Study I: 0, 1.75, 3.50, 5.00, 7.00, 10.00, 14.00, 20.00 mg/mL Study II: 0, 5.00, 6.25, 8.00, 10.00, 12.50 mg/mL (dissolved in DMSO) Purity: 93.9%	Boer W. C. den 1985(b)
Chromosome aberration mammalian cells of Cycloxydim sodium salt Chinese hamster ovary (CHO) cells (OECD 473)	weakly positive without S-9 mix at cytotoxic concentrations negative with S-9 mix	0, 2.0, 3.3, 4.0, 5.0 mg/mL (dissolved in DMSO) Purity: 93.9%	Taalman R. D. 1985(a)
Chromosome aberration mammalian cells of	weakly positive without S-9 mix at	0, 0.017, 0.05, 0.167, 0.50, 1.667	Taalman R. D. 1985(b)

Cycloxydim free acid Chinese hamster ovary (CHO) cells (OECD 473)	cytotoxic concentrations negative with S-9 mix	mg/mL (dissolved in DMSO) Purity and batch: not stated in the study	
Unscheduled DNA synthesis *in vitro* of Cycloxydim sodium salt Rat primary hepatocytes (OECD 482)	Negative	0, 0.906; 1.81; 3.63; 9.06; 18.1; 36.3; and 90.6 µg/mL (dissolved in DMSO) Purity: 93.9%	Cifone M. A., Brusick D. J. 1985
Unscheduled DNA synthesis in vitro of Cycloxydim free acid Rat primary hepatocytes (OECD 482)	Negative	0, 0.1, 0.25, 0.375, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/mL (dissolved in DMSO) Purity and batch: not stated in the study	Cifone M. A., Myhr C. B. 1985
In vivo studies			
Micronucleus test of Cycloxydim sodium salt NMRI mice (OECD 474)	Negative	Oral (gavage – 1 application): 0, 225, 450, 900 mg/kg bw (in aqua dest.) Purity: 93.9%	Engelhardt G., Gelbke H.-P. 1985(a)
Chromosomal aberration test of Cycloxydim sodium salt Chinese hamster (OECD 475)	Negative	Oral (gavage – 1 application): 0, 500, 1700, 5000 mg/kg bw (in aqua dest.) Purity: not stated in the study	Taalman R. D. 1987

4.9.1 Non-human information

4.9.1.1 In vitro data

Point mutation assay with bacteria

Cycloxydim Na-salt

No bacteriotoxic effects were observed in any of the Salmonella typhimurium strains after treatment with Cycloxydim sodium salt in any experiment and at any concentration tested. The test substance was completely soluble in DMSO.

The test substance did not increase the number of his^+ revertants at any concentration of the two experiments with or without metabolic activation. The mutagenic response of the positive controls indicates that the test system was able to detect mutagens.

Cycloxydim sodium salt is not mutagenic in the bacterial reverse mutation assay with *S. typhimurium* under the experimental conditions chosen.

Cycloxydim free acid

No bacteriotoxic effects were observed in any of the Salmonella typhimurium strains after treatment with Cycloxydim sodium salt in any experiment and at any concentration tested. The test substance was completely soluble in DMSO.

The test substance did not increase the number of his^+ revertants at any concentration of the two experiments with or without metabolic activation. The mutagenic response of the positive controls indicates that the test system was able to detect mutagens.

Cycloxydim sodium salt is not mutagenic in the bacterial reverse mutation assay with *S. typhimurium* under the experimental conditions chosen.

Gene mutation assay with mammalian cell

Cycloxydim Na-salt

1. study

There was no evidence of cytotoxicity up to 10 mg/mL (= 0.73 mg/mL Cycloxydim sodium salt), while the highest concentration of 40 mg/mL (= 2.92 mg/mL Cycloxydim sodium salt) was extremely cytotoxic in this experiment, both with and without S-9 mix. In the main experiment test substance concentrations were used ranging from 5 to 40 mg/mL with and without metabolic activation. No distinct reduction in the survival of cells was observed up to 40 mg/mL. However, a reduction in the relative cell growth as compared to the controls was observed both with and without metabolic activation.

The mutant frequencies with and without metabolic activation varied randomly and were comparable to the vehicle control cultures both with and without metabolic activation.

The positive controls proved that the system is able to detect known mutagens.

2. study

The results of the 3 experiments are compiled in Table below. In the first study, an increase of the mutation rates was observed with beginning cytotoxicity in the presence as well as in the absence of metabolic activation. The effect was not detectable at lower concentrations. For the interpretation of this result, it has to be taken into account that dose levels of > 1 mg/mL may have led to a precipitation of the test material (probably in form of the free acid). This may have occurred, because an alkaline pH-value, as indicated by the indicator color, was encountered by superposition of CO_2 gas, and a slight clouding of the solution was observed. The precipitated particles may enter the cell via phagocytosis and thereby casue an activation of lysosomes and production of oxygen radicals. It is pointed out, that this concentration range was also the onset of cytotoxicity (as determined by decreased cloning efficiencies).

Whether the increased number of mutants in the first study was caused by such kind of artefacts and might therefore be regarded as a false positive result, could not be stated from a single study alone.

In the following 2 experiments, CO_2 gassing was omitted and clear negative results with respect to mutation frequency were obtained at all concentrations including those being positive (mutation frequency above 15×10^{-6} cells) in the first experiment.

Tests with both positive control compounds showed that the system is able to detect known mutagens.

Table 47: Cloning efficiency and mutation frequency in the CHO HPRT Assay

Experiment 1				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	**Cloning efficiency ***	**Mutation rate****	**Cloning efficiency ***	**Mutation rate****
0	76%	4.4	93%	0
0.215	69%	4.8	80%	5.0
0.464	71%	0	77%	9.5
1.0	66%	65.6	48%	77.4
2.15	48%	15.2	10%	0
4.64	11%	53.3	0%	-
10.0	1%	0	0%	-
21.5	2%	0	0%	-
Positive controls	16%	2,908	27%	343
Experiment 2				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	**Cloning efficiency ***	**Mutation rate****	**Cloning efficiency ***	**Mutation rate****
0	78%	0	102%	0
0.1	67%	0	70%	0
0.215	64%	0	55%	0
0.464	56%	0	55%	1.2
0.681	55%	8.5	54%	0
1.0	59%	0	36%	0
2.15	41%	0	11%	0
4.64	No sufficient cell growth			
Positive controls	43%	143	51%	214
Experiment 3				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	**Cloning efficiency ***	**Mutation rate****	**Cloning efficiency ***	**Mutation rate****
0	73%	0	98%	0

0.215	63%	0	91%	0
0.464	55%	0	85%	0
0.681	64%	0	44%	0
1.0	58%	0	49%	0
2.15	47%	0	12%	0
4.64	0%	0	0%	-
Positive controls	47%	349	61%	141

* % of cloning efficiency in relation to seeded cells (i.e., 200 cells)

** Mutation rate is expressed as number of mutants per 10^6 cells (corrected for cytotoxicity)

Cycloxydim sodium salt is not mutagenic in the *in vitro* mammalian cell (CHO/HPRT) test with or without metabolic activation under the experimental conditions chosen.

3. study

The results of the experiments are compiled in Table below.

Experiment 1 (with and without metabolic activation): Without S-9 mix, concentration-dependent toxic effects (i.e., reduced viability) were observed. No mutation frequency exceeding 74 x 10^{-6} units was observed in any of the test groups, which would indicate a positive effect in this test. In the presence of metabolic activation, concentration-dependent toxic effects were observed. Only at the highly cytotoxic concentration of 20 mg/mL (relative growth of 12%), the mutation rate (86 x 10^{-6} units) was just above the criteria for a positive response (75.6 x 10^{-6} units) with respect to mutant frequency.

Experiment 2 (with metabolic activation): In the repeat experiment, the mutation rate (122 x 10^{-6} units) again exceeded the figure for a positive response (56.5 x 10^{-6}) at the highest concentration that could be evaluated (cytotoxicity was indicated by only 4% relative growth when compared to solvent controls). The negative controls were in the normal ranges. The positive controls demonstrated that the system is able to detect known mutagens.

Table 48: Cytotoxicity and mutation rates in the mouse lymphoma forward mutation assay

Experiment 1				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	**Viability ***	**Mutation rate****	**Viability ***	**Mutation rate****
1.75	84	35	83	44
3.50	70	31	82	42
5.00	67	40	74	42
7.00	53	40	51	49
10.00	18	38	29	46
14.00	24	38	19	50
20.00	10	60	12	86

Experiment 1				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	**Viability ***	**Mutation rate****	**Viability ***	**Mutation rate****
Solvent controls	100	38 - 49	100	40 - 46
Positive controls	28 - 67	361 - 685	35 – 59	302 - 457
Experiment 2				
Concentration	**Without** metabolic activation		**With** metabolic activation	
(mg/mL)	Not tested		**Viability ***	**Mutation rate****
5.00			47	26
6.25			26	33
8.00			8	45
Experiment 2				
Concentration	**Without** metabolic activation		**With** metabolic activation	
10.00			10	54
12.50			4	122
Solvent controls			100	25 – 34
Positive controls			43 - 49	259 - 322

* relative cell growth (compared to solvent control = 100 %) in %,
** mutant frequency x 10^{-6} units

An increased mutation rate was found in the presence of metabolic activation at cytotoxic doses of 20 mg/mL test substance (first experiment, 12% relative growth when compared to control) and 12.5 mg/mL (2nd experiment, 4% relative growth compared to control). According to current OECD guideline recommendations (OECD 476 adopted in 1997), the maximum test concentration to be used in mammalian cell gene mutation tests should not exceed 5 mg/mL; furthermore cytotoxicity at the maximum concentration should be in the range about 10 - 20% relative cell survival (not less than 10% relative cell survival).
Since positive results were obtained at test concentrations that were 2.5 - 4 fold higher than the recommended maximum concentration according to current OECD guidelines, the relevance of the increased mutation rate observed in the presence of cytotoxic effect is questionable. At test concentrations within the recommended range of the non-excessive cytotoxicity, reproducible negative test results were obtained.
Overall, cycloxydim sodium salt is considered not to be mutagenic without metabolic activation and weakly mutagenic only at cytotoxic concentrations (which exceeded by a factor of 2.5 - 4 the maximum concentration as required by guidelines) in the presence of metabolic activation when tested in the mouse lymphoma TK locus assay.

Chromosomal mutation assay with mammalian cell

Cycloxydim Na-salt

In the range-finding experiment without metabolic activation, a reduction of the monolayer confluency of 60 % was noted at 5000 µg/mL, with a decrease in mitotic cells occurring at this concentration in the metaphase of the first mitosis. In the range-finding experiment with metabolic activation no toxicity was observed with the exception of the highest concentration, which showed a slight decrease in the number of mitotic cells. Cell cycle progression was not affected.

The results of the chromosome aberration analysis are summarised in Table below.

Table 49: Cytogenetic assay in CHO cells with Cycloxydim sodium salt

Test **without** metabolic activation (15 hour exposure time)						
	Culture 1 and 2 (../..)			**Pooled** cultures		
	Aberrations per cell	**% cells with aberrations**	**% cells with more than 1 aberration**	**Aberrations per cell**	**% cells with aberrations**	**% cells with more than 1 aberration**
Untreated control	0.01	1.0	0.0	0.005	0.05	0.0
Solvent control	0.00	0.0	0.0			
Test substance						
2000 µg/mL	0.03 / 0.03	3.0 / 3.0	0.0 / 0.0	0.030	3.0	0.0
3333 µg/mL	0.05 / 0.04	5.0 / 3.0	0.0 / 1.0	0.045	4.0*	0.5
4000 µg/mL	0.03 / 0.06	3.0 / 6.0	0.0 / 0.0	0.045	4.5*	0.0
5000 µg/mL	0.03 / 0.11	3.0 / 9.0	0.0 / 2.0	0.070	6.0*	1.0
Positive control	0.24	20.0	4.0	0.24	20.0*	4.0
Test **with** metabolic activation (2 hour exposure time)						
	Culture 1 and 2 (../..)			**Pooled** cultures		
	Aberrations per cell	**% cells with aberrations**	**% cells with more than 1 aberration**	**Aberrations per cell**	**% cells with aberrations**	**% cells with more than 1 aberration**
Untreated control	0.01	1.0	0.0	0.005	0.5	0.0
Solvent control	0.00	0.0	0.0			

Test substance						
2000 µg/mL	0.00 / 0.02	0.0 / 2.0	0.0 / 0.0	0.010	1.0	0.0
3333 µg/mL	0.01 / 0.02	1.0 / 2.0	0.0 / 0.0	0.015	1.5	0.0
4000 µg/mL	0.00 / 0.00	0.0 / 0.0	0.0 / 0.0	0.000	0.0	0.0
5000 µg/mL	0.01 / 0.02	1.0 / 2.0	0.0 / 0.0	0.015	1.5	0.0
Positive control	0.24	24.0	0.0	0.24	24.0*	0.0

Statistical evaluation: * $p < 0.05$ when compared to solvent control (Chi-square test)

Without metabolic activation, a statistically ($p < 0.05$) significant increase in the percentage of cells with aberrations was seen at concentrations of 3333 µg/mL and above, also showing a concentration dependent effect. However, it is noteworthy that mainly chromatid-type aberrations were observed and that the aberrations were seen only in one each of the duplicate cultures. With metabolic activation, there was no increase in aberration frequency at any of the concentrations tested. While aberration frequencies in the negative and solvent controls were within the normal limits, both positive controls showed that the system is able to detect known genotoxic agents causing chromosomal damage.

However, evidence for a clastogenic effect was seen only at high test concentrations (3333 - 5000 µg/mL), which, according to the current revision of the OECD test guideline 473, exceeded the maximum concentration of 0.01 M (= 3255 µg/mL) recommended for testing of non-toxic test substances. In this study, signs of cytotoxicity were seen already at 2000 µg/mL.

No information on the percentage of cells with gaps is present in the study.

Cycloxydim sodium salt caused a weak chromosomal damaging (clastogenic) effect in the absence of metabolic activation at cytotoxic concentrations. With metabolic activation no clastogenic effect of the compound was noted under the test conditions chosen.

Cycloxydim free acid

Cytotoxicity range finding tests

In the range-finding experiment without metabolic activation a precipitation of the test compound was noted at 5000 µg/mL only. This concentration was also highly cytotoxic. A reduction of the monolayer confluency by approx. 60% compared to solvent control was noted at 1666.6 µg/mL, and a decrease in mitotic cells occurred at this concentration. The cell cycle kinetics showed complete mitotic delay at 1666.6 µg/mL with all metaphases still in the first mitosis (see Table below). Therefore, a delayed harvest time of 18 hours was chosen for the aberration test without metabolic activation.

Table 50: In vitro cytogenetic assay with cycloxydim

Test **without** metabolic activation					Test **with** metabolic activation				
	%Cells*			% Confluence		%Cells[a]			% Confluence
	M1	M1+	>M2			M1	M1+	>M2	
Untreated control	25	2	77	70	Untreated control	25	0	75	60
DMSO control	58	0	42	70	DMSO control	20	0	80	60

Test substance					**Test substance**				
16.6 µg/mL	55	0	45	60	500.0 µg/mL	13	0	87	50
50.0 µg/mL	67	0	33	60	1666.6 µg/mL	21	0	79	50
166.6 µg/mL	70	0	30	60	5000.0 µg/mL	16	0	84	10
500.0 µg/mL	63	0	37	55					
1666.6 µg/mL	100	0	0	30					
Mitomycin-C (400 ng/mL)	100	0	0	50	Cyclophosphamide (20 µg/mL)	100	0	0	55

* = cells that have completed one (M1), two (M2) or between one and two (M1+) cycles in BrdUrd

In the assay with metabolic activation confluency of the monolayer at 5000 µg/mL was reduced by 85% when compared to the untreated or solvent control, and a decrease in mitotic cells was noted at this concentration. Because no cell cycle delay was observed, it was decided to use a normal harvest time of 10 hours for the aberration assay with metabolic activation.

Aberration assays with and without metabolic activation

Evaluation of clastogenicity without metabolic activation was performed from cultures treated with 500 µg/mL to 3000 µg/mL. The results of the chromosome aberration analysis are summarised in Table below.

Table 51: In vitro cytogenetic assay with cycloxydim

Test **without** metabolic activation (15 hour exposure time)						
	Culture 1 and 2 (../..)			**Pooled** cultures		
	Aberrations per cell	**% cells with aberrations**	**% cells with more than 1 aberration**	**Aberrations per cell**	**% cells with aberrations**	**% cells with more than 1 aberration**
Untreated control	0.00	0.0	0.0	0.01	1.0	0.0
Solvent control	0.02	2.0	0.0			
Test substance						
500 µg/mL	0.01 / 0.00	1.0 / 0.0	0.0 / 0.0	0.005	0.5	0.0
1000 µg/mL	0.01 / 0.01	1.0 / 1.0	0.0 / 0.0	0.010	1.0	0.0
1500 µg/mL	0.03 / 0.04	3.0 / 4.0	0.0 / 0.0	0.035	3.5	0.0
3000 µg/mL	0.08 / 0.04	8.0 / 4.0	0.0 / 0.0	0.060	6.0*	0.0
Positive control	0.32	28.0	4.0	0.32	28.0*	4.0
Test **with** metabolic activation (2 hour exposure time)						
	Culture 1 and 2 (../..)			**Pooled** cultures		
	Aberrations per cell	**% cells with aberrations**	**% cells with more than 1 aberration**	**Aberrations per cell**	**% cells with aberrations**	**% cells with more than 1 aberration**

Untreated control	0.03	3.0	0.0	0.015	1.5	0.0
Solvent control	0.00	0.0	0.0			
Test substance						
2000µg/mL	0.04 / 0.02	4.0 / 2.0	0.0 / 0.0	0.030	3.0	0.0
3333µg/mL	0.02 / 0.02	2.0 / 2.0	0.0 / 0.0	0.020	2.0	0.0
4000µg/mL	0.05 / 0.00	5.0 / 0.0	0.0 / 0.0	0.025	2.5	0.0
5000µg/mL	0.00 / 0.04	0.0 / 4.0	0.0 / 0.0	0.020	2.0	0.0
Positive control	0.24	20.0	4.0	0.240	20.0*	4.0

Statistical evaluation: * $p < 0.05$ when compared to solvent control (Chi-square test)

In the absence of metabolic activation, a decrease in the number of metaphases was seen from 1000 µg/mL cycloxydim onwards, and a reduction of monolayer confluency of up to 60% was noted at 3000 µg/mL. At a concentration of 3000 µg/mL a statistically significant increase in percent of cells with aberrations (chromatid breaks) was observed. A statistically non-significant increase in the percentage of aberrant cells was observed at the next lower test concentration of 1500 µg/mL, suggesting a dose-related response.

In the presence of metabolic activation, a 50% reduction of the monolayer confluency and a decrease in mitotic cells was observed at the highest concentration of 5000 µg/m; however, there was no increase in aberration frequency at any of the concentration evaluated (2000 to 5000 µg/mL).

Aberration frequencies in the negative and solvent controls were within the normal limits, and both positive controls showed that the system is able to detect known genotoxic agents causing chromosomal damage.

No information on the percentage of cells with gaps is present in the study.

Based on the results of this *in vitro* cytogenetic experiment in Chinese Hamster Ovary (CHO) cells Cycloxydim (acid) caused a weak chromosome damaging (clastogenic) effect in the absence of metabolic activation, statistically significant at a concentration of 3000 µg/mL. The compound is considered <u>weakly positive under the non-activation conditions</u> of this assay in a concentration of 3000 µg/mL.

DNA effect assay with mammalian cell

<u>Cycloxydim Na-salt</u>

No test substance precipitation was noted in this study. Toxic effects with respect to survival and morphology of the hepatocytes were noted at Cycloxydim sodium salt concentrations above 36.3 µg/mL in a dose dependent manner. Strong toxic effects leading to non-scorable slides were noted at the highest concentration of 90.6 µg/mL of Cycloxydim sodium salt. None of the concentrations of the test material caused nuclear labelling significantly different from the solvent control. Furthermore no dose related trend was observable. The number of heavily labelled nuclei representing undergoing DNA replication as opposed to DNA repair was low and did not interfere with the assay. The increase observed with 2-AAF indicated that the test system is able to detect compounds known to induce unscheduled DNA synthesis. A summary of hepatocyte UDS assy results can be found in Table below.

Table 52: Summary of hepatocyte UDS assay results

Test condition	Concentration	*UDS grains / nucleus	**Avg. % nuclei with > 6 grains	**Avg. % nuclei with > 20 grains	***% Survival at 20 hrs.
Negative control	-	0.47	0.7	0.0	100.0
Positive control (2-AAF)	0.1 µg/mL	13.94	90.0	22.7	93.6
Test substance	90.6 µg/mL	Insufficient cells for grain analysis			
	36.3 µg/mL	0.80	1.3	0.0	46.9
	18.1 µg/mL	0.50	2.0	0.0	86.0
	9.06 µg/mL	0.37	0.0	0.0	99.8
	3.63 µg/mL	0.45	0.0	0.0	99.8
	1.81µg/mL	0.45	0.0	0.0	95.3
	0.906 µg/mL	0.37	0.0	0.0	N.D.

*USD is the average value of net nuclear grain counts on triplicate coverslips (150 total cells).
**Average values for triplicate coverslips
***Survival is the number of viable cells per unit area relative to the negative control x 100%

Cycloxydim sodium salt did not induce unscheduled DNA synthesis in primary rat hepatocytes under the test conditions chosen.

Cycloxydim free acid

Concentrations of 250 µg/mL and above caused a transient yellowish precipitation in the culture medium. A permanent precipitation was noted above 1000 µg/mL. Toxic effects with respect to survival and morphology of the hepatocytes were noted at Cycloxydim concentrations above 250 µg/mL in a concentration dependent manner. Strong toxic effects leading to non-scorable slides were noted at the highest concentration of 2000 µg/mL. None of the concentrations of the test material caused nuclear labelling significantly different from the solvent control. Furthermore no concentration-related trend was observed. The number of heavily labelled nuclei representing undergoing DNA replication as opposed to DNA repair was low and did not interfere with the assay. The increase observed with 2-AAF indicated that the test system is able to detect compounds known to induce unscheduled DNA synthesis. Summary of results from rat hepatocyte UDS assay are presented in Table below.

Table 53: Summary of hepatocyte UDS assy results

Test condition	Concentration	*UDS grains / nucleus	**Avg. % nuclei with > 6 grains	**Avg. % nuclei with > 20 grains	***% Survival at 21 hrs.

Negative control (absolute ethanol)	1%	0.54	0.0	0.0	100.0
Positive control (2-AAF)	0.1 µg/mL	8.5	58.7	5.3	102.6
Test substance	2000 µg/mL	Excessively toxicity, grain analysis not possible			27.7
	1500 µg/mL	0.53	0.0	0.0	21.8
	1000 µg/mL	0.69	0.7	0.7	48.6
	750 µg/mL	0.65	0.0	0.0	46.1
	500 µg/mL	0.66	0.0	0.0	60.8
	375 µg/mL	0.94	0.0	0.0	68.7
	250 µg/mL	0.43	0.0	0.0	112.2
	100 µg/mL	0.52	0.7	0.0	101.2

*USD = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
**Average values for triplicate coverslips
***Survival = Number of viable cells per unit area relative to the negative control x 100%

Cycloxydim (acid) is a potent cytotoxic compound, but did not induce unscheduled DNA synthesis in primary rat hepatocytes under the test conditions chosen.

4.9.1.2 In vivo data

Micronucleus test in male and female NMRI mice

Negative and positive control animals did not show any clinical symptoms. Distinct toxic effects were observed at the highest dose level and less pronounced at the lower dose levels. A dose of 900 mg/kg bw of the test substance caused irregular respiration, piloerection, and in some cases squatting posture, spastic gait, excitation and apathy. The general state of the animals was poor and some symptoms lasted up to 1 day after dosing. At the lower dose levels the symptoms were less pronounced and lasted up to 3 hours after dosing.

The administration of cycloxydim at dose levels from 225; 450 and 900 mg/kg bw did not lead to an increase in the number of polychromatic erythrocytes containing micronuclei. The micronuclei rate was comparable to the negative control.

The positive controls induced increases in the micronuclei rates indicating that the test system is able to detect chromosome damaging (clastogenic) or spindle poisoning compounds. Data are summarised in Table below.

Table 54: Summary of NMRI mouse micronucleus test

Cycloxydim mg/kg	**Interval 16h**				**Interval 24h**				**Interval 48h**			
	*PCE investigated	Normocytes / 10000 PCE	Cells with micronuclei		*PCE investigated	Normocytes / 10000 PCE	Cells with micronuclei		*PCE investigated	Normocytes / 10000 PCE	Cells with micronuclei	
			Per 1000 PCE	Per 1000 **NCE			Per 1000 PCE	Per 1000 **NCE			Per 1000 PCE	Per 1000 **NCE
0	-				10000	4256	1.4	1.41	-			
900	10000	4515	1.6	1.33	10000	3127	1.3	1.28	1000	4294	0.7	0.47
450	-				10000	3354	1.6	2.98	-			
225	-				10000	3398	1.1	0.59	-			
***+	-				10000	3830	25.7	0.52	-			

* PCE = Polychromatic erythrocytes
** NCE = Normochromatic erythrocytes
*** + = positive control (cyclophosphamide 40 mg/kg bw)

Cycloxydim as sodium salt did not induce micronuclei in polychromatic erythrocytes of mice when treated orally up to toxic doses of 900 mg/kg bw by gavage.

Chromosomal aberration test in Chinese hamster

No clinical symptoms were noted even at the highest dose (5000 mg/kg body weight). There was no indication of any increase in chromosome aberrations over control levels in the groups treated with the test substance in either sex at any of the preparation intervals.

The positive control Cyclophosphamide induced an increased rate of chromosomal aberrations indicating that the test system is able to detect such effects. The results of the clastogenic evaluation data of the individual animals at the 24 hour intervall in the Chinese hamster bone marrow cytogenetic assay are given in Table below.

Results after 6 and 48 hours (only high dose group animals have been evaluated) are given in Table below.

Table 55: Clastogenic evaluation data of the individual animals at the 24 hour intervall in the Chinese hamster bone marrow cytogenetic assay

Treatment	♂			Treatment	♀		
	No. of cells		Mitotic index %		No. of cells		Mitotic index %
	With aberrations	With >1 aberrations			With aberrations	With >1 aberrations	

Treatment	♂			Treatment	♀		
	No. of cells		Mitotic index %		No. of cells		Mitotic index %
	With aberrations	With >1 aberrations			With aberrations	With >1 aberrations	
- control animals	0.4	0.2	4.5	- control animals	0.6	0.0	6.4
+ control animals	18	12	6.2	+ control animals	24.8	18.2	6.8
animals dosed with 0.5 g/kg	0.6	0.0	5.7	animals dosed with 0.5 g/kg	0.2	0.0	6.3
animals dosed with 1.7 g/kg	0.4	0.0	8.5	animals dosed with 1.7 g/kg	0.6	0.0	7.2
animals dosed with 5.0 g/kg	0.9	0.0	4.1	animals dosed with 5.0 g/kg	0.6	0.0	6.3

Table 56: Clastogenic evaluation data of the individual animals after 6 and 48 hours in the Chinese hamster bone marrow cytogenetic assay (5 mg/kg bw)

Treatment	♂			Treatment	♀		
	No. of cells		Mitotic index %		No. of cells		Mitotic index %
	With aberrations	With >1 aberrations			With aberrations	With >1 aberrations	
after 6 hours	0.0	0.0	4.3	after 6 hours	0.0	0.0	4.5
after 48 hours	0.6	0.2	3.8	after 48 hours	0.4	0.0	3.5

Cycloxydim did not induce chromosomal aberrations under the conditions of this assay and is therefore considered negative in the chromosomal aberration test *in vivo* when Chinese hamsters were treated orally by gavage up to 5000 mg/kg of the sodium salt of Cycloxydim.

4.9.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.9.3 Other relevant information

No other information.

4.9.4 Dossier submitter's summary and discussion of mutagenicity

Cycloxydim was tested in a range of in vitro and in vivo assays measuring different mutagenic endpoints such as gene mutation in bacterial and mammalian cells, chromosomal mutation and unscheduled DNA synthesis in vitro as well as in vivo in the micronucleus test in mice and in the Chinese hamster bone marrow chromosomal aberration test.

The substance has been found to be strongly cytotoxic (without S-9 activation); e.g. in mouse lymphoma cells cytotoxic effects were already found at concentrations of 8-10 mg/mL; Chinese hamster ovary cells were even more sensitive showing toxic effects at 1.67 mg/mL. In addition, in some *in vitro* tests, indications of genotoxic potential were found at cytotoxic concentrations: mouse lymphoma forward mutation test was positive at 20 mg/mL, and the Chinese hamster ovary cells showed increased aberrations at a concentration of 3.0 mg/mL.

Nevertheless, in both *in vivo* studies which also investigated chromosomal aberrations, clear negative results were obtained. In the ADME studies it was shown that cycloxydim was detected in the bone marrow from 5 to 120 hours post-administration. Therefore, the systemic availability of cycloxydim in the *in vivo* genotoxicity studies is considered to be plausible.

4.9.5 Dossier submitter's comparison with criteria

Effects observed in the *in vitro* and *in vivo* mutagenicity studies do not trigger the criteria for classification and labelling for mutagenicity.

4.9.6 Dossier submitter's conclusions on classification and labelling

There is no evidence of genotoxic potential of cycloxydim, therefore, no classification is proposed.

4.9.7 Comments received during public consultation

No specific comments were received on this endpoint.

4.9.8 The RAC assessment – comparison with criteria

In vitro and in vivo testing indicated that cycloxydim has no genotoxic potential. A proposal for classification is not warranted.

4.10 Carcinogenicity

Table 57: Summary table of relevant carcinogenicity studies

Method	NOAEL/Effects	Dose levels	Reference
18-month chronic toxicity Wistar rat drinking water study (OECD 452)	7 mg/kg bw/d (males and females) <u>Main effects at 28 mg/kg bw/d</u>: - body weight ↓ - triglycerides ↓	0, 100, 400, 1600 and 2700 ppm equivalent to 0, 7.0, 28, 103 and 171mg/kg bw/d Purity: 93.9%	Kuehborth B. et al.; 1988(c)
24-month carcinogenicity Wistar rat drinking water study (OECD 451)	6.4 mg/kg bw/d <u>Main effects at 26 mg/kg bw/d</u>: - body weight ↓ - triglycerides ↓ - creatinine ↑ - liver weight ↑ - bile duct proliferation **no treatment related increase in tumor responses**	0, 100, 400 and 1600 ppm equivalent to 0, 6.4, 26 and 99 mg/kg bw/d Purity: 93.9%	Kuehborth B. et al.; 1988(a)
24-month carcinogenicity B6C3F1 mouse drinking water study (OECD 451)	32 mg/kg bw/d - no adverse effects up to the highest dose level **no treatment related increase in tumor responses**	0, 10, 20, 60 and 240 ppm equivalent to 0, 1.3, 3.0, 8.4, and 32 mg/kg bw/d Purity: 93.9%	Kuehborth B. et al.; 1988(b)

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

The long term toxicity and carcinogenicity of Cycloxydim has been investigated in rats and mice. Because of the insufficient stability of the free acid in food and low water-solubility, the sodium salt of Cycloxydim was orally administered either via diet or drinking water. The data in ppm or mg/kg bw/d correspond to Cycloxydim as acid.

Rat:

18 Month chronic rat toxicity study, *Kuehborth B. et al.; 1988(c)*

Cycloxydim sodium salt (batch: N 88; purity: 93.9%) was administered for 18 months in the drinking water to 20 male and 20 female Wistar rats (source: Dr. Karl Thomae GmbH,

Germany) per dose level. The dose levels in the drinking water were 0, 100, 400, 1600 and 2700 ppm. The drinking water solutions were prepared twice a week; stability of the test substance and concentrations were confirmed analytically. Feed consumption was determined once a week for the first 14 weeks. Thereafter, it was determined at 3-month intervals. Body weight and drinking water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week. Ophthalmological examinations were carried out before the start of the study and after 3, 6, 12 and 18 months of the administration.
Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumine, glutamate-pyruvate transaminase, alkaline phosphatase, glutamate-oxalacetate transaminase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean haemoglobin content per erythrocyte, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) as well as urinalyses (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, sediment) were carried out after about 3, 6, 12 and 18 months of administration.
After 18 months of administration all animals were assessed by gross-pathology and histopathology (brain, pituitary, thyroid, parathyroids, thymus, lungs, trachea, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, ln. mesenterialis, ln. mandibularis, pancreas, testes/ovaries, epididymides, prostate, seminal vesicle, female mammary gland, skin, sciatic nerve, skeletal muscle, cervical-, thoriacic- and lumbal cord, sterum, femur, eyes, all gross lesions). Organ weights of selected organs (brain, liver, kidneys, adrenals and testes/ovaries) were determined.
The animals received the concentrations of the test substance as stated in table below:

Table 58: Test substance intake (as free acid)

Drinking water concentration (ppm)	Test substance intake (mg/kg bw/d) Males and females
100	7.0
400	28
1600	103
2700	171

General observations: Survival rates at quaterly intervals were determined on days 91, 182, 273, 364, 455 and 546 of the study (details are listed in table below).

Table 59: Survival rates of animals (absolute and in %)

Day of study	91	182	273	364	455	546
			♂			
0 ppm	19	19	19	19	19	19
100 ppm	95%	95%	95%	95%	95%	95%
400 ppm	20	20	20	19	19	19
1600 ppm	100%	100%	100%	95%	95%	95%
2700 ppm	20	20	20	20	20	20
	100%	100%	100%	100%	100%	100%
	20	20	20	20	20	20

	100% 20 100%	100% 20 100%	100% 20 100%	100% 20 100%	100% 20 100%	100% 19 95%
			♀			
0 ppm 100 ppm 400 ppm 1600 ppm 2700 ppm	20 100% 20 100% 20 100% 20 100% 20 100%	20 100% 20 100% 20 100% 20 100% 20 100%	20 100% 20 100% 20 100% 20 100% 20 100%	20 100% 20 100% 20 100% 20 100% 20 100%	20 100% 18 90% 20 100% 20 100% 20 100%	19 95% 18 90% 20 100% 20 100% 19 95%

Food consumption was reduced by approx. 8 – 9 % throughout the study in both sexes at 2700 ppm and in males at 1600 ppm compared to the control group. Food consumption in treatment groups 400 or 100 ppm were comparable to the amount consumed by the controls.
Drinking water consumption was reduced in males and females of the 2700 ppm and 1600 ppm groups. In males, the reduction of drinking water consumption was more pronounced during the early phases of the study (2 months: 2700 ppm males: 80 % of control level, females: 76 %; 1600 ppm males: 81.5 %, females: 87.5 %; 18 months: 2700 ppm males: 94.5 %, females: 74 %; 1600 ppm males: 94.5 %, females: 80.2 %).
After 18 weeks of treatment, body weight and body weight gains were reduced in males and females of the 2700 ppm; 1600 ppm and 400 ppm groups compared to the respective control values (see tables below).

Table 60: Body weight after 18 months (Day 546)

Dietary dose level	Male body weight		Female body weight	
(ppm)	(g)	(% control)	(g)	(% control)
0	749.9	(100%)	399.0	(100%)
100	711.3	(95%)	380.4	(95%)
400	678.7*	(91%)	357.3*	(90%)
1600	617.9**	(82%)	319.7**	(80%)
2700	589.5**	(79%)	314.1**	(79%)

Statistical evaluation: * $p < 0.05$, ** $p < 0.01$ (ANOVA + Dunnett's tests)

Table 61: Body weight gain within 18 months (Day 0–546)

Dietary dose level	Male body weight		Female body weight	
(ppm)	(g)	(% control)	(g)	(% control)
0	553.5	(100%)	247.9	(100%)

Dietary dose level	Male body weight		Female body weight	
(ppm)	(g)	(% control)	(g)	(% control)
100	513.6	(93%)	230.2	(93%)
400	482.2	(87%)	207.1	(84%)
1600	422.2	(76%)	169.5	(68%)
2700	393.2	(71%)	163.9	(66%)

Hematological changes were rare and females seemed to be more susceptible than males. After the 3-months blood collection, females revealed statistically significant decrease in number of erythrocytes, associated with decreased haematocrit at concentrations of 400 ppm and 1600 ppm, but not at 100 ppm or 2700 ppm, suggesting effects on haematopoetic system. Total bilirubin was measured with the adapted analysis method using sulfanilic acid. No effect on bilirubin content could be observed.
Clinicochemical examinations revealed increased plasma creatinine content after 6 and 18 months in male rats and at all time points measured in female rats. With exception of the final blood collection (18 months after beginning of the study) a concentration of 100 ppm did not show any effect on plasma creatinine concentration (see table below).

Table 62: Creatinine values at 3, 6, 12 and 18 months

Dietary dose level	Creatinine (mmol/l)			
(ppm)	3 months	6 months	12 months	18 months
		♂		
0	56.08 (100%)	55.28 (100%)	59.82 (100%)	53.18 (100%)
100	56.21 (100%)	56.37 (102%)	56.44 (94%)	56.66 (107%)
400	53.84 (96%)	58.68* (106%)	57.71 (96%)	58.54* (110%)
1600	61.13 (109%)	60.04** (109%)	59.93 (100%)	60.17** (113%)
2700	57.30 (102%)	60.86* (110%)	59.89 (100%)	59.17* (111%)
		♀		
0	57.39 (100%)	55.79 (100%)	48.46 (100%)	46.99 (100%)
100	58.83 (103%)	56.42 (101%)	53.17 (110%)	54.62** (116%)
400	61.02 (106%)	61.85* (111%)	58.28** (120%)	55.41** (118%)
1600	62.26* (108%)	60.42* (108%)	53.95* (111%)	54.71** (116%)
2700	62.56* (109%)	60.75 (109%)	55.48* (114%)	54.37** (116%)

Statistical analysis ANOVA / Dunnetts test *P < 0.5, **P < 0.01 two sided

Further clinicochemical examinations revealed a reduction of triglycerides in females of the 2700 ppm; 1600 ppm and 400 ppm groups (see table below). This reduction was noted in all of the blood samples taken after 3, 6, 12 and 18 months. The statistically significant decrease of triglycerides noted in females at 100 ppm after 18-month treatment was considered to have resulted from an exceptionally high control group value of 7.82 mmol/l and not considered toxicological relevant.
In males, a statistically significant decrease of the triglyceride concentration was observed only in the mid-high dose group of 1600 ppm after 12 and 18 months of treatment. This effect was due to decreased triglyceride values of few individual animals.

Table 63: Triglycerides values at 3, 6, 12 and 18 months

Dietary dose level	Triglycerides (mmol/l)							
(ppm)	3 months		6 months		12 months		18 months	
♂								
0	3.67	(100%)	4.90	(100%)	5.24	(100%)	6.28	(100%)
100	3.95	(108%)	5.52	(113%)	6.15	(117%)	5.82	(93%)
400	4.29	(117%)	5.52	(113%)	5.20	(99%)	6.04	(96%)
1600	2.75	(75%)	3.24	(66%)	2.92**	(56%)	3.75*	(60%)
2700	3.45	(94%)	3.99	(81%)	4.05	(77%)	5.03	(80%)
♀								
0	3.74	(100%)	5.27	(100%)	5.89	(100%)	7.82	(100%)
100	3.45	(92%)	4.16	(79%)	5.06	(86%)	5.13**	(66%)
400	2.18*	(58%)	3.30*	(63%)	3.05**	(52%)	4.59**	(59%)
1600	2.68	(72%)	2.73**	(52%)	3.40**	(58%)	4.24**	(54%)
2700	2.02*	(54%)	2.71**	(51%)	2.77**	(47%)	2.59**	(33%)

Statistical evaluation** $p < 0.01$ (t -Test)

Urinalysis did not reveal any test substance-related changes.
There were no ophthalmological changes related to the administration of the test substance.
Organ weight determinations revealed liver weight changes in males and females. Females appeared more succeptible than male rats, because absolute liverweight reduction was already significant at a concentration of 400 ppm (see table below).The reduction of absolute liver weights were considered to be a consequence of reduced body weights at dose levels of 400 ppm and above.

Table 64: Liver weight alteration after 18 months cycloxydim administration

Dietary dose level (ppm)	Liver weight	
	♂	♀

Dietary dose level (ppm)	Liver weight			
Terminal bw (g) 0	749.93	(100%)	399.03	(100%)
100	711.28	(95%)	380.40	(95%)
400	678.73**	(91%)	357.30*	(90%)
1600	617.92**	(82%)	319.66**	(80%)
2700	589.48**	(79%)	314.35**	(79%)
Absolute (g) 0	19.646	(100%)	11.544	(100%)
100	18.776	(96%)	10.836	(94%)
400	18.776	(96%)	10.271*	(89%)
1600	16.791**	(85%)	9.746**	(84%)
2700	17.518*	(89%)	10.147*	(88%)
Relative (g/100 g bw) 0	2.625	(100%)	2.918	(100%)
100	2.647	(101%)	2.854	(98%)
400	2.773	(106%)	2.883	(99%)
1600	2.713	(103%)	3.069	(105%)
2700	2.934**	(112%)	3.220*	(110%)

Pathology/Histopathology: In the liver, bile duct proliferation was more frequently recorded in treated males at 1600 and 2700 ppm than in controls. The incidence across the increasing dose groups was 20% (control), 20, 15, 35 and 40 % in males and 0%, 5, 5, 10 and 0 % in females. The relative number of males with basophilic foci in liver tissue was 10% (control), 5, 35, 10 and 10 %, indicating no dose-relationship.
All other macroscopic as well as microscopic findings were found to be not treatment-related. A number of spontaneous non-neoplastic and neoplastic findings were encountered in various organs of both control and treated rats. The type, incidence and severity of these gross lesions were considered to be similar in all groups. Most lesions occurred in the liver and kidneys of male rats and in pituitary gland, adrenal glands, ovaries, uterus and mammary glands in female rats.

The administration of Cycloxydim via drinking water for 18 months resulted in a significant reduction of body weight in both sexes at and above concentrations of 400 ppm. Body weight was significantly reduced beginning at a concentration of 400 ppm in males and female rats. Drinking water consumption was reduced in the 1600 ppm and 2700 ppm groups. Some significant changes in clinicochemical parameters (reduction of triglycerides; increase of creatinine) were also noted at 400 ppm and above. In addition, effects on liver weight were observed at these dose levels and histopathological changes (bile duct proliferations) in the liver were evident at 1600 and 2700 ppm. However, bile dict proliferation was without dose-response in females and of very doubtful dose response in males. The NOAEL in this study was 100 ppm (7.0 mg/kg bw/d) in males and females. There was no indication of treatment related increase in tumor response.

24 months carcinogenicity rat study, *Kuehborth B. et al.; 1988(a)*

Cycloxydim sodium salt (batch: N 88; purity: 93.9%) was administered in the drinking water to 50 male and 50 female Wistar rats (source: Dr. Karl Thomae GmbH, Germany) per dose level for 24 months. The treatment levels in the drinking water were 0, 100, 400 and 1600 ppm. The controls consisted of 100 males and 100 females.
A stock solution was prepared at the beginning of the study and subsequently at 3-weeks intervals diluted in 2N NaOH. Drinking water solutions were generally prepared twice a week. At the beginning of the study, and after about 4, 8, and 12 weeks and subsequently about every 3 months, samples of the drinking water solution were reanalysed of substance stability. Concentrations were stable at all time-points tested.
Feed consumption was not determined.
Body weight and drinking water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week.
Clinicochemical and hematological examinations (total bilirubin, creatinine, urea, sodium, potassium, total protein, glucose, inorganic phosphate, calcium, chloride, triglycerides, cholesterol, albumin, total protein, glutamate-pyruvate transaminase, glutamate-oxalacetate transaminase, alkaline phosphatase, clotting analysis; haemoglobin, erythrocyte count, hematocrit, mean cell volume, mean corpuscular haemoglobin concentration, platelet count, leukocyte count) as well as urinalysis (pH, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen, sediment) were carried out on 10 animals per sex and dose group towards the end of the administration period. In addition, creatinine, triglycerides and cholesterol levels were determined on all surviving animals at the end of the treatment period.
After 24 months of administration all animals were assessed by gross pathology and histopathology (brain, pituitary, thyroid, parathyroids, thymus, lungs, trachea, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, ln. mesenterialis, ln. cervicalis, pancreas, testes/ovaries, prostate, seminal vesicle, female mammary gland, skin, peripheral nerve, skeletal muscle, cervical-, thoriacic- and lumbal cord, sterum, femur, eyes, all gross lesions). Organ weights of selected organs were determined.

Animals received the intended concentrations of the test substance as stated in table below.

Table 65: Test substance intake (free acid)

Drinking water concentration (ppm)	Test substance intake (mg/kg bw/d)
	Males and females
100	6.4
400	26
1600	99

General observations: There were no test substance-related mortalities or signs of clinical toxicity related to treatment in any of the treatment groups. Survival rates at quaterly intervals are given in table below.

Table 66: Survival rates of animals (absolute and in %)

Day of study / Dosage	91	182	273	364	455	546	637	728
♂								
0 ppm	100	100	100	100	98	92	76	52

Day of study / Dosage	91	182	273	364	455	546	637	728
100 ppm	100%	100%	100%	100%	98%	92%	76%	52%
400 ppm	50 100%	50 100%	50 100%	50 100%	50 100%	48 96%	45 90%	35 70%
1600 ppm	50 100%	49 98%	48 96%	48 96%	47 94%	43 86%	35 70%	24 48%
	50 100%	50 100%	50 100%	49 98%	49 98%	47 94%	38 76%	29 58%
				♀				
0 ppm	100 100%	100 100%	99 99%	99 99%	98 98%	93 93%	81 81%	61 61%
100 ppm	50 100%	50 100%	50 100%	50 100%	49 98%	45 90%	32 64%	27 54%
400 ppm	50 100%	50 100%	50 100%	50 100%	49 98%	48 96%	37 74%	31 62%
1600 ppm	50 100%	50 100%	50 100%	50 100%	50 100%	48 96%	42 84%	34 68%

Drinking water consumption was reduced in males and females of the 1600 ppm group. In females, the extent of the reduction of drinking water consumption was more pronounced during the early phases of the study (see table below).

Table 67: Water consumption

Dose	Treatment duration (months)											
	0–3		0–6		0–9		0–12		0–18		0–24	
	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl
						♂						
0 ppm	28.1	100%	28.4	100 %	28.2	100 %	28.4	100 %	29.9	100%	32.3	100 %
100 ppm	27.6	98%	28.1	99%	27.7	98%	27.8	98%	29.0	97%	31.4	97%
400 ppm	27.2	97%	27.7	97%	27.4	97%	27.6	97%	29.0	97%	31.2	97%
1600 ppm	25.4	90%	26.2	92%	25.7	91%	25.3	89%	27.0	90%	29.4	91%
						♀						
0 ppm	23.6	100%	25.5	100 %	26.1	100 %	27.1	100 %	29.2	100%	31.1	100 %
100 ppm	23.4	99%	24.8	98%	25.5	98%	26.5	98%	29.0	99%	30.9	99%

Dose	Treatment duration (months)											
	0–3		0–6		0–9		0–12		0–18		0–24	
	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl	g/d	%ctrl
400 ppm	22.8	97%	24.9	98%	25.4	97%	26.0	96%	28.9	99%	30.9	99%
1600 ppm	18.3	77%	20.0	79%	20.6	79%	21.2	78%	23.9	82%	26.0	83%

No statistical analysis provided

Body weight gain was reduced in males and females of the 1600 ppm group (table below). At a dose level of 400 ppm and above the reduction of body weight was statistically significant during the first year of treatment in particular in females.

Table 68: Body weight after 12 and 24 months

Drinking water concentration	Body weight (g) – 12 months				Body weight (g) – 24 months			
(ppm)	♂		♀		♂		♀	
0	725.1	100%	410.3	100%	836.5	100%	495.4	100%
100	713.6	98%	402.1	98%	809.2	97%	511.1	103%
400	692.3	95%	373.6**	91%	828.9	99%	457.4	92%
1600	645.9**	89%	339.3**	83%	771.3	92%	405.5**	82%

Statistical evaluation: * $p < 0.05$, ** $p < 0.01$ (ANOVA + Dunnett's Test)

There were no hematological changes that could be related to the administration of the test substance. Clinicochemical examinations revealed a reduction of triglycerides in females of the 1600 ppm and 400 ppm groups, but an increase in male rats in the same dose groups. However, statistical significance was not obtained in both sexes due to the high standard deviation in the control group.

Table 69: Triglycerides values at 24 months

Drinking water concentration	Triglycerides (mmol/l)			
(ppm)	♂		♀	
0	4.39	100%	11.17	100%
100	4.82	110%	13.73	123%
400	7.59	173%	9.51	85%
1600	6.79	155%	5.24	47%

Statistical evaluation: ANOVA + Dunnett's test

Urinalysis did not reveal any test substance-related changes.
Organ weight determinations showed reduced absolute liver weights in females of the 1600 ppm group (see table below). Relative liver weights were significantly decreased in high-dose group females when compared with brain weight, which was similar between treatment groups, but relative liver weights were increased in the same group when related to body weight, indicating that the absolute liver weight decrease was a result of the overall reduction in body weight rather than an organ-specific effect.

Table 70: Absolute and relative liver weights

Dose level	Body wt (g)		Abs. liver wt (g)		Liver wt. relative to body wt. (%)		Liver wt. relative to brain wt. (%)	
♂								
0 ppm	836.5	100%	22.33	100%	2.67	100%	1028	100%
100 ppm	809.2	97%	21.37	96%	2.64	99%	986	96%
400 ppm	828.9	99%	21.08	94%	2.54	95%	991	96%
1600 ppm	771.3	92%	21.90	98%	2.84	106%	1020	99%
♀								
0 ppm	495.4	100%	15.31	100%	3.09	100%	754	100%
100 ppm	511.1	103%	15.52	101%	3.04	98%	757	100%
400 ppm	457.4	92%	14.07	92%	3.08	100%	712	94%
1600 ppm	405.5**	82%	13.52**	88%	3.33	108%	673*	89%

Statistical evaluation: * $p < 0.05$, ** $p < 0.01$ (Dunnett's test, 2-sided); no statistics available for liver wt rel. to body wt)

Gross and histopathological examinations did not show test substance-related effects (except liver) at any dose level. A number of spontaneous non-neoplastic and neoplastic findings were encountered in various organs of both control and treated rats. The type, incidence and severity of these gross lesions were considered to be similar in all groups. Most lesions were age-associated and degenerative, inflammatory or proliferative in character. They mainly affected the large parenchymatous, endocrine or reproductive organs, e.g. kidneys, testes/ovaries as well as adrenals and pituitary gland, but there was no treatment relation.
In the liver, bile duct proliferation was more frequently recorded in treated males than in controls. The incidence across the increasing dose groups was 2% (control), 8%, 16% and 24%, in females 8%, 12%, 4% and 12%. However, all incidences for bile duct proliferation were below comparable historical data from 4 studies (from 1981 to 1986) from the same laboratory, ranging from 14% to 48% incidences of bile duct proliferation in males and up to 18% in females.
Foci or areas of various types of hepatocellular alteration were more frequently recorded in males than in females. The relative number of males with basophilic foci was 7% (control), 22%, 28% and 28%. However, the occurrence of hepatocellular alterations (at the time of studies the alterations were not distinguished in different types) in historical controls (male rats) was even higher (up to 80%) than for cycloxydim treated male rats (28% of basophilic foci in the highest treated group). Since for the historical controls in the years 1981 – 1986 no differentiation in different hepatocellular alteration types was made, no adequate comparison to the effects of cycloxydim on basophilic foci can be made. However, historical control data of

later years (from 1987 to 1992, differentiation in different hepatocellular alteration types) shows even 65% basophilic foci occurrence in the control male animals. The effects in the rat long term study with cycloxydim were therefore below the historical control data from slightly later years. Finally, there was no evidence for the involvement of these or other foci in a carcinogenic process in the liver.

There was neither an evidence of any increase in the incidence of tumour-bearing animals nor any specific tumour type suggestive of a carcinogenic effect attributable to treatment with cycloxydim in Wistar rats.

In a 24-month carcinogenicity study in rats, administration of cycloxydim via the drinking water resulted in a reduction of body weight at concentrations of 400 ppm and 1600 ppm. After 24 months, body weights were decreased by 8% in males and 18% in females of the 1600 ppm group. Drinking water consumption was reduced in the 1600 ppm group. In females, there was a reduction of triglycerides at concentrations of 400 ppm and 1600 ppm. There were no organ weight changes findings at any dose level that would have indicated an organ-specific effect of cycloxydim. Observed bile duct proliferation and basophilic hepatic foci were evident and incidences increased dose-dependently in male rats. However, the incidence of these histopathological findings (bile duct proliferation and basophilic foci), in particular in males, was below the comparable historical control data. Treatment with cycloxydim did not indicate any treatment related increase in tumor responses.

Mouse:

24 month carcinogenicity mouse study, *Kuehborth et al., 1988(b)*

Cycloxydim (batch: N 88; purity: 93.9%) was administered as sodium salt to 50 male and 50 female B6C3F1 mice (source: Charles River Breeding Lab., USA, aged: 42 days and weighing 23 g and 20 g for male and female rats, respectively) per dose level for 24 months via drinking water. The dose levels in the drinking water were 10, 20, 60 and 240 ppm.
A stock solution was prepared at the beginning of the study and subsequently at 3-weeks intervals diluted in 2N NaOH. Drinking water solutions were generally prepared twice a week. Stability of Cycloxydim-Na salt in drinking water over a period of 14 days was confirmed analytically. At the beginning of the study, and after about 4, 8, and 12 weeks and subsequently about every 3 months, samples of the drinking water solution were reanalysed of substance stability. Concentrations were stable at all time-points tested.
Two groups of untreated controls were maintained in parallel for comparison, each with 50 males and 50 females
Drinking water consumption and body weight was determined once a week. Food consumption was not measured. The animals' health was checked at least daily. Detailed clinical examinations were performed once a week.
At the end of the administration periods, differential blood counts were determined in all surviving animals of the control and high dose groups. Differential blood counts were also determined in all animals that died during the study.
After 24 months of administration, all animals were assessed by gross pathology and histopathology (brain, pituitary, thyroid, parathyroids, thymus, lungs, trachea, heart, aorta, salivary glands, liver, spleen, kidneys, adrenals, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, uterus, urinary bladder, ln. mesenterialis, ln. cervicalis, pancreas, testes/ovaries, prostate, seminal vesicle, female mammary gland, skin, sciatic nerve, skeletal muscle, cervical-, thoriacic- and lumbal cord, sterum, femur, eyes, gallbladder, all gross lesions). Organ weights of selected organs (brain, liver, kidneys and testes) were determined.
Animals received the intended concentrations of the test substance via drinking water as stated in table below.

Table 71: Test substance intake (Cycloxydim free acid)

Drinking water concentration (ppm)	Test substance intake (mg/kg bw/d)
10	1.3
20	3.0
60	8.4
240	32

General observations: There were neither test substance-related increased mortalities nor clinical signs of toxicity. Survival rates are given in table below.

Table 72: Survival rates of animals (absolute and in %)

Day of study	91	182	273	364	455	546	637	728
				♂				
0 ppm C1 0 ppm C2 10 ppm 20 ppm 60 ppm 240 ppm	50 100% 50 100% 50 100% 50 100% 50 100% 50 100%	50 100% 50 100% 50 100% 50 100% 50 100% 50 100%	50 100% 50 100% 50 100% 50 100% 49 98% 50 100%	50 100% 49 98% 50 100% 50 100% 49 98% 50 100%	49 98% 49 98% 50 100% 49 98% 48 96% 49 98%	46 92% 49 98% 50 100% 46 92% 47 94% 49 98%	43 86% 46 92% 49 98% 40 80% 44 88% 46 92%	39 78% 38 76% 49 98% 37 74% 40 80% 41 82%
				♀				
0 ppm C1 0 ppm C2 10 ppm 20 ppm 60 ppm 240 ppm	50 100% 50 100% 50 100% 50 100% 50 100% 50 100%	50 100% 50 100% 49 98% 50 100% 50 100% 50 100%	50 100% 50 100% 49 98% 50 100% 50 100% 50 100%	50 100% 49 98% 49 98% 50 100% 50 100% 50 100%	50 100% 47 94% 48 96% 50 100% 50 100% 49 98%	48 96% 46 92% 48 96% 48 96% 48 96% 48 96%	43 86% 43 86% 47 94% 45 90% 47 94% 47 94%	35 70% 35 70% 35 70% 37 74% 39 78% 39 78%

There were no effects on drinking water consumption. Body weight and body weight gain were not affected at any dose level (table below).

Table 73: Body weight after 24 months

Dietary dose level	Body weight (g)	
	♂	♀
0 ppm (control 1)	42.8	41.3
0 ppm (control 2)	40.1	39.6
10 ppm	42.4	39.6
20 ppm	41.8	40.3
60 ppm	40.0	40.3
240 ppm	41.3	39.5

Statistical evaluation: ANOVA + Dunnett's test

There were no test substance-related changes found in the examination of the differential blood counts at any dose level. There were also no test substance-related changes in organ weights (liver weights are given in table below).

Table 74: Liver weights

Dietary dose level (ppm)	♂		♀	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
0 (control 1)	1.498	100	1.677	100
0 (control 2)	1.620	115	1.664	103
10	1.537	104	1.632	101
20	1.422	97	1.628	99
60	1.409	101	1.628	99
240	1.469	102	1.648	103

Statistical evaluation: ANOVA + Dunnett's test

Gross- and histopathological examinations as well did not show any test substance-related effects at any dose level. Most non-neoplastic lesions were age-related and degenerative, inflammatory, or proliferative in character. Although, numerical differences occurred between the groups, the type, incidence, and severity of all lesions did not indicate an organotoxic effect of the test article.
The tumours that occurred corresponded to those of the spontaneous range of the animal strain used so that no carcinogenic potential of the test substance was apparent.

As a result of a 24-month carcinogenicity study in mice, no test substance-related effects could be observed at any of the dose levels (10, 20, 60 and 240 ppm in the drinking water). In addition, detailed examination of pathological and histopathological data exhibited no convincing indication of any treatment related hyperplastic or oncogenic response. Therefore, the NOAEL in this study was 240 ppm, i.e. 32 mg/kg bw/d for male and female B6C3F1 mice.

Remark: According to the relevant guidelines, signs of minimal toxicity should be elicited at the highest dose level which was not the case. It was explained by the notifier that it was unexpected that no toxic effects occurred, since the selection of doses was based on the results of two mouse 28-day ranges-finding studies, where minimal toxicity was observed already at 100 and 300 ppm, and a dose of 240 ppm was expected to elicit signs of toxicity.

Nevertheless, although the validity of the study seems to be limited by this fact, data allow a valid interpretation of the potential oncogenicity of the test compound. In addition, the study was also accepted by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.10.3 Other relevant information

No other relevant information available.

4.10.4 Dossier submitter's summary and discussion of carcinogenicity

Based on the results of two submitted studies in rats (18- and 24 months) and one in mice (24 months study), there are no indications on treatment-related increases in non-neoplastic and neoplastic responses.

4.10.5 Dossier submitter's comparison with criteria

4.10.6 Dossier submitter's conclusions on classification and labelling

No oncogenic effects were observed in studies conducted with cycloxydim, neither in rat nor in mouse carcinogenicity studies (according to both DSD and CLP).

4.10.7 Comments received during public consultation

A Member State wished to have more information on tumour incidences. As there were no indications on any dose-related or treatment-related effect the Dossier submitter decided not to change the documentation.

4.10.8 The RAC assessment and comparison with criteria

There was no indication on any treatment-related increased tumour rates in two oral carcinogenicity studies in rats and in one mouse carcinogenicity study. An increased incidence of bile duct proliferation (without any tumour response originating from the bile duct) was observed in male rats in both rat studies.

The RAC agreed with the proposal that classification with regard to carcinogenicity is not required.

4.11 Toxicity for reproduction

Reproductive toxicity was investigated in rats and rabbits in several studies. Cycloxydim sodium salt was used as test substance instead of the free acid, which could not be used in the feed because of its insufficient stability and could also not be administered in the drinking water on account of its low solubility. All dose levels given in ppm or mg/kg bw/d correspond to cycloxydim as free acid.

Table 75: Summary table of relevant reproductive toxicity studies

Method	NOAEL	Dose levels	Reference
Two-generation study Wistar rats in the drinking water (OECD 416)	Parental: 9.7 mg/kg bw/d Fertility: 129 mg/kg bw/d Offspring: 38 mg/kg bw/d Main effects: Parental: - bw & bw gain ↓ Offspring: - reduced pup survival↓ - growth and developmental retardation	0, 100, 400 and 1600 ppm*, corresponding to 9.7; 38; 129 mg/kg bw/d Purity: 93.9%	Hellwig J. et al., 1988
Prenatal toxicity in Wistar rats (OECD 414)	Maternal: 200 mg/kg bw/d Embryo/fetotoxicity: 200 mg/kg bw/d Main effects at 400 mg/kg bw/d: Maternal: - bw & bw gain ↓ Fetal: - fetal weight ↓ - skeletal retardations and variations (altered ossification centers mainly of thoracic vertebrae bodies)	0, 100, 200 and 400 mg/kg bw/d* day 6 - 15 post coitum in water by gavage Purity: 93.9%	Hellwig J., Hildebrand B.; 1987(a)
Supplemental prenatal toxicity in Wistar rats with special attention to maternal toxicity (OECD 414)	Maternal: 200 mg/kf bw/d No fetal NOAEL questioned Main maternal effects: - bw & bw gain ↓ - changes on clinicochemical & hematological parameters	0, 200, 400 and 600 and 800 mg/kg bw/d* day 6 - 15 post coitum in water by gavage Purity: 93.9%	Hellwig J., Hildebrand B.; 1987(c)
Supplemental pre-, peri-, postnatal toxicity study in Wistar rats (OECD 414)	Maternal: Not established Embryo-/fetotoxicity: Not established Main effects: - skeletal variations in fetuses - effects partially	0 and 400 mg/kg bw/d* day 6 - 15 post coitum in water by gavage Purity:93.9%	Hellwig J., Hildebrand B.; 1987(b)

	reversible in pups - perinatal pup mortality		
Prenatal toxicity in Himalayan rabbits (OECD 414)	Maternal: 100 mg/kg bw/d Embryo-/fetotoxicity: 200 mg/kg bw/d Main effects: Maternal: - food intake ↓ - bw & bw gain ↓ Fetal: - ↑ incidence of fetal external variations	0, 100, 200 and 400 mg/kg bw/d* day 6 - 18 post insemination in water by gavage Purity: 93.9%	Merkle J., Hildebrand B.; 1985 Hellwig J.; 1986 (amendment)

* test substance prepared as sodium salt stock solution, concentrations given as Cycloxydim acid

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Two-generation study Wistar rats in the drinking water, *Hellwig et al., 1988*

Cycloxydim sodium salt (batch N 88; purity: 93.9%) was administered to groups of 24 male and 24 female Wistar rats (source: Karl Thomae, Germany) weighing 247 g and 168 g male and female rats, respectively via the drinking water at concentrations of 0, 100, 400 and 1600 ppm (F_0 parental generation).
A stock solution of the test substance was prepared by dissolving Cycloxydim in 2N NaOH. The end concentrations were achieved by dilution of the stock solution with drinking water. The pH value of the drinking water solutions was adjusted with 0.5N HCl solution.
The doses were selected based on the results of a 4-week study, a 3-month study, and preliminary results of a chronic study in the same rat strain and the same route of administration (drinking water studies with the sodium salt). The doses and test substance intake are calculated as Cycloxydim free acid. The age of the F_0 Generation animals at the beginning of treatment was 55 days. At least 70 days after the beginning of treatment, one female was mated with one male overnight for a period of maximum 3 weeks to produce the F_1 litter (see Table B.6.6.1-1). The F_0 generation was re-mated at least 10 days after the last weaning to produce a second litter. From the F_{1a} pups, 24 males and 24 females/dose group were selected as F_1 parent generation to produce the F_2 generation (one litter). The F_1 generation parental animals received the test substance at least 98 days before mating.
The examination of parental animals included monitoring for clinical symptoms/mortalities, food consumption, body weight development, mating and reproductive performances. Pathological examination was performed by gross inspection as well as extensive histopathological examination with special attention to the reproductive organs. Pups were sexed and monitored with respect to their developmental stages and behaviour in certain tests. All pups were examined macroscopically at necropsy (external and organ findings), stillborn pups and pups that died intercurrently were additionally examined for any skeletal findings.

Table 76: Study design: 2-generation study in Wistar rats

Dose levels	0 (Control)	100 ppm	400 ppm	1600 ppm
F_0 generation – parent animals:	Treatment period at day 55 *post partum* for at least 70 days prior to mating to produce F_{1a} and F_{1b} pups until sacrifice. Infertile animals were re-mated with a fertile partner.			
No. of males	24	24	24	24
No. of females	24	24	24	24
F_1 generation – parent animals:	Treatment at least 98 days prior to mating to produce F_2 pups (only 1 litter) until sacrifice. Infertile animals were re-mated with a fertile partner.			
No. of males	24	24	24	24
No. of females	24	24	24	24
$F_{1a/b}$ and F_{2a} generation pups:	Reared until day 4 *post partum* where 4 males and 4 females were selected for further rearing until day 21 *post partum*. The remainder were sacrificed and examined macroscopically (external and organs). Stillborn pups or pups that died as well as pups showing remarkable findings during rearing or abnormalities during macroscopic inspection were examined according to the method of Wilson (head) and Kimmel (modified) or X-rayed for their skeleton.			

The test substance uptake in the dose groups and in the different generations is given in table below.

Table 77: Test substance intake in - 2-generation study in Wistar rats

Test substance intake (mg/kg bw/d) at drinking water concentrations (ppm)	100 ppm	400 ppm	1600 ppm
F_0 generation animals:			
F_0 males, pre-mating period (days 0–70)	7.46	30.66	113.26
F_0 females, pre-mating period (days 0–70)	9.81	38.12	128.65
Average intake males/females	8.64	34.39	120.96
F_0 females, (F_{1a} litter) gestation period	10.59	43.37	149.09
F_0 females, (F_{1a} litter) lactation period	21.23	85.31	253.15
F_0 females, (F_{1b} litter) gestation period	10.19	37.85	120.82
F_0 females, (F_{1b} litter) lactation period	23.93	83.99	242.99
F_1 generation animals:			
F_1 males, pre-mating period (days 0–98)	9.68	38.19	132.18
F_1 females, pre-mating period (days 0–98)	11.93	44.11	142.06

Test substance intake (mg/kg bw/d) at drinking water concentrations (ppm)	100 ppm	400 ppm	1600 ppm
Average intake males/females	10.81	41.15	137.12
F_1 females, (F_{2a} litter) gestation period	10.36	42.38	149.90
F_1 females, (F_{2a} litter) lactation period	26.58	107.54	368.19

Parental toxicity:
There were no clinical symptoms or mortalities at any dose level in parental animals treated with Cycloxydim sodium salt that could be attributed to the test compound. Effects on food consumption, water consumption, body weight development are summarized in table below.

Table 78: Effects on food consumption, water intake and body weight gain (% control)

	F_0 gen.→ F_{1a} litter			F_0 gen.→ F_{1b} litter			F_1 gen.→ F_{2a} litter		
Dose level (ppm)	100	400	1600	100	400	1600	100	400	1600
Pre-mating period (F_0; days 0 - 70; F1: days 0 - 98)									
Food consumption [a)] ♂	102	101	99	N.A.	N.A.	N.A.	98	98	87
♀	100	98	96	N.A.	N.A.	N.A.	98	94	86
Water consumption [a)] ♂	102	103	95	N.A.	N.A.	N.A.	99	96	71
♀	103	100	81	N.A.	N.A.	N.A.	94	82	56
Body weight gain ♂	102	96	95	N.A.	N.A.	N.A.	96	142	85
♀	98	96	85	N.A.	N.A.	N.A.	95	94	83
Gestation period (days 0 - 20 p.c.)									
Food consumption [a)] ♀	96	93	90	95	91	87	96	96	93
Water consumption [a)] ♀	100	102	83	99	90	67	93	92	72
Body weight gain ♀	96	91	77**	93	85**	73**	104	102	92
Lactation period (days 0 - 21 p.p.)									
Food consumption [a)] ♀	86	90	76	90	87	71	99	100	86
Water consumption [a)] ♀	94	93	65	102	86	56	101	100	77
Body weight gain ♀	76	62*	23**	106	91	78	127	146	111
Body weight (d. 21 p.p.) ♀	97*	95**	88**	98	95**	87**	100	99	88**

Statistical evaluation: * $p < 0.05$, **$p < 0.01$ (Williams test), [a)] no statistical analysis performed;
N.A. = not applicable

At the high dose level of 1600 ppm, the pattern of effects observed during the study differed between the F_0 and F_1 parents:

F_0 generation:

- Reduced food consumption in females during gestation and lactation;
- Reduced water consumption in females throughout the study, most pronounced during lactation of the F_{1a} and F_{1b} litters (65 % and 56 % of control value, respectively);
- Reduced body weight and/or body weight gain in females. Statistically significant during gestation and lactation.

F_1 generation:

- Reduced food consumption in males and females during the premating period, and in females during lactation;
- Reduced water consumption in males during the pre-mating period and in females throughout the study; more pronounced during the pre-mating than during the lactation period (56 % vs. 77 % of control value, respectively);
- Reduced body weight and/or body weight gain in males and females, statistically significant decrease in females during lactation.

At the mid dose level of 400 ppm these effects were less pronounced:

- Water consumption was slightly reduced in F_0 dams during F_{1b} lactation (86%) and in F_1 females during F_{2a} pre-mating (82% of control value).
- Body weight and body weight gain was temporarily reduced in F_0 females during gestation and especially during lactation, while F_1 female body weight development appeared to have been marginally affected during the premating period.

No effects on food consumption, drinking water uptake and body weights/body weight gain were noted at the low dose of 100 ppm that were considered treatment-related.
Concerning pathology/histopathology no treatment-related effects were observed at any dose level.

There were no effects on reproductive function such as mating performance, pregnancy rate, mean duration of pregnancy or littering/lactation of the offspring at any dose level. The relative low fertility index in (F_{1a} litter) at 1600 ppm must be regarded as incidental as all F_0 parents (sires and dams) proved to be fertile at the F_{1b} mating. In addition no such findings were noted for the F_1 parental animals / F_{2a} mating.

Offspring toxicity:
Treatment-related findings in pups (table below) were confined to the highest dose group of 1600 ppm:
On the day of birth (day 0 p.p.) the mean number of live pups/litter was reduced in all high-dose group litters (F_{1a}, -6 %, $p < 0.01$; F_{1b}, -10 %, not statistically significant (n.s.); and F_{2a}, -9 %, n.s.). For the F1a litters only, the reduction in the number of live pups/litter was accompanied by an increased mean number of dead pups/litter (0.84 vs. 0.09, $p < 0.01$); however the percentage of stillborn F_{1b} and F_{2a} pups of the high-dose group were lower when compared to the respective control groups. The viability index appeared to be slightly reduced in the case of the high-dose group F1b pups, but was of a similar range in the high dose group F_{1a} and F_{2a} pups when compared to the control group indices. The lactation index was clearly decreased with regard to the F_{1a} and F_{1b} pups compared to the control, but not in the case of the F_{2a} pups, where the highest incidence of intercurrent deaths (i.e. lowest lactation index) was established in the F_{2a} control group. Pup weight and growth was reduced/retarded for all generations (F_{1a}, F_{1b}, F_{2a}).
A lower mean pup weight was also observed in F_{2a} pups at dose levels of 100 and 400 ppm. These reductions in mean pup weight should not be considered test substance related, but can be explained by the higher number of pups that died during rearing in the F_{2a} controls. Thus, dams of the test substance groups 100 and 400 ppm had to nurse more pups in comparison with the control group. The increased litter size at 100 ppm and 400 ppm corresponds to a 10% higher mean litter weight, however causing a reduced weight development in the groups with high litter size.

Table 79: Pup findings 2-generation study in Wistar rats (F_{1a}/F_{1b} and F_{2a} pups)

Dose level (ppm)	0 (control)	100 ppm	400 ppm	1600 ppm
No. of live pups/litter (day 0 p.p.)				
F_{1a} litter	12.65 (100%)	12.26 (97%)	11.70 (92%)	11.89^{++} (94%)
F_{1b} litter	14.74 (100%)	14.29 (97%)	13.54 (92%)	13.22 (90%)
F_{2a} litter	12.74 (100%)	13.52 (106%)	13.76 (108%)	11.64 (91%)
Viability index (day 4 vs. day 0 p.p.)				
F_{1a} litter	95.94 (100%)	88.75 (93%)	94.98 (99%)	90.58 (94%)
F_{1b} litter	94.55 (100%)	94.97 (100%)	93.62 (99%)	85.77 (91%)
F_{2a} litter	94.10 (100%)	94.33 (100%)	94.12 (100%)	92.77 (99%)
Lactation index (day 21 vs. day 4 p.p.)				
F_{1a} litter	87.63 (100%)	86.83 (99%)	87.51 (100%)	70.74 (81%)
F_{1b} litter	80.39 (100%)	88.88 (111%)	83.17 (103%)	62.31^{+} (78%)
F_{2a} litter	84.10 (100%)	98.34 (117%)	97.91 (116%)	92.90 (110%)
Pup body weight males (g, day 0/21)				
F_{1a} litter day 0	6.12 (100%)	6.03 (99%)	6.13 (100%)	5.95 (97%)
day 21	44.08 (100%)	44.82 (102%)	44.65 (101%)	39.84 (90%)
F_{1b} litter day 0	5.97 (100%)	5.92 (99%)	6.07 (102%)	5.66** (95%)
day 21	41.09 (100%)	39.73 (97%)	38.58 (94%)	33.85** (82%)
F_{2a} litter day 0	6.11 (100%)	5.85 (96%)	5.89 (96%)	5.89 (96%)
day 21	46.84 (100%)	42.69 (91%)	42.07* (90%)	41.65* (89%)
Pup body weight females (g, day 0/21)				
F_{1a} litter day 0	5.74 (100%)	5.75 (100%)	5.72 (100%)	5.67 (99%)

Dose level (ppm)		0 (control)		100 ppm		400 ppm		1600 ppm	
	day 21	41.95	(100%)	40.81	(97%)	42.08	(100%)	36.23*	(86%)
F_{1b} litter	day 0	5.67	(100%)	5.58	(98%)	5.71	(101%)	5.30**	(93%)
	day 21	39.14	(100%)	38.10	(97%)	37.04	(95%)	34.08**	(87%)
F_{2a} litter	day 0	5.77	(100%)	5.63	(98%)	5.56	(96%)	5.64	(98%)
	day 21	46.21	(100%)	41.11*	(89%)	39.79*	(86%)	41.86*	(91%)
Litter size (day 21 p.p.)									
F_{1a} litter		10.65	(100%)	10.00	(94%)	9.91	(93%)	8.16	(77%)
F_{1b} litter		11.35	(100%)	12.46	(110%)	11.00	(97%)	7.39	(65%)
F_{2a} litter		10.39	(100%)	12.87	(124%)	12.95	(125%)	10.09	(97%)
Litter weight (day 21 p.p.)									
F_{1a} litter		469.5	(100%)	448.2	(95%)	442.5	(94%)	325.1	(69%)
F_{1b} litter		466.4	(100%)	495.0	(106%)	424.4	(91%)	250.2	(54%)
F_{2a} litter		483.3	(100%)	539.3	(112%)	530.0	(110%)	421.3	(87%)

Statistical evaluation: * $p < 0.05$ / ** $p < 0.01$ (Williams trend test); $^{+}$ $p < 0.05$ / $^{++}$ $p < 0.01$ (Krauth test)

In F_{1a} and F_{1b} pups of high-dose group dams, morphological development appeared to be retarded: Ear unfolding and opening was delayed in the F_{1b} litter, and eye opening was delayed in both F_{1a} and F_{1b} litters. In addition, an increased number of female F_{1a} pups failed in the gripping (holding) reflex test. These effects on morphological and behavioural development are considered to be related to the growth retardation noted for these pups.
There was no indication of any adverse effects on development in F_{2a} pups up to the highest dose tested. With respect to external, internal and skeletal examination, there were no treatment related effects on F_{1a}, F_{1b} or F_{2a} pups at any dose level.

Cycloxydim was administered as aqueous sodium salt via drinking water (0, 100, 400 and 1600 ppm) to rats in a 2-generation study. At 1600 ppm, clear signs of toxicity such as reduced feed consumption, depression of mean body weights and body weight gain were induced in parental animals. In addition, a pronounced reduction of drinking water consumption was observed. The number of live pups from high-dose group F1a, F1b and F2a litters was reduced at the day of birth, which only in the case of the F1a litter was also associated with an increased number of dead pups on day 0 post partum. Furthermore the lactation index was decreased for F1a and F1b pups (not for F2a pups). While F1a, F1b and F2a pups showed reduced weight and retarded growth rate, the morphological development

was impaired only in the case of F1 pups. Effects on high-dose group F1a and F1b pups observed during the lactation period (increased mortality, delayed growth and development) were most probably a consequence of drastically reduced water intake by the F0 dams (as low as 56% of the control level), which must have had an impact on milk quantity and hence resulted in malnutrition of their pups during lactation. At the mid dose level (400 ppm, corresponding to 38 mg/kg bw/d), signs of parental toxicity were confined to dams, which exhibited reduced feed and water consumption as well reduced body weights and/or body weight gain during some parts of the study. No effects were noted for the pups at this dose level. No adverse findings were observed in parents at 100 ppm (equivalent to 9.7 mg/kg bw/d). The fertility was not affected at any of the dose levels tested. Following NOAEL have been established:

NOAEL offspring toxicity: 400 ppm (corresponding to 38 mg/kg bw/d), based on reduced survival, growth and developmental retardation in pups at 1600 ppm.

NOAEL parental toxicity: 100 ppm (corresponding to 9.7 mg/kg bw/d), based on reductions in feed consumption, lower body weight and body weight gain in dams at $\geq$ 400 ppm.

Fertility was not affected by treatment up to 1600 ppm (about 129 mg/kg bw/d), the highest dose tested.

4.11.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Rat:

Prenatal toxicity in Wistar rats, *Hellwig & Hildebrand, 1987(a)*

Cycloxydim sodium salt (batch no. N 88; purity: 93.9%) was examined for its prenatal toxicity in Wistar rats (source: K. Thomae, Germany), weighing 212 g, aged between 11 and 12 weeks. Dams (25/test group) were treated from day 6 through day 15 post coitum (p.c.) with Cycloxydim sodium salt daily in doses of 0 (control treated with the vehicle double distilled water), 100, 200 and 400 mg/kg bw/d by gavage at a constant dosing volume of 10 mL/kg bw/d. Purity, homogeneity and stability of Cycloxydim were determined prior to initiation of the study. In addition the stability of the test substance in the vehicle (double distilled water) was investigated and homogeneity and correctness of dose preparations at the 100 and 200 mg/kg bw/d dose levels were examined.
The animals were observed for food consumption and body weight gain regularly throughout the study period. Their state of health was checked daily. On day 20 p.c. all females were sacrificed and assessed by gross pathology. Implantations, resorptions, corpora lutea were determined and pre-/post implantation loss and conception rate were calculated. The fetuses were dissected from the uterus, sexed and weighed. About 2/3 of the fetuses were subjected to assessment of the skeleton, about 1/3 of the fetuses were assessed for visceral abnormalities. The fetuses intended for assessment of the skeleton were also eviscerated and the organs were assessed (after being fixed in ethanol). The findings were differentiated between retardations, variations and anomalies.

Maternal findings: There were no clinical signs or mortalities noted in treatment-group dams. Food consumption was slightly reduced at the onset of dosing (days 7 and 8) gaining statistical significance (about 8% absolute level) at the high dose level of 400 mg/kg bw/d and at the mid and high dose level (4% and 8%, respectively) when compared on a mg/kg bw/d base. At

the end of the treatment period, body weight gain at the high dose level was 13% below the control (Day 13-15, no statistical significance). The assessment of the difference between the body weights of the dams at at the beginning of the study and at termination (without uterus) showed a decrease of 9% at 400 mg/kg bw/d, which was statistically significant compared to control values (table below). The slight statistically significant decrease in feed consumption at 200 mg/kg bw/d is considered incidental because it did not correspond with changes of the net body weight change.

Table 80: Maternal data: first prenatal toxicity gavage study in Wistar rats

Dose (mg/kg bw)	0	100	200	400
Food consumption:				
- Abs. (g) Day 7–8	47.6 (100%)	48.0 (101%)	45.8 (96%)	43.8* (92%)
- Rel. (g/kg bw) Day 7–8	196.0 (100%)	194.8 (99%)	187.9* (96%)	179.8** (92%)
Body weight gain				
Bw gain (g) day 6-15	43.35 (100%)	41.82 (96%)	39.83 (92%)	39.96$^{n.s}$ (92%)
Bw gain (g) day 13-15	11.04 (100%)	10.13 (92%)	10.67 (97%)	9.61$^{n.s}$ (87%)
Corrected body weight gain (net weight change during the study)	65.1 (100%)	69.2 (106%)	63.1 (97%)	59.5* (91%)

Statistical evaluation: * p<0.05, **p<0.01, (Williams test); n.s. – not statistically significant

Evaluation of Caesarean sectioning revealed no treatment-related effects on uterus or placental weights, number of corpora lutea, incidence of live or dead implantations, incidence of early, intermediate or late resorptions, pre- or postimplantation loss, or on the incidence of live or dead fetuses.

Fetal findings: At the 400 mg/kg bw/d level, a statistically significant reduction of the mean fetal weight (especially in the male fetuses: -5.2 %; overall: -4.3 %) was observed. No significant effects on fetal weight were observed at 200 or 100 mg/kg bw/d (see table below).

Table 81: Data at Caesarean section/fetal examination – first prenatal toxicity gavage study in Wistar rats

Dose (mg/kg bw/d)	0	100	200	400
Mean fetal weight (g)				
Males	3.85 (100%)	3.82 (99.2 %)	3.74 (97.1 %)	3.65** (94.8 %)
Females	3.66 (100%)	3.66 (100%)	3.57 (97.5 %)	3.50* (95.6 %)

Statistical evaluation: fetal weights: * p<0.05, **p<0.01 (Williams test)

The assessment of external abnormalities did not reveal any substance-induced increase compared with the control even at the highest dose level.
The incidence of skeletal changes was not increased up to a dose of 200 mg/kg bw/d. At a dose level of 400 mg/kg bw/d, however, a pronounced increase of changes of the vertebral column and the sternebrae was observed (details are given in table below), which the study authors classified as "anomalies", "variations" and "retardations".

- Statistically significantly increased number of fetuses/litter with retardations (bipartite ossification centers of the vertebral bodies in the thoracic region, incomplete ossification of the sternebrae) of the skeleton. Although these findings are also seen in the controls, their increased incidence is clearly attributable to the test substance administration at 400 mg/kg bw/d and is in line with the reduced fetal body weight at this dose level.
- Statistically significant increase in the number of anomalies (dumbbell-shaped ossification centers of the thoracic region, bipartite-shaped ossification centers of the thoracic region) when compared to the untreated control. The majority of anomalies were mainly found in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage. According to current harmonized classification criteria, these skeletal findings termed "anomalies" in the study report are today classified as variations (bipartite ossification centers of the xyphoid process, insufficient cartilaginification of the 13th rib).
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Table 82: Incidence of skeletal effects in prenatal rat study

	Incidence at dose level [mg/kg bw/day]			
	0	100	200	400
SKELETAL ANOMALIES, TOTAL				
Fetal incidence	11/197 (5.6%)	13/174 (7.5%)	13/205 (6.3%)	67/199 (34%)
Litter incidence	8/23 (35%)	9/23 (39%)	10/24 (42%)	20/23 (87%)**
"Dumbbell"-shaped ossification centers, thoracic region, fetal incidence	5/197 (2.5%)	8/174 (4.6%)	9/205 (4.4%)	60/199 (30%)
"Bipartite"-shaped ossification centers, thoracic region, fetal incidence	0/197 (0.0%)	4/174 (2.3%)	2/205 (1.0%)	7/199 (3.5%)
SKELETAL VARIATIONS, TOTAL				
Fetal incidence	23/197 (12%)	20/174 (12%)	29/205 (14%)	42/199 (21%)
Litter incidence	15/23 (65%)	13/23 (57%)	14/24 (58%)	15/23 (65%)
"Bipartite" ossification centers of the xyphoid process, fetal incidence	2/197 (1.0%)	3/174 (1.7%)	5/205 (2.4%)	8/199 (4.0%)
Insufficient cartilaginification of the 13th rib (ossification centers present), fetal incidence	21/197 (11%)	15/174 (8.6%)	23/205 (11%)	33/199 (17%)

	Incidence at dose level [mg/kg bw/day]			
	0	100	200	400
SKELETAL RETARDATIONS, TOTAL				
Fetal incidence	88/197 (45%)	78/174 (45%)	95/205 (46%)	150/199 (75%)
Litter incidence	20/23 (87%)	22/23 (96%)	22/24 (92%)	23/23 (100%)
"Bipartite" ossification centers of the vertebral bodies in the thoracic region (without "cones" of cartilage), fetal incidence	0/197 (0.0%)	0/174 (0.0%)	0/205 (0.0%)	4/199 (2.0%)
Incomplete ossification of the sternebrae (cartilage present), fetal incidence	10/197 (5.1%)	14/174 (8.0%)	31/205 (15%)	66/199 (33%)

Statistical significance: */# = p < 0.05; ** = p < 0.01 (# Krauth Asymptotic Test; * Fisher Exact Test)

Taking into account also visceral findings (small thymus size, hydronephrosis, dilated kidney, and reduced size of testis) in fetuses, the following differences between the groups were noted:

Table 83: Summary of fetal examinations

	Incidence at dose level [mg/kg bw/day]			
	0	100	200	400
ANOMALIES, TOTAL				
Fetal incidence	11/293 (3.8%)	14/259 (5.4%)	14/309 (4.5%)	67/295 (23%)
Litter incidence	8/23 (35%)	10/23 (43%)	11/24 (46%)	20/23 (87%)**
% Fetuses per litter	4.94%	5.00%	4.38%	21.84%##
VARIATIONS, TOTAL				
Fetal incidence	23/293 (7.8%)	20/259 (7.7%)	29/309 (9.4%)	42/295 (14%)
Litter incidence	15/23 (65%)	13/23 (57%)	14/24 (58%)	15/23 (65%)
% Fetuses per litter	7.57%	12.03%	9.04%	13.75%#
RETARDATIONS, TOTAL				
Fetal incidence	98/293 (33%)	92/259 (36%)	101/309 (33%)	155/295 (53%)
Litter incidence	20/23 (87%)	22/23 (96%)	23/24 (96%)	23/23 (100%)

	Incidence at dose level [mg/kg bw/day]			
% Fetuses per litter	31.85%	35.24%	33.28%	51.76%$^{\#\#}$

Statistical significance: */# = p < 0.05; **/## = p < 0.01 (# Krauth Asymptotic Test; * Fisher Exact Test)

Cycloxydim when given as aqueous sodium salt from day 6 through 15 post coitum to pregnant Wistar rats via gavage caused no adverse effects on the maternal and foetal organism at 100 or 200 mg/kg bw/d. At the top dose of 400 mg/kg bw/d dams showed a statistically significant reduction on food consumption during onset of dosing (days 7–9 p.c.). Body weight gain at the end of the study (day 20) was 13% lower than control. In fetuses from high-dose group dams, reduced foetal weights correlated with an increase in the number of skeletal retardations. In addition, the incidence of skeletal variations (termed "anomalies" in the study report) was increased and were mainly found in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage. A NOAEL of 200 mg/kg bw/d was achieved for maternal- and embryo-/fetotoxicity in this study.

Supplemental prenatal toxicity in Wistar rats with special attention to maternal toxicity, *Hellwig & Hildebrand, 1987(c)*

The purpose of this supplementary study in Wistar rats (source: K. Thomae, Germany) weighing 219 g, aged between 10 and 14 weeks, was to determine the dose of Cycloxydim sodium salt (batch: N 88; purity: 93.9%) that elicits maternal toxic effects in pregnant animals. Investigation of the fetal toxicity was confined to assessment of resorption rates and to fetal weight determinations. 25 rats per group were treated from day 6 through day 15 post coitum (p.c.) with Cycloxydim doses of 0 (control treated with the vehicle double distilled water), 200; 400 and 600 and 800 mg/kg bw/d by gavage and at a constant dosing volume of 10 mL/kg bw/d. Half of the total daily dose was given twice a day at an interval of at least 4 hours to avoid test substance flocculation.
The dams were observed for food consumption and body weight gain regularly throughout the study period. Their health was checked daily. Hematology and clinicochemical parameters were determined on days 16 and 20 of the study in 10 animals to better understand maternal toxicity at these dose levels in pregnant rats (for details of parameters examined see table below).

Table 84: Scope of extended examination of dams - second prenatal toxicity gavage study in Wistar rats

End point	Parameters examined
Clinical findings	Clinical symptoms, food consumption, body weight, body weight gain, corrected body weight at study termination
Clinical chemistry	Enzymes: Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase Blood chemistry: Sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, iron, copper, zinc, manganese
Hematology	Leukocytes, erythrocytes, hemoglobin, hematocrit, mean cell volume, mean hemoglobin content per erythrocyte, mean corpuscular hemoglobin concentration, platelets

End point	Parameters examined
Organ weights	Liver, kidneys, adrenals (mean and absolute weights)

On day 20 p.c. all females were sacrificed and assessed by gross pathology. Caesarean parameters such as implantations, resorptions, corpora lutea were determined and pre-/post implantation loss and conception rate were calculated. The fetuses were dissected from the uterus, sexed, weighed and examined externally. After fixation they were examined macroscopically. No further fetal examinations were performed.

Maternal toxicity: There were no mortalities in treatment group rats. However, the following clinical symptoms were observed that could be related to treatment:

- 800 mg/kg bw/d: Two dams showed vaginal hemorrhages, three further dams were found to have fur smeared with urine.
- 600 mg/kg bw/d: Dam no. 79 temporarily showed reduced nutritional state, piloerection and intermittent respiration.

On days 7 - 8 there was a (substantially dose-dependent) reduction in feed consumption in all the treated groups. Whereas the marginal effect at the 200 mg/kg bw/d dose level was assessed as incidental in the absence of effect on body weight, the reduced feed intake at 400 mg/kg bw/d may have been test substance related since it was associated with body weight loss at the same time point of investigation. The effect on feed consumption at 600 and 800 mg/kg bw/d persisted throughout the treatment period.
Compared to control values, a statistically significantly decrease in body weight was noted at the 600 and 800 mg/kg bw/d dose levels, while there was no statistical significance and biological relevance at 200 or 400 mg/kg bw/d throughout treatment. Body weight gain was affected on days 6 - 8 at 400, 600 and 800 mg/kg bw/d. At later intervals only dose levels of 600 and 800 mg/kg bw/d were affected in some cases and overall body weight gain was reduced at 600 and 800 mg/kg bw/d (83.9 % and 84.4 %, respectively). The corrected body weight gain was significantly below the respective control value at 400 mg/kg bw/d and higher doses.

Table 84: Maternal toxicity: Effects on food consumption and body weight

Dose mg/kg bw/d	0	200	400	600	800
Food consumption [g] Day 7–8 (% control)	21.7[$] (100%)	20.2 (93.1%)	19.2 (88.5%)	16.5 (76.0%)	16.3 (75.1%)
Food consumption[g] Day 0–20 (% control)	22.7[$] (100%)	23.0 (101.3 %)	23.3 (102.6 %)	21.7 (95.6%)	21.6 (95.2%)
Body weight [g] Day 20 (% control)	342.8 (100%)	337.8 (98.5%)	340.0 (99.1%)	322.8** (94.1%)	323.1** (94.2%)
Body weight gain [g] Day 6–8	4.14	3.63	-0.78**	-4.54**	-4.95**
Body weight gain (g) Day 0–20 (% control)	126.0 (100%)	116.6 (92.5%)	119.3 (94.7%)	105.7** (83.9%	106.3** (84.4%)
Corrected body weight gain[a] [g] (% control)	58.6 (100%)	53.6 (90.8%)	52.0* (88.1%)	42.9** (72.7%)	45.6** (77.3%)

Statistical evaluation: * p < 0.05 - ** p< 0.01 (Williams test) [$] no statistics performed
[a] Corrected body weight = (Final body weight– uterus weight) – body weight at start

Clinicochemical parameters did show clear substance related effects (table below). The increase of creatinine at the high dose level is in line with the toxicological profile of Cycloxydim and has been observed in other toxicological studies. It is unclear whether the increase in reticulocytes observed at the second examination point at day 20 must be interpreted as a compensatory effect of the bone marrow due to blood sampling at day 16 post coitum or as a consequence of the reduced hemoglobin-, erythrocyte- and hematocrit values observed at the first sampling. Leucocytes were increased on day 16 p.c., in the highest does-group associated with increased polymorphonuclear neutrophilic granulocytes (2.96 $\pm$ 0.35 giga/l) compared to controls (1.71 $\pm$ 0.23 giga/l). Five days after test-substance withdrawal, some affected parameters could not be reproduced; this could be the consequence of increased feed consumption after substance administration had been stopped.

Table 85: Clinical chemistry and hematology results

Affected parameters	Sampling day 16 *post coitum*	Sampling day 20 *post coitum*
Creatinine	Control (µmol/l): 49.01 (100%) 800 mg/kg bw: 55.51** (113.3%)	No effect
Inorganic phosphate	Control (mmol/l): 2.53 (100%) 400 mg/kg bw/d: 2.31* (91.3%) 600 mg/kg bw/d: 2.24** (88.5%) 800 mg/kg bw/d: 2.18** (86.2%)	Control (mmol/l): 2.22 (100%) 800 mg/kg bw: 1.88* (84.7%)
Erythrocytes, hemoglobin, hematocrit and reticulocytes	Erythrocytes: Control (tera/l): 6.85 (100%) 400 mg/kg bw/d: 6.33** (92.4%) 600 mg/kg bw/d: 6.37* (93%) Hemoglobin: Control: (mmol/l): 8.01 (100%) 400 mg/kg bw/d: 7.41** (92.5%) 600 mg/kg bw/d: 7.46* (93.1%) 800 mg/kg bw/d: 7.46* (93.1%) Hematocrit: Control (l/l): 0.351 (100%) 400 mg/kg bw/d: 0.328* (93.4%) 600 mg/kg bw/d: 0.331* (94.3%)	Reticulocytes: Control ($^{o}/_{oo}$): 72 (100%) 600 mg/kg bw/d: 98** (136%) 800 mg/kg bw/d: 110** (153%)
Leucocytes	Control (giga/l): 7.21 (100%) 800 mg/kg bw/d: 9.02* (125%)	No effect

Affected parameters	Sampling day 16 *post coitum*	Sampling day 20 *post coitum*
Alkaline phosphatase	Control (µkat/l): 4.90 (100%) 600 mg/kg bw/d: 3.74** (76.3%) 800 mg/kg bw/d: 4.03* (82.2%)	No effect
Urea	Control (mmol/l): 8.57 (100%) 600 mg/kg bw/d: 11.41** (133.1%)	No effect
Triglycerides	Control (mmol/l): 4.10 (100%) 400 mg/kg bw/d: 2.31** (56.3%) 600 mg/kg bw/d: 2.54* (62.0%) 800 mg/kg bw/d: 2.42* (59.0%)	No effect
Copper	Control µmol/l): 26.43 (100%) 200 mg/kg bw/d: 30.83* (116.6%) 400 mg/kg bw/d: 30.79* (116.5%) 600 mg/kg bw/d: 30.76* (116.4%)	No effect

Statistical evaluation: * p<0.05 **p<0.01 (ANOVA + Dunnetts tests)

Selected organ weights measured (absolute and relative liver, kidney and adrenal weights) showed no difference comparable to the untreated control.

Fetal findings: Assessment of embryo-/fetomortality revealed no decrease in the number of live fetuses and no increase in prenatal mortality. Some macroscopic findings such as absent/filiform tail and or anal atresia and one skin defect in 2 resp. 3 litters were observed in a very small number of fetuses at 600 or 800 mg/kg bw/d. Fetal weight was statistically significantly decreased at 600 and 800 mg/kg bw/d (table below).

Table 86: Fetal weights (g)

Dose (mg/kg bw/d)	0	200	400	600	800
Males	3.76	3.69 (98%)	3.62 (96%)	3.34** (89%)	3.26** (87%)
Females	3.57	3.50 (98%)	3.47 (97%)	3.20** (90%)	3.11** (87%)
Males & females	3.66	3.62 (99%)	3.54 (97%)	3.26** (89%)	3.18** (87%)

Statistical evaluation: fetal weights: * p<0.05, **p<0.01 (Williams test)

A supplemental prenatal toxicity study using the same rat strain, the same route of administration and doses ranging from 200 to 800 mg/kg bw/d was conducted to elucidate maternal toxicity in more detail than normally done in a routine OECD 414 study. The maternal findings observed in the first study were confirmed. This holds for the NOAEL for maternal toxic effects (200 mg/kg bw/d), the effects on food consumption, body weight and body weight change and the corrected body weight gain. Additional examinations on clinical chemistry, hematology and selected organ weights revealed increased creatinine values at 800 mg/kg bw/d, decreased inorganic phosphate levels at 400 – 800 mg/kg bw/d and a weak effect on red blood cells – reduced hemoglobin, erythrocyte and hematocrit values at doses of 400 mg/kg bw/d and above. Furthermore, clinical findings (vaginal hemorrhages, fur smeared with urine) were observed in a few animals at 800 mg/kg bw/d. Foetal weights were reduced at 600 and 800 mg/kg bw/d.

Supplemental pre-, peri-, postnatal toxicity study in Wistar rats, *Hellwig & Hildebrand, 1987(b)*

The purpose of this supplementary study in rats was to investigate the persistence of the changes to vertebral bodies observed in the prenatal toxicity study at high dose levels of Cycloxydim. Cycloxydim sodium salt (batch N 88; purity: 93.9%) was tested with respect to pre-, peri-, postnatal toxicity in Wistar rats (source: K. Thomae, Germany) weighing 220 g, aged between 11 and 13 weeks.

The test substance was administered orally by gavage daily from day 6 through 15 of gestation at doses of 0 and 400 mg/kg bw/d. For details of dosing see table below. For assessment of prenatal toxicity, groups of 25 dams from the control and 400 mg/kg bw/d groups were killed on day 20 of gestation.

- Perinatal toxicity of Cycloxydim was investigated in control and treatment groups each consisting of 10 dams that were allowed to litter and killed on day 7 after birth of the offspring.
- Postnatal toxicity was investigated following treatments of 25 dams and study termination 3 weeks after birth of the offspring.

Table 87: Dosing scheme – pre-, peri, postnatal toxicity study in Wistar rats

Group number	Number of females mated	Concentration (mg/100 mL)	Dose volume (mL/kg bw)	Dosage level (mg/kg bw/d)
Prenatal experiment: Dams dosed from day 6 through 15 *post coitum* and sacrificed on day 20 *post coitum*				
1	25	-	10*	0*
2	25	4000	10	400
Postnatal experiment: Dams dosed from day 6 through 15 *post coitum* and sacrificed on day 7 (10 dams from group 3 and 4) or 21 (25 dams) after parturition				
3	35	-	10*	0*
4	35	4000	10	400

*Control dams received only the vehicle (double distilled water)

Investigations of the dams included monitoring of body weight, body weight change, corrected body weight gain (dams sacrificed day 20 p.c. only), clinical symptoms as well as pregnancy

and parturition parameters such as duration of pregnancy, number of live and dead pups at birth and litter size, fertility index and pregnancy index, determination of uterus weight, number of corpora lutea, live fetuses, conception rate, pre- and post-implantation loss.
Fetuses were examined with respect to their viability, weight, and sex ratio. Placental weights were recorded prior to fixation of fetuses for internal and skeletal examination.
Pups were examined for clinical signs daily including gross morphological anomalies, body weight and body weight gain on days 4, 7 and in the case of pups sacrificed at day 21 after birth also on days 14 and 21. Their sex ratio was determined and different developmental stages were monitored (erection of the auricles on day 4 after birth, opening of the auditory channel on day 13 after birth and opening of the eyes on day 16 after birth. Behavioural tests (righting test and swimming test) were also performed. Viability index, surviving rate and lactation index were calculated. Pups sacrificed on day 7 post partum (p.p.) were fixed in alcohol and their skeleton was stained and examined. Pups sacrificed on day 21 p.p. were first X-rayed before the skeletal fixation and examination with special attention to their vertebral column.

Prenatal study segment:
The following findings were noted in dams sacrificed on day 20 post coitum with respect to clinical examinations that were considered test substance related:

- Reduced body weight gain during treatment days 6–15 p.c. (-18% compared to controls, no statistical evaluation performed)
- Marked reduction with respect to body weight gain on day 6–8 (-64% compared to controls)
- Reduced corrected body weight gain (-9% compared to controls, no statistical significance).

Table 88: Maternal toxicity: Effects on body weight gain

Dose mg/kg bw/d	0 (Gr. 1)	400 (Gr. 2)	0 (Gr. 3)	400 (Gr. 4)
	N=22	N=23	N=32	N=28
Body weight gain [g] Day 6–8 (% control)	6.23 (100%)	2.22** (36%)	5.53 (100%)	2.54** (46%)
Body weight gain [g] Day 6–15 (% control)	44.02 (100%)	38.83 (88.2%)	47.47 (100%)	37.61 (79.2%)
Corrected body weight gain[a] [g] (% control)	72.45 (100%)	65.74 (91%)	N.A.	N.A.

Statistical evaluation: * p < 0.05 - ** p< 0.01 (Williams test)
[a] Corrected body weight = (Final body weight– uterus weight) – body weight at start

There were no significant and/or dose-related differences between the test groups with regard to uterus weights. No substance-related and/or significant differences were noted in the conception rate, mean number of corpora lutea, live fetuses, dead implantations, total implantations, or in the values calculated for the pre- and post-implantation losses. In the treatment-group, the mean fetal weight was slightly decreased (93% of control fetus weights). Furthermore, the number of runts (i.e. fetuses weighing 75% or less of the mean fetal weight per litter) was increased (see table below).

Table 89: Effect on fetal weights – prenatal study segment

Test group (dose level)	1 (control)	2 (400 mg/kg bw/d)

Test group (dose level)	1 (control)	2 (400 mg/kg bw/d)
Fetal weight in g: total (males/females)	3.82 (100.0%) (3.88/3.70)	3.54** (92.6%) (3.62**/3.46**)
Number of runts	1	6

Statistical evaluation: *p < 0.05, ** p < 0.01 (Williams test)

Further macroscopic examinations of the fetuses after Caesarean section showed no effects on sex distribution. Singular incidences of anomalies were recorded in the control (1x Brachygnathia superior) and in the treatment group fetuses (1x cleft palate, 1x caudal vertebrae absent, 1x shortened tail, 1x anasarca; 3 litters affected). Retardations of fetal organs (enlarged renal pelvis uni- or bilateral) were detected on both test groups but were found in the control group at higher incidences than in test group 2.

The skeletal findings in fetuses are summarised in the table below.

Table 90: Summary of skeletal findings – prenatal study segment

Test group / dose level		1 (control)	2 (400 mg/kg bw/d)
Total "Anomalies"			
Mainly dumbbell-shaped or bipartite ossification centers of vertebral body/bodies with involvement of cartilage (= variations according to current harmonised criteria)	Fetal incidence	38/237 (16.0%)	183/272 (67.3%)
	Litter incidence	12/22 (54.6%)	22/22** (100.0%)
	% Fetuses/litter	14.23%	68.01%$^{\#\#}$
Total Variations			
Vertebral column (notches of the cartilage but unchanged bony part of vertebral bodies or dumbbell-shaped vertebral bodies but unchanged cartilage), sternum (bipartited or dislocated ossification centers), rib (insufficient cartilaginification of 13th rib, ossification centers present)	Fetal incidence	68/237 (28.7%)	146/272 (53.7%)
	Litter incidence	18/22 (81.82%)	22/22 (100.0%)
	% Fetuses/litter	29.17%	55.72%$^{\#\#}$
Total Retardations			
Incomplete or missing ossification of sternebrae, cranial bones and vertebral column	Fetal incidence	141/237 (60.8%)	232/272 (85.3%)
	Litter incidence	20/22 (90.9%)	22/22 (100.0%)
	% Fetuses/litter	59.12%	85.39%$^{\#\#}$

* = p< 0.05; **/$^{\#\#}$ = p< 0.01 (*=Fisher Exact Test, $^{\#}$=Krauth test, asymptotic)

A marked increase of dumbbell-shaped or bipartite ossification centers of the vertebral bodies in thoracic region was observed in treatment group fetuses. The dumbbell-shaped changes occurred considerably more frequently than in the main study for both the control and treatment groups, while regarding the "bipartite" changes, similar results were obtained in the

main and in this study. In addition, a retarded ossification of the fetal skeleton was noted at increased incidence in the fetuses of dams administered Cycloxydim.

Postnatal segment findings:
The following test substance related findings were noted in test group 4 (dams sacrificed on days 7 or 21 after birth):

- Marked reduction with respect to body weight gain on day 6–8 p.c. (-54% compared to controls), no statistical significance at other 3-day intervals.
- Reduced body weight gain during treatment days 6–15 p.c. (-21% compared to controls, no statistical evaluation performed)

The marginal differences of the above-mentioned parameters during lactation being non-uniform between animals sacrificed on day 7 or 21 after birth are regarded as incidental. Litter data and macroscopic, internal and skeletal findings in pups are given in tables below.

Table 91: Fertility and Pregnancy indices (percentage of live / dead pups of test groups 3 and 4) - pre-, peri-, postnatal gavage toxicity study in Wistar rats

Test group/dose	Dams sacrificed day 7 after birth		Dams sacrificed day 21 after birth	
	3 (control)	4 (400 mg/kg bw/d)	3 (control)	4 (400 mg/kg bw/d)
Litters examined	10	10	22	18
Total number of live pups (Day 0 p.n.)	112	129	271	198
Mean number of live pups/dam (Day 0 p.n.)	11.2	12.9	12.3	11.0**
Total number of dead pups (Day 0 p.n.)	2	3	3	11
Mean number of dead pups/dam (Day 0 p.n.)	0.2	0.3	0.14	0.61**

Statistical evaluation: *p < 0.05, ** p < 0.01 (Fisher Exact test)

The perinatal mortality was slightly increased in pups from dams sacrificed on day 21 after birth. Mortality did not further increase. No effects were noted with respect to developmental stages examined and behavioural tests performed.
The skeletal examination of the fetuses showed the following findings (tables below).

Table 92: Pup skeletal examination sacrificed on day 7 after birth

Test group / dose level		3 (control)	4 (400 mg/kg bw/d)
Total anomalies			
Mainly dumbbell-shaped or bipartite ossifications on the vertebral bodies in the	Fetal incidence	8/110 (7.3%)	43/129 (33.3%)
	Litter incidence	5/10 (50%)	10/10* (100%)

Test group / dose level		3 (control)	4 (400 mg/kg bw/d)
thoracic region (variations according to current harmonised criteria)	% Fetuses/litter	8.88%	33.60%$^{\#\#}$
Total variations			
Mainly insufficient cartilaginification of the 13t^{h} rib (ossification centers present	Fetal incidence	8/110 (7.3%)	15/129 (11.6%)
	Litter incidence	6/10 (60%)	7/10 (70%)
	% Fetuses/litter	8.05%	12.15%
Total retardations			
Mainly "bipartite" (or notched) ossifications on thoracic vertebral bodies; incomplete ossification of the sternebrae (cartilage present)	Fetal incidence	1/110 (0.9%)	15/129 (11.6%)
	Litter incidence	1/10 (10%)	4/10 (40%)
	% Fetuses/litter	0.67%	10.59%$^{\#}$

*/$^{\#}$ = p< 0.05; **/$^{\#\#}$ = p< 0.01 (*=Fisher Exact Test, $^{\#}$=Krauth test, asymptotic)

Table 93: Pup external, skeletal examination sacrificed on day 21 after birth – pre-peri-, postnatal gavage toxicity study in Wistar rats

Test group / dose level		3 (control)	4 (400 mg/kg bw/d)
Total anomalies			
Mainly dumbbell-shaped or bipartite ossifications on the vertebral bodies in the thoracic region	Fetal incidence	12/263 (4.6%)	53/193 (27.5%)
	Litter incidence	9/22 (40.9%)	13/17* (76.5%)
	% Fetuses/litter	4.41%	26.56%$^{\#\#}$
Total variations			
Mainly insufficient cartilaginification of the 13t^{h} rib (ossification centers present)	Fetal incidence	44/263 (16.7%)	40/193 (20.7%)
	Litter incidence	16/22 (72.7%)	13/17 (76.5%)
	% Fetuses/litter	15.87%	19.82%
Total retardations			
Mainly "bipartite" (or notched) ossifications on thoracic vertebral bodies; incomplete ossification of the sternebrae (cartilage present)	Fetal incidence	1/263 (0.4%)	13/193 (6.7%)
	Litter incidence	1/22 (4.55%)	5/17* (29.4%)
	% Fetuses/litter	0.51%	5.65%

*/$^{\#}$ = p< 0.05; **/$^{\#\#}$ = p< 0.01 (*=Fisher Exact Test, $^{\#}$=Krauth test, asymptotic)
[1] classified as "variation" according to current criteria [see 2001/1021583 Solecki R. et al. 2001].

A supplemental pre-/peri-/postnatal study was performed in rats to investigate the extent of reversibility of the skeletal variations that were observed at increased incidences in fetuses of the main prenatal toxicity study at 400 mg/kg bw/d using the same rat strain. Cycloxydim sodium salt at a dose level of 400 mg/kg bw/d or the vehicle was administered by gavage to groups of pregnant rats from day 6 through 15 post coitum. Groups of dams were either killed on day 20 of gestation, or were allowed to rear their offspring for one or three weeks and killed thereafter. The effects noted in dams from the prenatal segment were comparable to the maternal toxicity observed in the main prenatal toxicity study. This also holds true for the pattern of foetal findings at Caesarean section (decreased fetal weight, marked increase in dumbbell shaped or bipartite vertebral body/bodies and retarded skeletal ossification). In the postnatal study segment, the same pattern of maternal effects was noted. Perinatal pup mortality (deaths on day 0-1) was slightly increased in pups from dams sacrificed on day 21 post partum (p.p.), but not in pups from dams killed on day 7 p.p., although treatment was identical. The same type of skeletal variations (termed "anomalies" in the study report) as in the main prenatal toxicity study was found. Specific effects in foetuses (mainly: dumbbell-shaped or bipartite ossification centers of vertebral bodies; insufficient cartilaginification of 13^{th} rib and missing or incomplete ossification of sternebrae) occurred. Examinations of pups 7 days and 21 days after birth showed that skeletal changes were only partially reversible. Insufficient cartilaginification of the 13^{th} rib was found to be reversible whereas dumbbell-shaped or bipartite ossification centers of vertebral bodies and missing or incomplete ossification of sternebrae were not reversed.

Rabbit:

Prenatal toxicity study in Himalayan rabbits, *Merkle & Hildebrand 1985 and Hellwig, 1986 (amendment)*

The sodium salt of Cycloxydim (batch: N 88; purity: 93.9%) in aqueous medium was administered to 15 female Himalayan rabbits (source: K. Thomae GmbH, Germany) with an average weight of 2.37 kg, aged: 25 to 31 weeks per test group by gavage from day 6 through 18 post insemination (p.i.). Doses selected were 0 (control), 100, 200 and 400 mg Cycloxydim /kg bw/d in a constant volume of 10 mL/kg bw/d. The control group achieved vehicle (distilled water) only.
Prior to initiation of the study the stability and homogeneity of the test substance were proven and a reanalysis was performed after the termination of the study. The stability and homogeneity of the test compound preparation were examined prior to study initiation. Analysis of intended dose concentrations and the homogeneity of the test compound in the carrier were carried out twice during the study.
Body weight and food consumption was monitored throughout the study. The animals were examined twice daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 29 (p.i.) and the fetuses were delivered by Caesarean section. Post mortem examinations, including gross macroscopic examinations of all internal organs. Caesarean section parameters such as number of corpora lutea, uterus weight, number of implantations, resorptions, and live/dead fetuses were determined. Fetuses were sexed, weighed and further examined for any external, soft tissue and skeletal findings. In addition to X-ray analysis the fetuses from the 400 mg/kg bw/d dose groups and individual fetuses from the control group were stained according to the method of Kimmel and assessed comparable to rats in order to determine whether vertebrae body effects observed in rats also occur in rabbits using similar examination techniques.

Maternal data: One dam from the low-dose (100 mg/kg) and one dam from the high-dose group (400 mg/kg) died shortly before the 8th and 10th treatment, respectively. Clinical signs were confined to one case of premature birth at 200 mg/kg bw/d and one case of abortion by a doe dosed with 400 mg/kg bw/d. Findings of premature birth and/or abortion are common in this rabbit strain and not considered treatment-related.

At 400 mg/kg bw/d, food consumption was statistically significantly reduced from days 7–21 p.i. ($p<0.01$, Williams Test), especially towards the end of test substance administration during days 15–18 p.i., where food consumption was reduced by more than 56% when compared to control food intakes. Food consumption of high-dose group rabbits increased after the end of the administration period and was even higher than in controls during days 22–29 p.i.
During the administration period, the dams continuously lost weight, amounting to a total of 100 g from days 6–18 p.i. (-4% of the initial body weight), while control rabbits gained weight by about 69 g (+3% of the initial body weight) during gestation days 6–18. Statistically significant reductions of body weight were observed on days 16–21 p.i. ($p<0.05$, Williams Test), thereafter, high-dose group rabbits gained body weight from days 21–29 p.i. (i.e. after the end of treatment). The mean net body weight gain [(bwend - uterus wt) - bwstart] at 400 mg/kg bw/d was -120 g compared to -68.5 g of control group dams; statistical significance was not attained for this difference.
At 200 mg/kg bw/d, mean daily food intakes were transiently reduced during days 12–16 p.i. (77–80% of control intakes, statistically not significant) but were similar to control levels for the other time points of investigation. Body weight gain was reduced on days 11–14 p.i. (6.86 g vs. 42.36 g; $p<0.05$, Williams Test) and increased on days 21-23 p.i. (36.14 g vs. 10.29 g; $p<0.05$, Williams Test). No other statistically significant effects on body weight or body weight gain were observed during the study.
At 100 mg/kg bw/d there were no effects observed that were suggestive of maternal toxicity.

Caesarean section/fetal toxicity
At the high dose level, uterus weights were decreased by -17% when compared to control values. The decreased uterus weight was considered to be the consequence of an increase of dead implantations in combination with a decrease of viable fetuses. Historical data were additionally supplied but were of limited relevance, because in no cited study (distilled) water was administered orally. No other parameters were affected at Caesarean section. Details of findings are given in tables below.

Table 94: Findings at Caesarean section

Parameter	Dose (mg/kg bw/d)				Historical control data[a]			
	0	100	200	400	A	B	C	D
Uterus weights (g)	341.86	322.92	343.23	284.58[+]	N.D.	N.D.	N.D.	N.D.
Implantations / pregnant animal	7.21	7.31	6.92	6.75	4.94	5.57	6.31	5.47
% viable implantations / p. animal	90.94	87.50	87.67	81.53[#]	68.75	90.00	91.01	83.0
% resorptions / pregnant animal	9.06	12.50	12.33	18.47[#]	31.25	10.00	9.00	17.0
- Early resorptions	9	10	9	8	N.D.	N.D.	N.D.	N.D.
- Intermediate resorptions	0	0	0	4	N.D.	N.D.	N.D.	N.D.
- Late resorptions	1	2	2	2	N.D.	N.D.	N.D.	N.D.
- Dead fetuses	0	0	0	1	N.D.	N.D.	N.D.	N.D.
Mean fetal weight (g)	39.3	37.3	41.2	36.5	N.D.	N.D.	N.D.	N.D.

[a] Historical control data compiled for Chbb: HM Himalayan-Rabbit; vehicle used: A: carboxymethylcellulose; B: 0.5% NaCl percutaneously, C: inhalation; D: oil
Statistical evaluation: [+] $p<0.05$ (William's test), [#] $p<0.05$ (Krauth asymptotic test)

Regarding tables below no clear dose-response relationship can be estimated for the presented findings. For fetal external and skeletal variations a NOAEL can be clearly set at 200 mg/kg bw/d. The incidences at this dose were all within HCD and were not statistically significant. For soft tissue variations there is a statistically significant increase at 200 mg/kg bw/d, but not at 400 mg/kg bw/d. Regarding specific variations, no treatment related effects on foetuses were observed below maternally toxic dose (below 200 mg/kg bw/d), except for fetal incidences of separated origin of the carotids. However, in this study the concurrent control had also higher incidences than historical control data and it seems that this finding is very common in this train (93.3% litter incidence in the concurrent control and in HCD). Additionally, no dose response could be observed in litter incidences from lowest to highest dose tested.

Table 95: Total incidence of variations

	Dose levels (mg/kg bw/d)				Historical control data		
Total variations							
	0	100	200	400			
Fetal incidence	58/91	67/83	70/79	62/66			
Litter incidence	13/14 (92.86%)	13/13 (100%)	13/13 (100%)	12/12 (100%)			
% fetuses/litter	63.20	80.13	87.40#	92.98##			
Fetal external variations ("Caesarean section")							
	0	100	200	400	Mean	Min	Max
Fetal incidence	0/91	2/83	1/79	8/66	24/1552 (1.5%)	0.0%	5.3%
Litter incidence	0/14 (0.0%)	2/13 (15.4%)	1/13 (7.7%)	4/12 (33.3%)	18/247 (7.3%)	0.0%	15.4%
% fetuses/litter	0.0	3.53	1.10	9.62	1.4%	0.0%	4.1%
Soft tissue ("organ") variations							
	0	100	200	400	Mean	Min	Max
Fetal incidence	58/91	65/83	69/79	57/66	434/1552 (28.0%)	12.1%	58.7%
Litter incidence	13/14 (92.9%)	13/13 (100%)	13/13 (100%)	12/12 (100%)	182/247 (73.7%)	46.2%	100%
% fetuses/litter	63.20	77.75	86.30#	85.24	28.6%	11.3%	58.0%
Fetal skeletal variations							
	0	100	200	400	Mean	Min	Max
Fetal incidence	3/91	4/83	1/79	10/66	181/1552 (11.7%)	4.1%	19.8%
Litter incidence	2/14 (14.3%)	3/13 (23.1%)	1/13 (7.69%)	7/12 (58.3%)	117/247 (47.4%)	14.3%	80.0%
% fetuses/litter	3.06	4.33	0.96	16.79	12.0%	4.1%	24.7%

= p< 0.05; ## = p< 0.01 (Krauth test, asymptotic)

[a] x / y = Number affected / Number examined

Historical control data: 17 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986-1993

Table 96: Specific variations

Test group / dose level	Dose (mg/kg bw/d)				Historical control (20 prenatal rabbit studies from 1986-1993)		
	0	100	200	400	Mean	Min	Max
External variations detected at Caesarean sectioning							
Pseudoankylosis							
Fetal incidence	0/91[a] (0%)	2/83 (2.4%)	1/79 (1.3%)	8/66 (12%)	24/1552 (1.5%)	0.0%	5.3%
Litter incidence	0/14 (0%)	2/13 (15.4%)	1/13 (7.7%)	4/12 (33.3%)	18/247 (7.3%)	0.0%	15.4%
Soft tissue variations							
Separated origin of the carotids							
Fetal incidence	58/91 (64%)	65/83 (78%)	69/79 (87%)	57/66 (86%)	315/1552 (20.3%)	5.3%	58.7%
Litter incidence	13/14 (93%)	13/13 (100%)	13/13 (100%)	12/12 (100%)	145/247 (58.7%)	15.4%	93.3%
Skeletal variations							
Asymmetrical sternebra(e)*							
Fetal incidence	3/91 (3.3%)	3/83 (3.6%)	1/79 (1.3%)	5/66 (7.6%)	38/1552 (2.4%)	0.0%	8.1%
Litter incidence	2/14 (14.3%)	2/13 (15.4%)	1/13 (7.7%)	4/12 (33%)	35/247 (14.2%)	0.0%	38.5%
Fused sternebra(e)							
Fetal incidence	0/91 (0.0%)	0/83 (0.0%)	0/79 (0.0%)	4/66 (6.1%)	66/1552 (4.3%)	0.0%	8.8%
Litter incidence	0/14 (0%)	0/13 (0%)	0/13 (0%)	3/12 (25%)	46/247 (18.6%)	0.0%	38.5%

X/Y = No. fetuses(litters) with effect / no. fetuses (litters) examined
* Asymmetrical sternebrae are now summarised under the term "sternebra(e) with irregular shape
Historical control data: 17 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986-1993

Additional histopathological examination of the vertebrae did not indicate any substance-related changes.

In a prenatal toxicity study in Himalayan rabbits, cycloxydim sodium salt was administered via oral gavage at dose levels of 0, 100, 200 or 400 mg/kg bw/d from days 6–18 post insemination. Food consumption and body weight gain were clearly impaired in dams at 400 mg/kg bw/d and to a very small extent (body weight gain/food consumption) at 200 mg/kg bw/d. No effects on foetuses were observed below maternal toxic concentrations. At a dose of

400 mg/kg bw/d, foetuses showed a higher number of external variations which were above historical control data. Based on maternal effects at this dose (statistically significantly reduced food consumption from days 7 to 21, reduced utersus weight) these effects are considered as consequence of maternal toxicity. There were no teratogenic effects observed at any dose level tested.

4.11.2.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.11.3 Other relevant information

Table 97: Summary table of other relevant reproductive toxicity studies

Method	Effects	Dose range	Reference
Supplemental *in vitro* whole embryo culture study in rat embryos day 9.5 of gestation for 48 hours (no Guideline applicable, supplemental information)	No indication of a embryotoxic effect by cycloxydim or by the main metabolite TSO	300 µg/mL Cycloxydim 150 µg/mL TSO metabolite Purity: 90.14%	Neubert D.; 1987

This in vitro study has been performed to further elucidate if the anomalies on the skeleton (observed in the in vivo rat studies at a dose level of 400 mg/kg bw/d) were maternally induced.To investigate direct embryotoxic effects, rat embryos were exposed to concentrations of 300 µg/mL Cycloxydim (batch: N 88; purity 90.14%) or 150 µg/mL of the main metabolite TSO (batch: L28/145; purity: not reported). Based on evaluation of toxicokinetic data, the chosen Cycloxydim concentration was considered to correspond to serum peak levels that result from oral gavage treatment at 400 mg/kg bw/d (oral dose of at which the skeletal changes of the thoracic vertebra were observed). For TSO (the sulphoxide metabolite of Cycloxydim), the peak serum concentration was assumed to be less than half of the Cycloxydim peak serum level.
The rat embryos aged 9.5 days were exposed for 48 hours to the culture medium, with either addition of DMSO (vehicle control was used to dissolve the test compounds), Cycloxydim or the TSO. At least 30 embryos were used for the 3 sub-experiments carried out on each tested compound (table below). The following parameters were examined: yolk sac diameter, crown-rump length, number of somite pairs, protein content (µg/embryo), developmental score according to Klug and embryos showing abnormal development (macroscopic and histological evaluation).

Table 98: Study design: Wistar rat whole embryo culture study in vitro with Cycloxydim and main metabolite

Compound	Sub experiment	Total no. of embryos	No. of embryos examined by histology
Culture medium control	A	8	1
Culture medium control	B	13	2
Culture medium	C	12	1

Compound	Sub experiment	Total no. of embryos	No. of embryos examined by histology
control			
DMSO (solvent) control	A	6	1
DMSO (solvent) control	B	11	2
DMSO (solvent) control	C	13	1
Cycloxydim (300 µg/mL)	A	6	1
Cycloxydim (300 µg/mL)	B	15	4
Cycloxydim (300 µg/mL)	C	14	4
TSO (150 µg/mL)	A	4	1
TSO (150 µg/mL)	B	15	4
TSO (150 µg/mL)	C	15	4

The results of the study are compiled in table below. Following 48-h in vitro exposure of fetuses to TSO at 150 µg/mL no evidence of abnormal development was detectable using various parameters including histology. Fetal exposure to Cycloxydim at 300 µg/mL resulted in a slight, statistically significant growth retardation (evident as reduced protein content, and a very slightly reduced crown rump length that led to a very slightly reduced overall score). These changes can be interpreted as unspecific signs of cytotoxicity. However no abnormal morphogenetic differentiation could be observed when the embryos were examined with a stereomicroscope. The extensive histological investigations also revealed no evidence for a substance-related interference with morphogenetic differentiation. Some necroses observed were within the physiological range of developing embryonic tissues.

Table 99: Results – Wistar rat whole embryo culture study in vitro with Cycloxydim and main metabolite

Test group	Conc. (µg/mL)	No. of embryos	Yolk sac diameter mm	Crown-rump length mm	No. of somites	Protein µg/embryo	Develop-mental score	No. of abnormal embryos
Control	0	33	4.7	4.1	27	237	38	0
			4.6	4.0	26	212	37	
			4.3	3.8	26	195	35	
DMSO	5 µL DMSO/ 7 mL medium	30	4.6	4.2	27	244	38	0
			4.4	3.9	27	216	37	
			4.2	3.7	26	180	36	
Cycloxydi	300	35	4.5	4.0	27	199	37	0

Test group	Conc. (µg/mL)	No. of embryos	Yolk sac diameter mm	Crown-rump length mm	No. of somites	Protein µg/embryo	Develop-mental score	No. of abnormal embryos
m	µg/mL		4.4	3.8*	26	175**	36*	
			4.3	3.7	26	162	35	
TSO	150 µg/mL	34	4.5	4.1	27	221	38	0
			4.4	3.9	26	205	37	
			4.4	3.8	26	192	35	

Statistical evaluation: * p>0.01 and < 0.05 / ** p < 0.01 (Mann-Whitney Test)

No abnormal morphogenetic differentiation was observed in vitro using whole embryo culture technique in 9.5 day old Wistar rat embryos by exposing them to concentrations of Cycloxydim or the TSO metabolite of 300 µg/mL and 150 µg/mL, respectively. The study was made available as a supplemental investigation but not for assessing a NOAEL.

4.11.4 Dossier submitter's summary and discussion of reproductive toxicity

In a two-generation drinking water study in rats (Hellwig et al., 1988), no effects on fertility were observed at any dose tested. At 400 ppm, maternal toxicity included reduced feed and water consumption as well as reduced body weight and/or body weight gain. Therefore, the maternal NOAEL was set at 100 ppm. The developmental NOAEL was set at 400 ppm, based on reduced survival, growth and developmental retardation in pups at 1600 ppm (at a maternally toxic dose).

In a prenatal toxicity rat study (Hellwig & Hildebrand, 1987a), no effects on foetuses were observed below the level at which maternal toxicity occurred, i.e. 400 mg/kg bw/d (measured as a statistically significant reduced body weight and body weight gain of dams). The foetuses of the 400 mg/kg bw/d group showed delayed ossifications which contributed to lower foetal weight/slower development as the consequence of maternal effects (lower body weight/gain). No malformations were observed.

In a prenatal toxicity study with special attention to maternal toxicity (Hellwig & Hildebrand, 1987c), dams showed lower food consumption, statistically significantly reduced corrected body weight gain, statistically significantly decreased RBC, haemoglobin and haematocrit, all at 400 mg/kg bw/d. No detailed foetus examination was conducted, since the emphasis of the study was on maternal toxicity. However, at 400 ppm, no effects on foetal weight were observed.

In a supplemental pre-, peri-, postnatal toxicity study in rat (Hellwig & Hildebrand, 1987b), the persistence of changes to vertebral bodies caused at 400 mg/kg bw/d was investigated. Insufficient cartilaginification of the 13th rib was observed but was reversible at 7 and 21 day p.p., while dumbbell-shaped or bipartite ossification centers of vertebral bodies and missing or incomplete ossification of sternebre were still present at day 7 and 21 p.p. However, it should be noted that at 400 mg/kg bw/d in this study, the dams had very marked reduction of body weight gain from days 6 to 8 (-64%) and marked reduction in body weight gain during treatment days 6 to 15 (-18%).

In an *in vitro* whole embryo culture study in rat, no indication of embryotoxic effects by cycloxydim or by the main metabolite TSO were observed. The effects observed in rat foetuses in other studies were therefore considered to be a consequence of maternal toxicity.

In a prenatal rabbit toxicity study (Merkle & Hildebrand, 1985 and Hellwig, 1986 (amendment)), no effects on rabbit foetuses were observed below the level at which maternal toxicity (at 100 mg/kg bw/d) was observed. Maternal toxicity at 200 mg/kg bw/d was reported as a reduced mean daily food intake (-20% from days 12 to 16), statistically significantly reduced body weight gain from days 11 to 14 and from days 21 to 23. The effects on foetuses at 200 mg/kg bw/d were either without any dose response or within historical control data. No teratogenic effects were observed.

4.11.5 Dossier submitter's comparison with criteria

No treatment related effects on fertility or development were observed in studies conducted with cycloxydim, neither in rat multigeneration study, nor in rat and rabbit developmental studies (according to both DSD and CLP). All observed effects on foetuses were below maternally toxic dose, which was mainly obvious in reduced food consumption, reduced body weight and body weight gain. No malformations were observed in any of the studies, but primarily delayed ossifications contributed to the maternal toxicity. It is considered that there is no evidence of developmental effects of cycloxydim given and that no classification for reproduction for this substance is needed.

4.11.6 Dossier submitter's conclusions on classification and labelling

There is no evidence of effects on reproduction and development caused by cycloxydim, therefore, no classification is proposed.

4.11.7 Comments received during public consultation

There were no specific comments received on reproductive toxicity during the public consultation of the CLH proposal. However, during a targeted expert consultation (5-19 November 2012), one Member State Competent Authority and one expert provided information on the interpretation of some data concerning maternal and developmental toxicity effects of cycloxydim and the relevance of the findings for classification. For further details see *"Overall conclusion of the rat developmental toxicity studies (Hellwig & Hildebrand, 1987a-c)"* below.

4.11.8 Additional key elements: Consultation of RAC members and experts from public

Further clarification on the observed effects was requested by rapporteurs. Members were asked for their views on whether a proposal for classification of developmental effects should be considered. Dossier submitter's proposal on non-classification had been distributed for puplic consultation. According to the RAC procedures on CLH a second consultation is needed if deviating higher classification categories as distributed for public consultation are proposed for agreement in RAC. RAC members were asked for answers on specific questions on the interpretation of the observed skeletal effects in pubs and on the weighing of maternal toxicity at doses that caused pup effects and whether classfication should be discussed as alternative option in RAC. Six members sent their views (see ORCOM) and agreed that discussion of non-classification and classification on developmental toxicity should take place.

One member suggested to consider the dumbbell-shaped or bipartite ossification on the vertebral bodies in the thoracic region that remain until 21 days after birth should not be considered as minor developmental changes and should be considered relevant for classification. In the Solecki et al., 2001 dumbbell-shaped ossification is described as a variant, which in general would not justify classification.

Consequently ECHA launched a second public consultation that was targeted on this endpoint from 5-19 Nov 2012. One Member State (Spain) and one expert sent their comments. One comment suggested that dumb-bell shaped ossification centers with the cartilage affected can

be evaluated as a malformation. The other comment considered dumbbell shaped ossification centers in the thoracic region as variation or retardation but not as a malformation. The latter did not discriminate between two dumbbell effects with or without cartilage involvement.

4.11.9 The RAC assessment and comparison with criteria

Fertility

The RAC agreed with the Dossier submitter's view that in the absence of treatment-related effects on reproductive function, a proposal for classification could not be justified.

Developmental toxicity

2-generation drinking water rat study (Hellwig et al., 1988)

Cycloxydim had smaller effects on F1 pup mortality and viability at Day 0 until Day 4 at 1600 ppm, which was 90% to 94% of control values, corresponding to reductions in food and water consumptions (Table 79) and lower body weight gain in F0 dams (Table 78).

No statistically significant adverse effect on pup survival and development was observed in the F2-pups. The only effect observed was a lower body weight in high dose F2 pups at postnatal day 21 (Table 79). Corresponding findings in F1 dams were significantly reduced body weight gain and significantly lower absolute weight as compared to controls at postnatal day 21 (-13%) and reduced food and water consumption during the lactation phase (-14 % and -23%, resp., Table 78,), In general, reduction in food consumption and reduction in body weight gain during gestation and lactation was less prominent in F1 dams compared to F0 dams (see Table 78).

Effects on survival, growth and development (retardations of ear unfolding and eye opening) in F1a and F1b pups of the high dose groups can be associated with effects on F0-dams during the gestation and lactation phases. In F0 dams, the observed reductions in food and water consumption and reduced body weight gain were most prominent during lactation. This can be related to a higher test substance uptake of F0 dams on a mg/kg bodyweight basis during lactation (1600 ppm in drinking water corresponds to ≈250 mg/kg bw/d during lactation versus 120-150 mg/kg bw/d during gestation phase, Table 77, BD) and can be interpreted to cause the reduced F1 pup survival and litter weight at postnatal day 21 (BD Table 79).

A consistent relationship between the observed reductions in food/water consumptions resulting in lower body weight gains and the effects on pup weights and survival was found in both generations. When dams consumed significantly lower amounts of food/water, the effects on pup weight and survival were evident (e.g. at the end of the lactation phase). In general effects were less prominent in F2-pups, this is in line with the observation that F1 dams showed also less severe reductions in food and water consumption.

It is noted that effects on pup weight are also reported in F2 at 100 and 400 ppm but litter size in these two groups is elevated, which may explain in part the reduced individual pup weight compared to the controls. Besides, fetal weight in F2 controls was quite high compared to controls in previous generations so that the significance of the effect in F2 at 100 and 400 ppm is questionable.

No malformations were observed. (Data on delayed or abnormal ossifications are not available.)

Conclusion on the 2-generation study:

Effects seen on pups in the 2-generation study are not relevant to justify classification mainly because they are most likely secondary to maternal toxicity.

Prenatal toxicity study in rabbits - Merkle & Hildebrand, 1985 and Hellwig, 1986 (amendment)

A significantly increased rate of resorptions was observed in rabbits that received 400 mg/kg bw/d on gestation days (GDs) 6-18. At this dose, the rabbits lost body weight during GDs 6-18 (-4% of initial body weight, in controls +3% of initial body weight) and food consumption was reduced by more than 56%. Body weight loss is considered a severe maternal effect and the observed increase in resorptions and the increased incidences of external variations (pseudoankylosis) and skeletal variations (fused sternebrae) were considered to be secondary to non-specific toxicity.

Conclusion on the pre-natal development rabbit study:

In the absence of treatment-related effects on pup development which could be considered not to be a secondary non-specific consequence of maternal toxicity and in the absence of teratogenic effects, no classification for developmental effects is warranted based in this study.

In contrast to the two foregoing studies, the developmental studies in rats (Hellwig & Hildebrand, 1987a-c) caused the RAC to consider whether cycloxydim could be regarded as a suspected reproductive toxicant:

Prenatal toxicity in Wistar rats (Hellwig & Hildebrand, 1987a)

- Increased foetal incidences of dumbbell-shaped ossification centers in the thoracic region were observed at 400 mg/kg bw/d (30% vs. 2.5% in controls). No increase was seen at 100 and 200 mg/kg bw/d. The dossier submitter considered the effect as an anomaly consisting of dumbbell-shaped or bipartite vertebral bodies with the involvement of cartilage (combined incidence 34% vs. 5.6%). The dossier submitter stated that according to current harmonised terminology and classification of rat fetal skeletal effects, these skeletal findings termed 'anomalies' in the study report are today classified as variations.
- Body weight gain in dams receiving 400 mg/kg bw/d during gestation was only mildly affected, on the GD 6-15 it was 92% of the control value and the corrected body weight gain (net weight change during the study) was 91% of controls (see Table 80). A lower foetal weight (around 5%) may be related to the lower body weight gain in dams. The assumptions remains uncertain, as only body weight gain data are available and no absolute values on body weight.
- It is concluded by the Dossier submitter that the foetal weight reductions were correlated with an increase in the number of skeletal retardations. However, mean foetal weight was only slightly lower than the control values (male pups 94.8%, female pups 95.6%, see Table 81). It appears questionable whether a 4-5% lower foetal body weight causes significant increases in skeletal retardations (dumbbell-shaped ossification centers of thoracic region, incomplete ossification of sternebrae; see Table 82).
- Chernoff et al. (2008) underlined that a reduction in food intake and the resultant under-nutrition would be more likely to induce foetal growth retardation, if these effects occur late in gestation when foetal growth is the greatest. The effect of cycloxydim on maternal food consumption is mainly reported at GD 7-8, which is quite early in the embryo-foetal development and it questions its impact on reduced foetal weight.

Supplemental prenatal toxicity in Wistar rats with special attention to maternal toxicity (Hellwig & Hildebrand 1987c)

- This supplementary study confirmed a mild reduction in body weight gain in dams receiving 400 mg/kg bw/d from GD 6-15. Body weight gain from GD 0-20 was 94.7% of the control value (corrected 88.1%, see Table 84b). Absolute body weight at GD 20 reached 99.1% of control values. Foetal weights at 400 mg/kg bw/d were 96% of controls for male pups and 97% for female pups (Table 86).

The study confirmed mild effects on body weight of dams and pups. No information on skeletal effects was given.

Supplementary pre-peri-postnatal toxicity study in Wistar rats (Hellwig & Hildebrand 1987 b)

The effects were summarised by the Dossier submitter as follows: 'The insufficient cartilagination of the 13th rib was reversible (7 and 21 day p.p.), while dumbbell-shaped or bipartite ossification centers of vertebral bodies and missing or incomplete ossification of sternebre were still present at day 7 and 21 p.p. However, it should be noted that at 400 mg/kg bw/d in this study, the dams had very marked reduction of body weight gain from days 6 to 8 (-64%) and marked reduction in body weight gain during treatment days 6 to 15 (-18%)'.

- Dumbbell-shaped of vertebral body/bodies with involvement of cartilage (cartilaginous bone precursor) in the thoracic region was observed at GD 20 at a foetal incidence of 16% in the control group and 67.3% in the 400 mg/kg bw/d treatment group, with litter incidence of 100% compared to 54.6 % in the control group (see Table 90, prenatal study segment).
- The finding of significantly increased dumbbell-shaped or bipartite ossifications of the vertebral bodies in the thoracic region was confirmed in the postnatal segment study at 400 mg/kg bw/d on postnatal day (PND) 7. The incidences remained significantly elevated on PND 21, i.e.in the same range as on PND 7 (foetal incidence: 27.5 vs. 4.6% in controls, % foetuses/litter: 26.56% vs. 4.41%. see Table 93).
- The finding of significantly increased dumbbell-shaped or bipartite ossifications on the vertebral bodies in the thoracic region is consistent with the findings in the other prenatal toxicity study in rats (Hellwig & Hildebrand, 1987a). In the other study foetal incidences were separately documented for each effect. At 400 mg/kg bw/d the Dossier submitter described in the text significantly increased foetal incidences of 'dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage', while in the table 82 it says 'dumbbell-shaped ossification centers, thoracic region' at foetal incidence of 30% vs. 2.5% in controls. As this effect was judged as an anomaly, the terms are considered to describe the same effect. The persistence of the effect as seen in the postnatal segment study from Hellwig & Hildebrand (1987b) corresponds to the proposed classification as dumbbell-shaped vertebral bodies with involvement of cartilage.
- The summary of maternal body weights effects (see Table 88) highlights that maternal body weight gain was significantly lower (36% of control value) during the first days of treatment (GD 6-8). For the whole treatment period, dams treated with 400 mg/kg bw/d on GD 6-15 gained body weight at 88.2% compared to body weight gain of controls. No data are given on the absolute body weights. Foetal weight was at 92.6% of the control value (see Table 89).
- It remains questionable whether a 12% reduction of maternal body weight gain during GD 6-15 as compared to the controls and a 7% lower total weight of the foetus are sufficiently severe effects to explain the significant increases in skeletal retardations (see Table 90). The marked reduction during GD 6-8 and the mild to moderate reduction of body weight gain over the total period of GD 6-15 indicates that animals are mainly affected during the first days of treatment and have adapted during the following days.
- The second part of this study (postnatal segment) demonstrated that pup survival on PND 21 was significantly lower than in controls (Table 91). Mean number of live pups/dam was also significantly lower (11 live pups/dam) compared to 12.3 in control dams. As the treatment of dams stopped at GD 15 treatment during lactation period was not the cause of pup deaths.
- The body weight gain was reduced in dams at GD 15 (79.2% of the control values, Table 88, Group 4). However no data on absolute body weight and on body weight at PND 21 was reported in this dossier.
- Examinations of pups on PND 7 and PND 21 after birth showed that the observed skeletal anomalies had a continuous tendency towards lower incidences at PND 7 and

PND 21 However, two of the three reported skeletal effects (dumbbell-shaped or bipartite ossifications on vertebral bodies in the thoracic region and incomplete ossification of sternebrae) remained at significantly higher level (at PND 21% fetus/litter 26.56% vs. 4.41% in controls, Table 93).

Overall conclusion of the rat developmental toxicity studies (Hellwig & Hildebrand, 1987a-c):

The observed adverse effects on pup survival, growth and development in rats could not completely be attributed to maternal toxicity. According to the classification criteria (3.7.2.4.3, Annex I CLP), cycloxydim was not so toxic that maternal death or severe inanition was the result, nor were they prostate and incapable of nursing the pups. Therefore it is not reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and so to discount the developmental changes.

The following key events as reported in the rat studies support this view.

Two developmental rat studies (Hellwig and Hildebrand, 1987a,b) revealed significantly increased rates of skeletal effects consisting of dumbbell-shaped ossification centers of thoracic vertebrae with involvement of cartilage, incomplete ossification of the sternebrae and (only in one study) insufficient cartilaginification of 13th rib at 400 mg/kg bw/d cycloxydim.

Postnatal follow-up demonstrated a tendency towards lower incidences at PND 7 and PND 21. However the incidences of skeletal effects were still significantly higher for incomplete ossification in the thoracic region and the sternebrae, only the delayed cartilaginification of the 13th rib reached similar incidences as control animals.

The observed skeletal abnormalities were identified as malformations in accordance with the harmonised classification system on foetal skeletal terminology (Solecki et al., 2001, Makris et al., 2009). The effects may also occur spontaneously. The observation that their incidences in decreases from the day of Caesarean section (i.e. 5 days after finalisation of treatment), PND 7 to PND 21, but remained elevated compared to controls, is in agreement with the interpretation of a persistent structural change (malformations).

The Dossier submitter assessed the most critical effect 'dumbbell-shaped or bipartite ossification centers of vertebral bodies, thoracic region, with involvement of cartilage' as an anomaly and noted that according to the current terminology and classification harmonized criteria this effect is classified as a variation.

Although one expert commented during the targeted expert consultation (5-19 November, 2012), that dumbbell-shaped ossification centers in the thoracic region should be regarded as a variation or retardation, the other commenting expert (MSCA) provided a proposal for the interpretation of the dumbbell-shaped vertebral bodies as malformations, if the cartilaginous bone precursor is affected as well as the subsequent process of ossification. The involvement of the cartilage has been documented by the dossier submitter.

As an outcome of a harmonization workshop on terminology and classification of foetal abnormalities Solecki et al. (2001) distinguished between "dumb-bell" and "dumb-bell ossification", with the former being considered as a malformation and the latter a variation. It is stated that 'The term dumbbell implies that the bone precursor is affected as well as the ossification site and the change is likely to be permanent. Therefore the condition would classify it as a malformation. Dumbbell ossification would suggest that only the ossification site is abnormally shaped and, as with bipartite ossification, this alteration would be classified as a variation.'

In the update of the harmonisation activity on developmental abnormalities, Makris et al. (2009) proposed that a dumbbell-shaped thoracic centrum was a structural abnormality involving the bone precursor (cartilage), while dumbbell ossification or bipartite ossification was a change of the ossification state.

Thus the RAC concluded that the effect 'dumbbell-shaped' or 'bipartite ossification centers of vertebral bodies' in the thoracic region, with involvement of cartilage is a malformation. Taking into account its persistence until PND 21 it was considered as evidence for developmental toxicity.

Comparison with the criteria for classification

(Criteria in 3.7.2.4.3 Annex I, CLP)

"Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such cases, classification in Category 2 may be considered more appropriate than Category 1.

However, when a substance is so toxic that maternal death of severe inanition results, or the dams are prostate and incapable of nursing the pugs it is reasonable to assume that developmental toxicity is produced solely as a secondary consequences of maternal toxicity and discount the developmental effects."

Cycloxydim did not cause severe disturbance of general health conditions of treated dams at doses which caused reduced pup weights, reduced survival and developmental variations and retardations.

As maternal toxicity at 400 mg/kg bw/d in the developmental studies consisted of lower bw gain (-9% in Hellwig and Hildebrand 1987a, 5.3% % in Hellwig and Hildebrand, 1987c, -9% and -21% in prenatal and postnatal segment in Hellwig and Hildebrand, 1987b) with most of this reduction observed during the first few days of the exposure period and no other sign of general health disturbance, it is unlikely to contribute the foetal skeletal findings to lower maternal bw gain.

The reduction of bw gain was mainly observed during the day 6-8. As ossification occurs during the late gestation period, it is unlikely that the skeletal effects were caused as nonspecific consequence of mild-moderate lower bw gain.

Lower maternal growth may have affected foetal growth. However, the magnitude of the reduction foetal weight reduction was only small (5-7% in three studies, Hellwig & Hildebrand 1987a, b in which pup data were available).

Only for the prenatal toxicity study on maternal toxicity (Hellwig & Hildebrand 1987c) data on absolute maternal bw were given. Dams receiving 400 mg/kg bw/d during GD 6-15 showed 5.3% reduction in bw gain and bw at GD 20 was 99.1% compared to controls.

In conclusion, the RAC considered that the level of maternal toxicity was not sufficiently severe to explain the reduced pup weights, lower pup survival and skeletal anomalies as only secondary to maternal toxicity. The observed tendency of reduced incidences of the skeletal anomalies after birth and after termination of exposure, appears to confirm the retardational nature of the effects. However persistence of significantly increased incidences of skeletal effects until postnatal day 21 cannot be interpreted in a way that these effects are only minor developmental changes that do not justify classification as for developmental toxicity. The criteria (3.7.2.4.3., Annex I, CLP) say that *"Classification is not necessarily the outcome in case of minor developmental changes, when there is only small effects on foetal/pup body weights, or retardation of ossification when seen in association with maternal toxicity".*

Consistent evidence of significantly increased incidences of 'dumbbell-shaped or bipartite ossification centers of vertebral bodies, thoracic region, with involvement of cartilage' from several rat studies is considered as a malformation. The persistence of this skeletal anomaly until postnatal day 21 counter argues against the interpretation of a minor developmental change. Information on potential functional impairment related to this skeletal anomaly that may occur during life stages later than postnatal day 21 is not available.

The fact that this lesion occurred spontaneously (although at significantly lower rates) in control animals and its overall incidences decreased by postnatal day 21 was considered as indicative for a less serious effect that does not justify classification in Cat. 1B.

In conclusion, the RAC was of the opinion that there is evidence of developmental effects that could not solely be attributed to maternal effects and therefore the observed developmental effects fulfil the requirements for classification with Repr. 2, H361d (CLP).

As the criteria for DSD are very similar to the CLP criteria, classification with Repro Cat 3, R63, is warranted according to the DSD.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

There were no clinical signs suggesting neurotoxic potential in any species tested, therefore studies on the potential neurotoxicity of cycloxydim were not carried out.

4.12.1.2 Immunotoxicity

According to the available acute, subchronic and chronic studies, there was no indication of an immunotoxic potential of cycloxydim.

4.12.1.3 Specific investigations: other studies

No other data available.

4.12.1.4 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Note: no comments were received in the public consultation in relation to environmental hazard assessment.

5.1 Degradation

Table 100: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: US EPA Subdivison N, § 161-1, BBA Merkblatt 55	DT50 (pH 5, 24°): 8.3 d (SFO r^2 = 0.987) DT50 (pH 7, 24°): 172 d (SFO r^2 = 0.831) DT50 (pH 9, 24°): 206.3 d (SFO r^2 = 0.723)		Keller (1985b)
Hydrolysis EPA Subdivision N, § 161-1; SETAC 1995; CD 94/37/EG; OECD 111	**pH** / **DT_{50} [days]** / **DT_{90} [days]** **4** / 2.1 / 7.0 **5** / 12.2 / 40.5 **7** / 264 / 875 **9** / 958 / >1000		Hassink, J., (2009)
Photolysis Guideline: EPA 161-2	DT50 (pH 5, 22°): 5.8 h DT50 (pH 7, 22°): 17.6 h DT50 (pH 9, 22°): 22.3 h		Goetz N. von (2000a)
Screening tests	No data submitted, substance considered not readily biodegradable.		
Water/Sediment Study Guideline: BBA IV 5-1, EPA 162-4, SETAC Europe Part 8. and Recommendations of the Report of the FOCUS Working Group on Surface Water Scenarios	Whole system: DT50 (System A: pH 8.8, 20°): 30.6 d DT50 (System B: pH 7, 20°): 14.1 d DT50 (both systems, geomean): 20.8 d	Calculation of DT50 values: Cyclocydim showed a biphasic degradation behaviour, therefore, the observations of the study (Ebert 2000) were also fitted with the Hockey-stick model (Jene 2006)	Ebert D. (2000) Jene B. (2006)
Field Studies	No data submitted.		

5.1.1 Stability

Hydrolysis

Reference: Keller E. (1985b): Hydrolysis of ^{14}C-BAS 517 H at pH 3, 5, 7 and 9.
Report No: 1985/0446.
Guideline: US EPA Subdivison N, § 161-1, BBA Merkblatt 55
GLP: No, studies were conducted prior to the implementation of GLP

Test item:
Cyclohexene-4(6)-^{14}[C] labelled cycloxydim (BAS 517 H)
Batch: not stated
Radiochemical purity: ≥ 98 %(by radio TLC and radio HPLC)

Material and methods:
The rate of hydrolysis of cycloxydim (test concentration ~ 10 mg/L) was investigated in sterile aqueous buffer solutions at four pH levels (pH 3, 5, 7 and 9) at 24 ± 2°C. In addition, the formation of possible hydrolysis products was investigated.
Following buffer solutions were used: 0.1 M potassiumhydrogenphthalate and 0.1 M HCl (pH 3), 0.1 M potassiumhydrogenphthalate and 0.1 M NaOH (pH 5), 0.1 M KH_2PO_4 and 0.1 N NaOH (pH 7) and 0.1 M boric acid in 0.1 M KCl and 0.1 N NaOH (pH 9).
The incubation period was 32, 14 and 6 days at pH 7 and 9, pH 5 and pH 3, respectively. All test samples were maintained in the dark under constant stirring or shaking.
During the incubation time repeatedly test samples were taken and analysed by LSC (determination of total radioactivity). Further on cycloxydim and hydrolysis products were separated and identified by TLC, HPLC and MS. The radioactivity of each compound were determined and the concentrations were calculated.

Findings:
Recovery rates ranged from 101.5 – 109.4 % at pH 3, 84.7 – 108.1 % at pH 5, 100.5 – 107.3 % at pH 7 and 100.1 – 107.8 % at pH 9. Sterile conditions were maintained during the incubation.

Table 101: Hydrolysis of cycloxydim and its degradation products at pH 7

Sample	Cycloxydim		BH 517-T2SO		BH 517-TSO		BH 517-T1S		BH 517-T2S	
	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR
0	7.97	78.5	-	-	1.34	13.2	0.64	6.3	-	-
4 days	8.32	77.3	-	-	1.32	12.2	0.49	4.5	0.34	3.2
7 days	7.8	73.1	0.02	0.2	1.49	14	0.53	5	0.53	5
14 days	7.45	71	-	-	1.42	13.5	0.62	**5.9**	0.72	6.9
21 days	7.8	72	-	-	1.43	13.2	0.37	3.4	0.93	8.6
28 days	7.41	70.7	-	-	1.4	13.4	0.5	4.8	0.9	8.6
32 days	7.09	67.2	-	-	1.65	**15.6**	0.53	5	0.91	**8.6**

TRR – Total radioactive residues

Assuming a pseudo first order reaction kinetic half lives for cycloxydim were calculated by linear regression. Results are summarised in table B.8.4.1.1-5

Table 102: Calculated DT50 values of cycloxydim, assuming a pseudo first order reaction kinetic

pH	DT_{50} in days	correlation coefficient
3	1.1	0.988
5	6.7	0.987

7	104	0.734
9	102	0.651

The DT_{50} values were calculated with log-transformed data. Therefore, the RMS recalculated the DT_{50} values with untransformed data according SFO kinetics. The results are summarized in the table Table 103.

Table 103: Recalculated DT50 values of cycloxydim, assuming SFO kinetics (ModelMaker v4)

pH	**DT_{50} in days**	**r^2**
3	1.7	0.989
5	8.3	0.971
7	172.3	0.831
9	206.3*	0.723

* value of day 4 was excluded as outlier

Conclusion:
Cycloxydim was hydrolytically instable under acidic conditions (pH 3 and 5) with DT_{50} values of 1.7 and 8.3 days. Under neutral and alkaline conditions (pH 7 and 9) cycloxydim was rather stable with half lives of 172.3 days (pH 7) and 206.3 days (pH 9). The major hydrolysis degradation products under acid conditions (pH 3, 5) were BH 517-TSO (at the test initiation) and BH 517-T2S (at the end of incubation). The predominant metabolite BH 517-T2S was the result of a Beckman rearrangement. At neutral and more alkaline conditions BH 517-TSO was mainly found due to oxidation of the active substance. BH 517-TSO and BH 517-T2S are considered as hydrolytical stable under test conditions.

Reference:	**Aqueous hydrolysis of BAS 517 H**
Author(s), year:	Hassink, J., 2009
Report/Doc. number:	2008/1090871
Guideline(s):	EPA Subdivision N, § 161-1; SETAC 1995; CD 94/37/EG; OECD 111
GLP:	Yes
Deviations:	No
Validity:	Fully valid

MATERIAL AND METHODS:

Test compound:	[Cyclohexene-4(6)-^{14}C]-Cycloxydim (BAS 517 H), batch no. 529-5301, 4.97 MBq/mg, radiochem. purity > 95 %
Reference compounds:	Cycloxydim (unlabelled), BH 517-T2S, BH 517-T2SO
Test conditions:	0.35 mg/L of test compound in sterile buffer solutions at 25 °C for 30 days in the dark
Buffer solutions:	pH 4 (Citrate-HCl) pH 5 (Citrate - NaOH) pH 7 (Phosphate) pH 9 (Boric acid/KCl – NaOH) All Titrisol, Merck (diluted 1:9 with bidest. water)
Analytical methods:	LSC, HPLC-RAD, HPLC-MS (ESI)
Kinetic analysis:	Simple first order (SFO) for the parent, KinGUI 1.1

FINDINGS:

Recovery rates ranged from 100 – 110.2 % at pH 4, 99.7 – 101.7 % at pH 5, 94.5 – 100.5 % at pH 7 and 100.0 – 101.4 % at pH 9. Sterile conditions were maintained during the incubation.

Table 104: Distribution of radioactivity [% of AR] following hydrolysis of cycloxydim in sterile buffered water at pH 4 (25 °C).

DAT	BH 517 H (Z-isomer)	BH 517 H (E-isomer)	BH 517 H (Sum)	BH 517-T2S	BH 517-TSO	Sum of others[a]	Total
0	6.1	81.0	87.1	10.4	2.5	nd	100.0
1	7.9	63.0	70.9	28.1	2.0	nd	101.0
2	4.1	41.2	45.3	47.0	2.6	5.8	100.7
3	3.9	35.7	39.6	59.5	1.3	nd	100.4
8	1.2	9.8	11.0	87.3	0.3	2.5	101.0
15[b]	-	-	-	-	-	-	-
30	nd	nd	nd	99.7	1.7	8.8	110.2

nd denotes not detected
[a] Individual peaks < 3 % of AR
[b] Recovery > 180 % of AR indicate application mistake, sample not considered

Table 105: Distribution of radioactivity [% of AR] following hydrolysis of cycloxydim in sterile buffered water at pH 5 (25 °C).

DAT	BH 517 H (Z-isomer)	BH 517 H (E-isomer)	BH 517 H (Sum)	BH 517-T2S	BH 517-TSO	Sum of others	Total
0	3.1	94.6	97.7	nd	2.3	nd	100.0
1	8.2	77.3	85.5	13.3	1.0	nd	99.8
2	7.7	73.2	80.9	17.9	0.9	nd	99.7
3	6.1	66.1	72.2	26.8	0.8	nd	99.8
8	6.0	50.1	56.1	44.5	0.8	nd	101.4
15	6.0	36.2	42.2	56.4	1.1	2.1	101.7
30	nd	19.4	19.4	78.1	1.3	2.5	101.3

nd denotes not detected

Table 106: Distribution of radioactivity [% of AR] following hydrolysis of cycloxydim in sterile buffered water at pH 7 (25 °C).

DAT	BH 517 H (Z-isomer)	BH 517 H (E-isomer)	BH 517 H (Sum)	BH 517-T2S	BH 517-TSO	Sum of others	Total
0	4.3	93.7	98.0	nd	2.1	nd	100.0
1	4.1	94.4	98.5	0.9	1.1	nd	100.4
2	4.6	93.4	98.0	1.2	1.3	nd	100.4
3	4.9	86.8	91.7	1.3	1.4	nd	94.5
8	4.6	90.9	95.5	3.3	1.4	nd	100.2
15	5.6	89.0	94.6	3.5	1.8	nd	100.0
30	4.9	85.7	90.6	7.1	2.8	nd	100.5

nd denotes not detected

Table 107: Distribution of radioactivity [% of AR] following hydrolysis of cycloxydim in sterile buffered water at pH 9 (25 °C).

DAT	BH 517 H (Z-isomer)	BH 517 H (E-isomer)	BH 517 H (Sum)	BH 517-T2S	BH 517-TSO	Sum of others	Total
0	4.2	89.9	94.1	nd	5.9	nd	100.0
1	3.7	96.5	100.2	nd	1.3	nd	101.4
2	3.9	95.5	99.4	nd	1.4	nd	100.8
3	4.2	95.0	99.2	nd	2.1	nd	101.3
8	5.1	93.2	98.3	nd	2.2	nd	100.5
15	4.2	92.2	96.4	1.1	2.9	nd	100.5
30	3.7	92.4	96.1	0.7	3.6	nd	100.3

nd denotes not detected

Table 108: Hydrolysis half-life [days] of cycloxydim in sterile buffered aqueous solutions (25 °C).

pH	DT_{50} [days]	DT_{90} [days]	C_0 [% of AR]	Chi^2 error [%]	Kinetics
4	2.1	7.0	98.6	5.6	SFO
5	12.2	40.5	93.3	4.2	SFO
7	264	875	97.7	1.8	SFO
9	958	>1000	98.3	1.6	SFO

CONCLUSION:
Cycloxydim is most labile under acidic conditions (DT50 at pH 4 = 2 days, DT50 at pH 5 = 12 days) and rather stable at neutral and alkaline conditions. The predominant hydrolysis product at pH 4 and 5 was BH 517-T2S, the result of a Beckman rearrangement of the parent compound. No other metabolite occurred above 5 % of AR. The metabolite BH 517-TSO was detected with maximum 5.9 % of AR in the pH 9 sample of 0 DAT but should not be considered a hydrolytic product but rather as an unspecific oxidation product. No significant formation of the Z-isomer of either the test item or any metabolite during the study was observed.

COMMENTS (RMS):

- The results of this study are in close agreement with findings from the earlier aqueous hydrolysis study from Keller (1985b). However, in the earlier study BH 517-TSO was found above 10 % of AR at 0 DAT samples whereas it reached max. 5.9 % at 0 DAT samples in the new study.

Photolysis:

Reference: Goetz N. von (2000a): Aqueous photolysis of Cycloxydim (BAS 517 H).
Report No: 2000/1000143.
Guideline: EPA 161-2
GLP: Yes

Test item:
[Cyclohexene-4(6)-14 C] cycloxydim (BAS 517 H), Batch: 529-2102 and 529-2201, Radiochemical purity: 92.6 % (pH 7)

Material and methods:

The photodegradation of cycloxydim in aqueous solutions was studied, because the molar absorption coefficients of cycloxydim at wavelength ≥ 290 nm were > 10 L mol^{-1} cm-1 (Sarafin 1991 b).
The initial test concentration was 0.4 – 0.5 mg/L in sterile buffer solutions at pH 5 (citrate-sodium hydroxide buffer), pH 7 (phosphate buffer) and pH 9 (boric acid/potassium chloride/sodium hydroxide buffer). Test solutions were continuously irradiated with light of about 3 mW/cm³ and wavelength > 290 nm (simulating a clear summer day) of a xenon burner in a SUNTEST CPS apparatus. The temperature was maintained at 22 ± 1°C. Test vessels with chemical actionometer solution for the determination of the quantum yield were incubated under the same conditions like the irradiated test samples. In order to collect volatile degradation products a closed flow-through system with suitable absorption traps were used. Dark control samples were stored in a climatic chamber at 22 ± 1°C (without traps for volatile compounds). The incubation period was 15 days. At appropriate time intervals irradiated samples, dark control samples and the content of volatile traps were taken and analysed by LSC (quantification), TLC and HPLC (analysis of photoproducts). Additionally major peaks were characterised by MS. Sterility of buffer samples were checked by plate count technique for each sampling date.

Findings:
All test samples were sterile during the course of the study.
Total recovery rates of irradiated samples ranged from 99.1 – 107.0 % at pH 5, 87.7 – 100 % at pH 7 and 92.2 – 106.4 % at pH 9.
The maximal amounts of volatiles, whereas the radioactivity was found only in NaOH-traps, were 5.3 % (pH 5), 0.2 % (pH 7) and 0.7 %(pH 9) after 15 days.

Table 109: DT50 and DT90 values for cycloxydim and its metabolites during aqueous photolysis at the different pH's and under continuous irradiation

	pH 5 (r^2= 0.919)		**pH 7 (r^2= 0.973)**		**pH 9 (r^2= 0.977)**	
	DT_{50} [h]	**DT_{90} [h]**	**DT_{50} [h]**	**DT_{90} [h]**	**DT_{50} [h]**	**DT_{90} [h]**
Cycloxydim	5.8	19.4	17.6	58.5	22.3	73.9
BH 517-TSO	32.2	106.9	33	109.5	55.6	184.7
BH 517-T1S	4.9	16.4	-	-	58.2	193.3
BH 517-T2S	6.0	19.8	-	-	24.6	81.8
BH 517-T1SO	202.3#**	671.9#**	n.c. #	n.c. #	1202.8* #	3995.6* #
BH 517-T2SO	94.5	314.0	50.5	167.7	-	-
BH 517-TGSO	93.0	309.1	n.c. #	n. c. #	n.c. #	n.c. #

* values given although they exceeded twice the study duration
** values seems to be not valid
# considered as stable
n.c.: not calculated due to lack of data

Conclusions:
Under test conditions in sterile aqueous buffer solutions cycloxydim was rapidly photolytically degraded in all tested buffer solutions with DT_{50} values of 5.8 h, 17.6 h and 22.3 h at pH 5, pH 7, and pH 9, respectively. The main product was BH 517-T1SO with > 60 % in all test solutions.

5.1.2 Biodegradation information provided the dossier submitter

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

No data submitted.

5.1.2.3 Simulation tests

Reference: Ebert D. (2000): Degradation of BAS 517 H (Cycloxydim) in water/sediment-systems under aerobic conditions.
Report No. 2000/1000138
Guideline: BBA IV 5-1, EPA 162-4, SETAC Europe Part 8.2
GLP: Yes

Test item:
Cyclohexene-4(6)-^{14}[C] labelled cycloxydim (BAS 517 H), Batch: 529-2102, Radiochemical purity: ≥98 %
Material and methods:
The aerobic aquatic metabolism and degradation of Cyclohexene-4(6)-14 [C] labelled cycloxydim was studied in two different water/sediment systems. The water/sediment systems were treated with 72 µg test substance (corresponding to 0.5 mg/L) and test samples were incubated at 20°C under aerobic conditions in darkness for up to 100 days.
The sediment with associated water was sampled at two sites in natural environment:
System A: „Kellmetschweiher", pond in the area Kastenbergheide, west of Schifferstadt, DE
System B: „Berghäuser Altrhein", pond like side arm of the river Rhine, south of Speyer, DE

The characterisations of both systems are given in table below:
Table 110: Physical and chemical properties of the two test systems:

		System A: "Kellmetschweiher"	**System B: "Berghäuser Altrhein"**
water phase:			
Temperature*		9.7°C	8 °C
pH*		8.75	8.55
O2-concentration *		10 mg/l (87 %)	5 mg/l (50 %)
total hardness		0.93 mmol/L	1.18 mmol/L
total nitrogen	beginning: end:	2 mg/L 29 mg/L	2 mg/L 2 mg/L
total phosphorous	beginning: end:	< 3 mg/L < 3 mg/L	< 3 mg/L < 3 mg/L l
total organic carbon	beginning: end:	10.6 mg/L 7.3 mg/L	8.6 mg/L 8.8 mg/L
sediment phase:			
pH		7.7	7.4
Corg		0.4 %	4.3 %
total nitrogen	beginning: end:	0.03 % 0.02 %	0.38 % 0.38 %
total phosphorus	beginning: end:	0.006 % 0.005 %	0.10 % 0.11 %
redox potential*		-145 mV	-267 mV
CEC		6 meq Ba/100 g	25 meq Ba/100 g

		System A: "Kellmetschweiher"	**System B: "Berghäuser Altrhein"**
ATP	beginning: end:	219 µg/kg 118 µg/kg	1236 µg/kg 1043 µg/kg
Bacteria	beginning: end:	3.8 x 106 cfu/g 2.3 x 106 cfu/g	3.3 x 107 cfu/g 4.7 x 107 cfu/g
Actionomycetes	beginning: end:	0 8.4 x 103 cfu/g	2.3 x 105 cfu/g 1.8 x 104 cfu/g
Fungi	beginning: end:	7.2 x 103 cfu/g 4.5 x 103 cfu/g	2.3 x 105 cfu/g 1.6 x 105 cfu/g
texture / particle size distribution (USDA)	sand [%]: silt [%]: clay [%]:	sand: 93 % 1 % 6 %	loam: 42 % 42 % 16 %

* determined at sampling

Two days after collection of the water/sediment systems the sediments were washed through a 2 mm sieve and the water was filtered through a 0.2 mm sieve. Per test sample 205 g (system A) or 145 g (system B) wet sediment and 290 mL water was added to glass incubation flasks. This corresponded to a sediment layer of 2.0 – 2.5 cm and a water layer of ca. 6 cm, with a total volume of 400 mL. All flasks were attached to the incubation system and were ventilated with moistened air. Each flask was connected with a series of three traps (1x ethylene glycol, 2x 2M potassium hydroxide solution). The test units were acclimatised to test conditions and were allowed to equilibrate for 21 days. The pH, the O2 content, the redox potential and the temperature were monitored during the acclimatisation period and at each sampling point.
Duplicate samples (flasks and associated trap solutions) were taken after 0, 0.25, 1, 2, 7, 14, 31, 60 and 100 days of incubation. Additionally one test vessel treated with a high application rate (~0.75 mg/L), the sterile assays and the control samples (treated with unlabeled test substance) were sampled after 81, 101 and 106 days, respectively.
Water and sediment phase were separated by decantation and the sediment was transferred to centrifuge tubes for extraction. The radioactivity content was analysed by TLC and HPLC. Additionally selected samples were analysed by HPLC/MS to verify the identity of test substance and metabolites.
Volatile trap solutions were removed for analysis at each sampling time and the radioactivity was determined by LSC. Due to low radioactivity in the traps during the study only in the 100 days sample the radioactivity in potassium hydroxide traps was confirmed as CO2 by acidifying (HCl) and expelling of the CO2 with nitrogen.
Non extractable residues (NER fraction) in the sediment at the last two sampling dates (60 d and 100 d) were further characterised by fractionation of sediment organic matter into humic- and fulvic acids and humin fraction.

Findings:

Table 111: Recovery of radioactivity in % AR and distribution of metabolites after application of cycloxydim to water/sediment system and incubation at 20°C - System A "Kellmetschweiher" (mean values)

	Incubation time in hours (h) / days (d)									
	0 h	6 h	1 d	2 d	7 d	14 d	31 d	60 d	100 d	101 d (steril)
Water										
as	90.9	87.5	85.6	83	73.9	68.8	55	5.9	-	35.8
BH 517-TSO*	6.4	7.7	8.6	9.6	13	11.4	21	66.5	62.1	40
BH 517-T1S	3.3	2.5	1.7	1.7	-	-	-	-	-	-
BH 517-T1SO	-	-	-	-	-	-	-	1.6	1.6	2.2
Total water	100	97.	95.	94.	89.	83.	78.	77.	71.	79.7

	Incubation time in hours (h) / days (d)									
	0 h	6 h	1 d	2 d	7 d	14 d	31 d	60 d	100 d	101 d (steril)
	.6	7	9	4	3	6	4	1	7	
Sediment										
as	-	-	-	5.6	7.8	9.2	6.1	2.1	-	7.6
BH 517-TSO*	-	-	-	0.7	1.7	0.5	1.3	9.7	10.4	11.4
BH 517-T1S	-	-	-	-	0.4	2.2	1.6	1.5	0.7	1.2
BH 517-T1SO	-	-	-	-	-	0.4	0.4	2.5	2.4	1.2
Total extractable	na	na	na	5.3	9.8	13.5	15.9	16.9	15.5	20.7
Non-extractable	0.3 **	2.3 **	4.8 **	1.1	1.3	2.2	3.4	4.8	6.1	2.6
Total sediment	0.3	2.3	4.8	6.4	11.1	15.7	19.3	21.7	21.6	23.3
Whole system										
as	90.9	87.5	85.6	88.6	81.7	78.0	61.1	8.0	-	43.4
BH 517-TSO*	6.4	7.7	8.6	10.3	14.7	11.9	22.3	76.2	72.5	51.4
BH 517-T1S	3.3	2.5	1.7	1.7	0.4	2.2	1.6	1.5	0.7	-
BH 517-T1SO	-	-	-	-	-	0.4	0.4	4.1	4.0	3.4
Total water and sed. extracts	100.6	97.7	95.5	100.8	99.2	97.2	95.1	93.9	87.7	101
CO2	-	0.0	0.0	0.0	0.3	0.6	2.1	2.9	4.8	ns
Total balance	100.9	100.0	100.6	100.7	100.7	99.9	99.9	101.8	98.1	103.1

* sum of 2 peaks (isomers) identified as BH 517-TSO by HPLC/MS

** No extraction of the sediment was performed and only the total radioactivity of the total fraction was determined.

na: not analyzed (no extraction, since all of the radioactivity is still in the water phase)

Table 112: Recovery of radioactivity in % AR and distribution of metabolites after application of cycloxydim to water/sediment system and incubation at 20°C - System B "Berghäuser Altrhein" (mean values)

	Incubation time in hours (h) / days (d)									
	0 h	**6 h**	**1 d**	**2 d**	**7 d**	**14 d**	**31 d**	**60 d**	**100 d**	**101 d (steril)**
Water										
as	89.8	84.9	80.8	72.6	57.6	38.9	1.0	-	-	24.3
BH 517-TSO*	7.6	8.8	12.3	15.7	21.1	27.2	52.1	44.9	31.1	24.0
BH 517-T1S	3.3	2.3	-	-	-	-	-	-	-	-
BH 517-T1SO	-	-	-	-	1.5	0.7	-	-	-	-
Total water	100.7	95.7	93.1	88.2	81.4	68.6	60.4	52.0	39.0	53.0
Sediment										
as	-	-	5.1	7.6	9.9	13.1	7.3	6.5	4.5	11.4
BH 517-TSO*	-	-	-	-	2.1	3.5	11.6	9.1	6.6	12.4
BH 517-T1S	-	-	1.7	2.7	2.1	4.8	5.6	8.1	10.8	8.8
BH 517-T1SO	-	-	-	-	0.7	1.4	4.9	5.4	5.6	5.0
Total extractable	na	na	6.8	10.2	17.0	24.7	30.1	30.5	34.6	40.5
Non-extractable	0.4**	4.0**	1.0	1.8	2.1	4.0	6.1	9.9	13.9	6.6
Total sediment	0.4	4.0	7.8	12.1	19.0	28.6	36.1	40.4	48.5	47.1
Whole system										
as	89.8	84.9	85.9	80.2	67.5	52.0	8.3	6.5	4.5	35.7
BH 517-TSO*	7.6	8.8	12.3	15.7	23.2	30.7	63.7	54.0	37.7	36.4
BH 517-T1S	3.3	2.3	1.7	2.7	2.1	4.8	5.6	8.1	10.8	8.8
BH 517-T1SO	-	-	-	-	2.2	2.1	4.9	5.4	5.6	5.0
Total water and sed. extracts	100.7	96	99.9	98.4	98.2	93.2	90.3	83.9	73.8	96.4
CO2	ns	0	0	0.1	0.3	1.4	2.9	5.2	7.6	ns
Total balance	101.1	99.8	100.9	100.4	100.5	98.9	99.5	97.8	95.1	100

* sum of 2 peaks (isomers) identified as BH 517-TSO by HPLC/MS
**No extraction of the sediment was performed and only the total radioactivity of the total fraction was determined.
na not analyzed (no extraction, since all of the radioactivity is still in the water phase)

Calculation of half-lives:
DT50 and DT90 were calculated for cycloxydim and its metabolites BH 517-TSO and BH 517-T1S assuming SFO kinetics by using the computer progam ModelMaker, version 3.0.4. The kinetic modelling was based on the single data points of the analysed replicates at each sampling point for both systems. The fate of cycloxydim and its metabolites was described in a multi-compartment model for each system. The determination of half lives is based on rate constants (k) determined in these models.
The coefficients of determination of the two models were $r^2 = 0.96$ ("Kellmetschweiher") and $r^2 = 0.98$ (Berghäuser Altrhein).
Results of calculated degradation times are summarised in table:

Table 113: Degradation times (DT50 and DT90) for cycloxydim and its metabolites in water/sediment system – ModelMaker vers. 3.0.4.

test system	substance	DT50 (days)		DT90 (days)		r^2
		water	sed	water	sed	Complete model
system A "Kellmetschweiher"	cycloxydim	28	10.5	93.0	35.0	0.96
	BH 517-TSO	n.c.*	-	n.c.*	-	0.96
system B "Berghäuser Altrhein"	cycloxydim	10.5	18.1	35.0	60.1	0.98
	BH 517-TSO	115.2	-	382.8	-	0.98
	BH 517-T1S	-	n.c.**	-	n.c.**	0.98

n.c. not calculated
* calculation not possible (the related rate constant was 0) -> considered as stable
**calculation not possible (the standard deviation of the related rate constant was too high) -> considered as stable

Conclusions:
In both systems cycloxydim was oxidized to the main metabolite BH 517-TSO reaching a maximum of 66.5 % AR in water phase (system A) after 60 days and 52.1 % AR in system B after 31 days. Two other metabolites, BH 517-T1S and BH 517-T1SO, were identified in both systems. In water phase both metabolites occurred in small amounts (< 3.5 % AR). BH 517-T1S occurred in sediment in system B "Berghäuser Altrhein" with maximal amounts of 10.8 % at the end of the study. All other metabolites detected in the chromatograms did not appeared > 10 % AR.
The mineralization to CO2 was low with maximal amounts of 4.8 % at day 100 in "Kellmetschweiher" system and 13.9 % at day 100 in "Berghäuser Altrhein" system. The formation of non-extractable radioactivity in NER-fraction was noted with max. 6.1 % in „Kellmetschweiher" system and with max. 13.9 % in "Berghäuser Altrhein" system, both maxima were found at the end of the study.

Reference: Jene, B. (2006): Predicted environmental concentrations of BAS 517 H - Cycloxydim and metabolites in surface water and sediment on a European level according to FOCUS
BASF AG, Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep.; unpublished, BASF RegDoc# 2006/1037685

Guideline: According to guidance given in "FOCUS (1997): Surface Water Models and EU Registration of Plant Protection Product. European Commission Document 6476/VI/96." and "FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp.
GLP: No (modelling study)

Material and methods:
For assessing risk to aquatic organisms predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED}) were calculated for cycloxydim and its metabolites BH 517-TSO, BH 517-TSO2, BH 517-T1S, BH 517-T1SO, BH 517-T2S, BH 517-T2SO, BH 517-T2SO2 and BH 517-TGSO, when applied to sugar beets or winter oilseed rape. For modeling purposes the entry routes via spray drift, run-off and drainage were considered. The calculations were conducted according to the tiered approach as proposed by FOCUS. For these calculations the model STEPS1-2 in FOCUS v1.1 was used.

Cycloxydim

The total DT_{50} of the parent compound cycloxydim in the water/sediment system was calculated in consideration of the recommendations of the FOCUS kinetic report. ModelMaker (v3.04) calculations were conducted based on the data of the residues of cycloxydim (sum of E and Z-isomer) in the two water/sediment systems "Kellmetschweiher" and "Berghäuser Altrhein". In a first step, the estimated data were fitted with SFO kinetic. However, the "Kellmetschweiher" system showed a biphasic degradation behaviour (slower degradation in the first phase and faster degradation in the second). Therefore, the observations were also fitted with the Hockey-stick model. Comparing the results (visual and statistic assessment) of both used kinetic models the Hockey-stick kinetic model fitted the observations significantly better than the SFO kinetic. The DT_{50} values derived from "Kellmetschweiher" system are summarized in the tables below:

Table 114: Estimated parameters (SFO and HS kinetics) of the "Kellmetschweiher" system

SFO			
		Standard Deviation	**type-I error rate**
k [d^{-1}]	0.0225 (DT_{50} = 30.8 d)	0.0032	<0.001
M0 [% AR]	92.8	3.39	not applicable
HS			
		Standard Deviation	**type-I error rate**
k1 [d^{-1}]	0.0097 (DT_{50} = 71.5 d)	0.0024	0.001
k2 [d^{-1}]	0.0704 (DT_{50} = 9.9 d)	0.0103	<0.001
M0 [% AR]	88.69	1.24	not applicable
tb [d]	29.8	1.23	not applicable

Table 115: DT50 and statistical results of the "Kellmetschweiher" system

	SFO kinetics	**Hockey stick kinetics**
DT_{50} [d]	30.8	35.6*
Error level Chi^2 - test [%]	11.2	1.9
R^2 [-]	0.92	0.99

* DT_{50} > tb: DT_{50} = tb+[ln(2)-k1*tb]/k2

Hence, the result of the Hockey-stick kinetic was considered for the risk assessment. As recommended in the FOCOS kinetic report the half-life from the slow phase (71.5 d) was considered.

For the "Berghäuser Altrhein" system no biphasic degradation behaviour of cycloxydim could be observed. The SFO kinetic model showed an acceptable fit of all data points. The results are summarized in the tables below:

Table 116: Estimated parameters (SFO kinetic) of the "Berghäuser Altrhein" system

SFO			
		Standard Deviation	**type-I error rate**
k [d^{-1}]	0.0484 d-1 (DT_{50} = 14.3 d)	0.0044	<0.001
M0 [% AR]	88.4	2.1323	not applicable

Table 117: DT_{50} and statistical results of the "Berghäuser Altrhein" system

	SFO kinetics
DT_{50} [d]	14.3
Error level Chi^2 - test [%]	7.5
R^2 [-]	0.98

Hence, for "Berghäuser Altrhein" system the SFO half-life of 14.3 d was considered.
The geometric mean DT_{50} value of both systems of 20.8 days was used for PEC_{SW} calculation.

Table 118: Re-evaluated $DegT_{50}$ values [days] for cycloxydim in the entire system (RMS assessment).

System	**$DegT_{50}$ [days]**	**$DegT_{90}$ [days]**	**Kinetics**	**Chi^2 error [%]**	**Visual fit**
System A "Kellmetschweiher"	30.6	102	SFO	11.2	Acceptable
System B "Berghäuser Altrhein"	14.1	46.7	SFO	7.5	Good
Geometric mean	**20.8**	**68.9**	**-**	**-**	**-**

The geometric mean $DegT_{50}$ of 20.8 days for cycloxydim in the entire water/sediment system is considered most appropriate for the surface water risk assessment.

5.1.3 Dossier submitter's summary and discussion of degradation

Aquatic hydrolysis
Cycloxydim was hydrolytically instable under acidic conditions (DT50 at pH 3 and 4 = 2 days, DT50 at pH 5 = 8 and 12 days) and rather stable at neutral and alkaline conditions (DT50 at pH 7 = 172 and 264 days, DT50 at pH 9 = 206 and 958 days) in two hydrolysis studies. The predominant hydrolysis product under acid conditions (pH 3, 4 and 5) was BH 517-T2S (max. 99.7 %), the result of a Beckman rearrangement of the parent compound. The metabolite BH 517-TSO was detected with maximum 19.3 % of AR in the pH 9 sample of 21 DAT but should not be considered a hydrolytic product but rather as an unspecific oxidation product. The metabolite T2SO which is a result of a Beckman rearrangement of the metabolite TSO reached a maximum of 9.6 % under acidic conditions. BH 517-TSO and BH 517-T2S are considered as hydrolytically stable under test conditions.

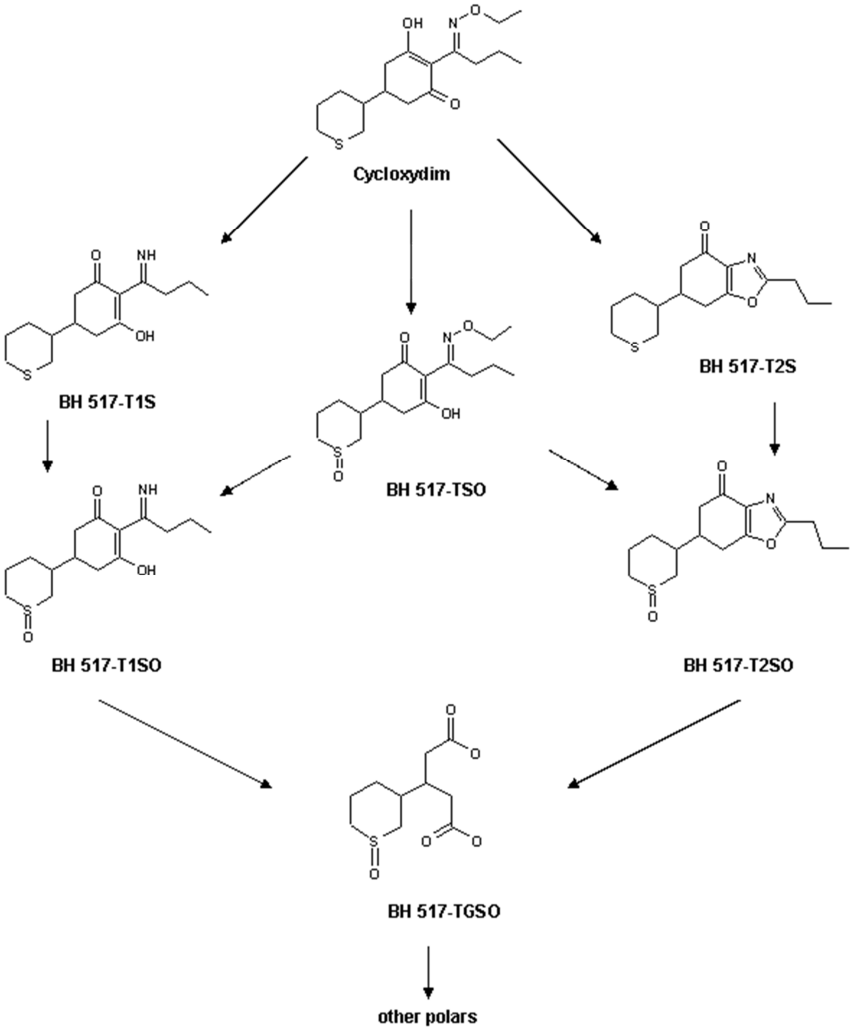
Aquatic photolysis
Under test conditions in sterile aqueous buffer solutions cycloxydim was rapidly photolytically degraded in all tested buffer solutions with DT_{50} values of 5.8 hrs, 17.6 hrs and 22.3 hrs at pH 5, pH 7 and pH 9, respectively. The main product was BH 517-T1SO with > 60 % of AR in all test solutions. The calculated DT_{50} of 8.4 days at pH 5 has to be dealt carefully as the last sampling point is very questionable. In both other solutions (pH 7 and pH 9) the metabolite BH 517-T1SO is considered stable. Furthermore BH 517-TGSO, BH 517-T2SO, BH 517-TSO and BH 517-T2S were found in amounts > 10 % of AR. BH 517-TGSO was formed rather at the last sampling points and in the solutions pH 7 and pH 9 the concentrations still increased. Therefore BH 517-TGSO is considered as stable. All the other metabolites were rapidly photolytically/hydrolytically degraded with calculated DT_{50} values in the range of 0.25 days to 3.9 days.

Biological degradation
No study was submitted for screening tests.

Water/sediment studies

In two water/sediment systems cycloxydim was degraded to the main metabolite BH 517-TSO. A second major metabolite, BH 517-T1S, was identified in sediment in system B "Berghäuser Altrhein" with maximal amount of 10.8 % of AR at the end of the study. The minor metabolite BH 517-T1SO was detected in both systems with maximum amounts of 5.6 % of AR in the total system of system B "Berghäuser Altrhein".
No total half-lives were estimated in this study but were calculated in the course of the PECsurface water calculation. Following resubmission, $DegT_{50}$ of cycloxydim in the entire system was recalculated by the RMS. For both systems degradation half-lives following SFO kinetics were calculated. The visual fit for "Kellmetschweiher" system was acceptable and it was good for "Berghäuser Altrhein" system, the Chi^2 error for both systems was below 15 %. For the "Kellmetschweiher" system a SFO DT50 of 30.6 days was calculated and for the "Berghäuser Altrhein" system a SFO DT50 of 14.1 days was calculated. The geometric mean $DegT_{50}$ of 20.8 days for cycloxydim in the entire water/sediment system is considered most appropriate for the surface water risk assessment.
The mineralization to CO_2 was low with maximal amounts of 4.8 % at day 100 in "Kellmetschweiher" system and 13.9 % at day 100 in "Berghäuser Altrhein" system. The formation of non-extractable radioactivity was noted with max. 6.1 % in „Kellmetschweiher" system and with max. 13.9 % in "Berghäuser Altrhein" system, both maxima were found at the end of the study.

Photolysis: Proposed degradation pathway of cycloxydim in sterile aqueous solutions



Proposed route of degradation of Cycloxydim (BAS 517 H) in water/sediment systems

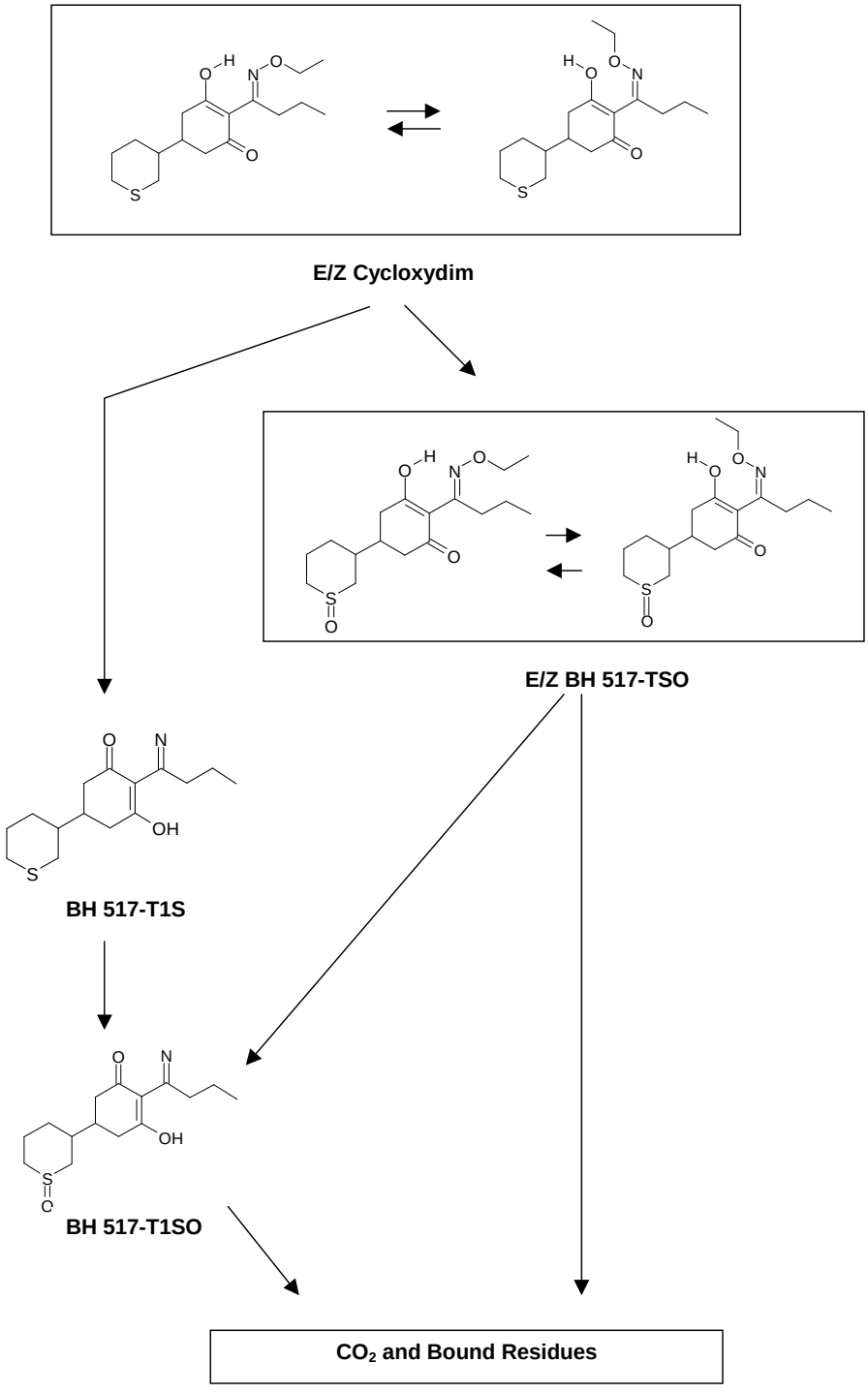


Table 119: Summary on DT_{50} and DT_{90} [days] for the degradation of cycloxydim (BH 517) in laboratory hydrolysis (SFO), photolysis (SFO) and water/sediment studies (SFO).

Hydrolysis			Photolysis				Water/sediment	
	DT50 [d]	**DT90 [d]**		**DT50 [h]**	**DT90 [h]**		**DT50**	**DT90**
pH 3	**2**	**-**				**Water**	**28/10.5**	**93/35**
pH 4	**2**	**7.0**				**Sediment**	**10.5/18.1**	**35/60**
pH 5	**8/12**	**40.5**	**pH 5**	**5.8**	**19.4**	**Entire system**	**30.6/14.1**	**102/46.7**
pH 7	**172/264**	**875**	**pH 7**	**17.6**	**58.5**	**Geo.mean**		
pH 9	**206/958**	**>1000**	**pH 9**	**22.3**	**73.9**	**entire system**	**20.8**	**68.9**

Table 120: Summary on maximum occurrence [% of AR] of cycloxydim and metabolites of cycloxydim in hydrolysis, photolysis and water/sediment studies (data stated in brackets give day and pH of maximum occurrence)

Compartment	Cycloxydim	T1S	T2S	TSO	T1SO	TGSO	T2SO
Hydrolysis			**99.7** (30 d, pH 4)	**19.3** (21 d, pH 9)			
Photolysis		**27.9** (1 d, pH9)	**12.4** (6 h, pH 5)	**53.1** (1 d, pH7)	**68.8** (6 d, pH 5)	**26.4** (9 d, pH 5)	**18.3** (1 d, pH 5)
Water	**91** (0 d)/ **90** (0 d)	<5		**67** (60 d)/ **52** (31 d)	<5		
Sediment	9 (14 d)/ **13** (14 d)	<5/ **10.8** (100 d)		**10** (100 d)/ **12** (31 d)	<6		
Entire system	**91** (0 d)/ **90** (0 d)	<5/ **11** (100 d)		**76** (60 d)/ **64** (31 d)	<5/ <6		

Summary: Degradation (evidence of rapid degradation)

<table>
<tr><th colspan="4">Data element: Degradation</th></tr>
<tr><td></td><td>Test guideline / design</td><td>GLP (y/n)</td><td>Reliability</td></tr>
<tr><td>Biotic degradation (% degradation in 28 davs (or, if absent, half- life in water (d)):
Water/sediment system (simulation test)

Whole ystem
DT50 (System A: pH 8.8, 20°): 30.6 d
DT50 (System B: pH 7, 20°): 14.1 d
DT50 (both systems, geomean): 20.8 d</td><td>Water/Sediment Study
Guideline: BBA IV 5-1, EPA 162-4, SETAC Europe Part 8.</td><td>y</td><td></td></tr>
<tr><td>Abiotic degradation

Hydrolysis:
<table>
<tr><th>pH</th><th>DT₅₀ [days]</th><th>DT₉₀ [days]</th></tr>
<tr><td>4</td><td>2.1</td><td>7.0</td></tr>
<tr><td>5</td><td>12.2</td><td>40.5</td></tr>
<tr><td>7</td><td>264</td><td>875</td></tr>
<tr><td>9</td><td>958</td><td>>1000</td></tr>
</table>
Photolysis
DT50 (pH 5, 22°): 5.8 h
DT50 (pH 7, 22°): 17.6 h
DT50 (pH 9, 22°): 22.3 h</td><td>EPA Subdivision N, § 161-1;
SETAC 1995;
CD 94/37/EG;
OECD 111

Guideline: EPA 161-2</td><td>y

y</td><td>y

y</td></tr>
</table>

Dossier submitter's conclusion: The criteria for rapid degradation are not fulfilled because

Cycloxydim was hydrolytically rather stable at neutral and alkaline conditions (DT50 at pH 7 = 172 and 264 days, DT50 at pH 9 = 206 and 958 days) in two hydrolysis studies.

In the photolysis study cycloxydim was rapidly photolytically degraded but the main product BH 517-T1SO, detected with > 60 % of AR in both solutions (pH 7 and pH 9) is considered stable. The maximal amounts of volatiles, whereas the radioactivity was found only in NaOH-traps, were 0.2 % (pH 7) and 0.7 %(pH 9) after 15 days.

No data are available with regard to the ready biodegradability of cycloxydim.

In water/sediment studies the substance was degraded with a DT50 whole system
System "Kellmetschweiher" (pH 8.8) = 30.6 d (SFO),
System "Berghäuser Altrhein"(pH 8.5)= 14.3 d (SFO)
arithmetic geomean = 20.8 d.

The mineralization to CO2 was low with maximal amounts of 4.8 % at day 100 in "Kellmetschweiher" system and 13.9 % at day 100 in "Berghäuser Altrhein" system.

5.1.4 The RAC assessment of degradation and comparison with criteria

See section 5.2.3.

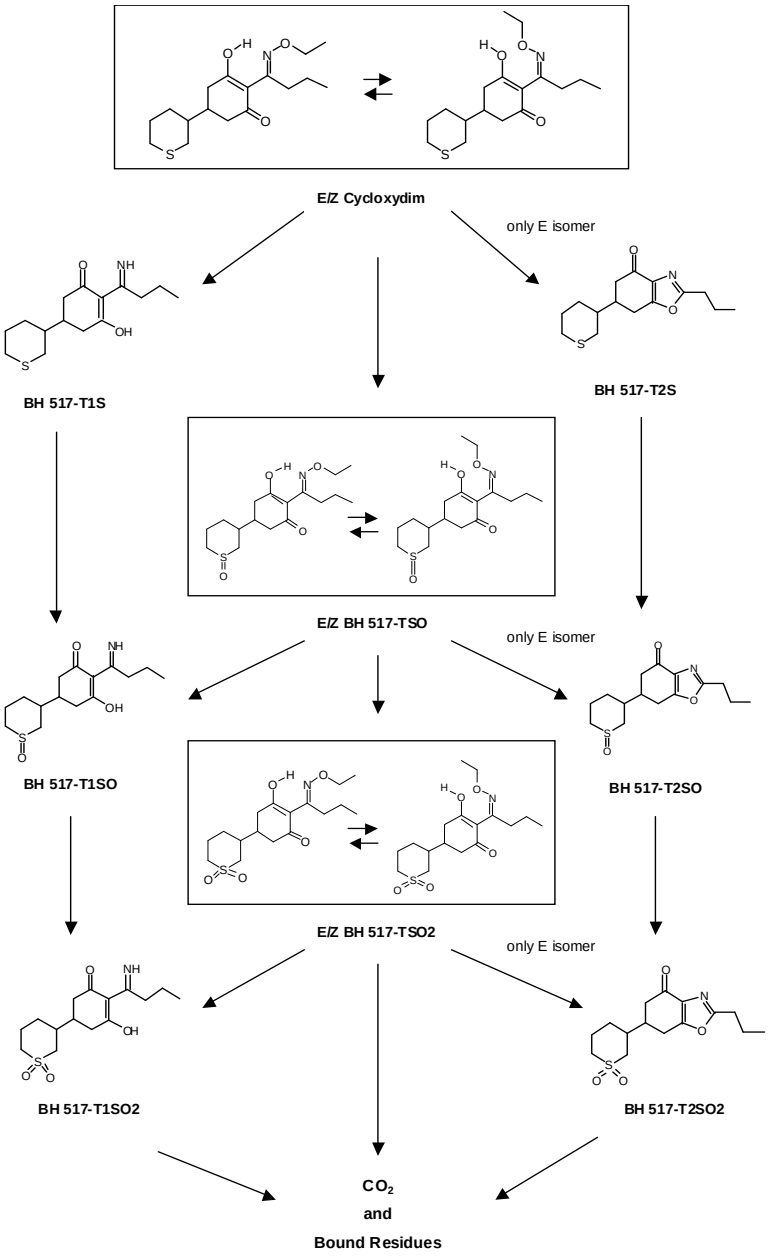
5.2 Environmental distribution

Information on route of degradation in soil provided by the dossier submitter

The route of degradation of cycloxydim in soil under dark aerobic conditions at 20 ± 2 ºC or 22 ± 2 ºC was investigated in three studies. A total of six soils were investigated covering a range of pH (5.4 – 7.5) and organic carbon (0.52 % - 3.5 %). In the first two conducted metabolism studies it was not possible to separate the peaks in each cluster and therefore a third study was conducted to investigate the metabolism of cycloxydim.

The **aerobic** metabolism studies showed that cycloxydim was oxidized at the sulfur atom immediately after application to the soil. The predominant metabolite is the corresponding sulfoxide BH 517-TSO, which occurs with a maximum of 90.4 % of AR by 1 day after application. BH 517-TSO degrades to a certain extent to the oxidation product BH 517-TSO2 (max. 9.5 % of AR by 14 days). The active substance and these two metabolites contain the ethoxyimino group. This ethoxyimino group includes a C=N double bond where an E/Z isomerization occurs frequently and easily. It was shown by dynamic NMR studies that the ratio of the isomers depends on the physical state of the compound and the polarity of the solvents. In acidic solution in polar protic solvents, the E form isomerizes to the Z form until equilibrium is reached after several hours. In alkaline solution, no E/Z isomerization was observed. It was not possible to isolate the Z isomer as it seems to be on a higher energy level. Therefore, all tests were started with the E isomer from the solid state or in formulation. This approach seems to be appropriate as the tests mirror the environmental conditions. For this reason all calculations were carried out with the sum of both isomers.

Six additional metabolites were formed under aerobic soil conditions. All these metabolites, BH 517-T1S (max. 0.7 % of AR), BH 517-T1SO (max. 4.2 % of AR by 7 days), BH 517-T1SO2 (max. 2.6 after 60 days), BH 517-T2S (max. 0.3 % AR at day 0), BH 517-T2SO (max. 8.1 % of AR by 30 days) and BH 517-T2SO2 (max. 9.9 % of AR by 90 days) were formed in amounts < 10 % AR. The final product of degradation was CO_2 which was formed in amounts up to 40 – 60 % AR in the investigated soils. Only moderate amounts of soil bound residues were formed; not more than 30 % of AR was found to be non-extractable.



Proposed degradation pathway of cycloxydim in soil.

During **anaerobic** incubation mineralization of cycloxydim to CO_2 was significantly reduced, the amount of bound residues increased up to 9.7 % of AR. Four degradation products could be identified as BH 517-TSO, BH 517-T2S, BH 517-T2SO and BH 517-T1SO, whereas BH 517-TSO (major) was observed with a maximum of 42.4 % of AR on day 30. All others were observed as minor metabolites. The pathway is the same as in the aerobic studies.

In a **soil photolysis** study cycloxydim was rapidly degraded under irradiated conditions. After 8 hours incubation period only 2 % AR of cycloxydim were recovered. In the dark samples after 8 hours 15 % of AR were still present. The half-life was stated to be < 1 hour. BH 517-TSO and BH 517-T2SO were identified as major metabolites (BH 517-TSO and BH 517-T2SO could not be separated: maximum occurrence of peak cluster: 81.4 % of AR by 8 hrs, ratio approx 1:1). Minor photolytic degradation products were BH 517-TSO2/BH 517-T2SO2 (max. 8.2 % of AR), BH 517-T1S (max. 1.9 % of AR) and BH 517-T2S (max. 5.3 % of AR). Amounts of polar compounds did not accumulate within the increasing incubation time (max. 7.5 % by 6

hours). Volatiles did not appear during the experiment. No other metabolites occurred under irradiated conditions than under aerobic dark conditions. The pathway was the same as in the aerobic metabolism studies. The study was conducted with a test temperature of about 30°C which is not appropriate with current guidelines. Due to the very fast elimination of the parent substance the test duration was very short. However, the results indicate that no other products will be formed and the metabolism scheme is the same like the metabolism scheme of the aerobic metabolism study in the dark. The degradation of cycloxydim seems to be accelerated under irradiation but as cycloxydim is rapidly degraded by soil microorganisms the photolytic degradation has no significant influence on the elimination of the active substance.

Dossier submitter's summary on rate of degradation in laboratory soil studies

The aerobic laboratory soil degradation rate of cycloxydim (BH 517 H) was investigated in 3 studies conducted on 6 soils with a representative range of properties (pH, organic carbon, texture, origin) using [cyclohexene-4(6)-^{14}C] labelled cycloxydim:

- pH 5.4 – 7.2
- organic carbon: 0.52 – 3.5 %
- clay content: 4 – 22 %

Under **aerobic conditions** cycloxydim rapidly degraded with a $DegT_{50}$ in a range of < 0.1 – 2.7 days (all SFO kinetics) with a geometric mean $DegT_{50}$ of 0.2 days. Normalization to 20 °C and pF 2 was not conducted; owing to the instantaneous transformation of cycloxydim to the sulfoxid metabolite BH 517-TSO the normalized $DegT_{50}$ for cycloxydim was set to 1.0 days for modelling.
Under **anaerobic conditions** (conducted on one soil) degradation of cycloxydim was significantly slower with a calculated SFO $DegT_{50}$ of 51 days.
Under **sterile/aerobic conditions** (conducted on one soil) cycloxydim degraded with a $DegT_{50}$ of 8.5 days (SFO).

In a **soil photolysis** study cycloxydim was rapidly degraded under irradiated conditions. After 8 hours incubation period only 2 % AR of cycloxydim were recovered. In the dark samples after 8 hours 15 % AR were still present. The half-life was stated to be < 1 hour. The degradation of cycloxydim seems to be accelerated under irradiation but as cycloxydim is rapidly degraded by soil microorganisms the photolytic degradation has no significant influence on the elimination of the active substance.

Kinetic evaluation of **metabolites** was only possible for one of the three aerobic parent degradation studies (Bayer, 2000) since metabolites could not be adequately separated in the other two studies. In addition, separate studies conducted with metabolites BH 517-TSO2 (Richter, 2006) and BH 517-T2SO2 (Simmonds and Early, 2005) are available.

The major soil metabolite **BH 517-TSO** (maximum occurrence 90.4 % of AR) degraded with a $DegT_{50}$ in a range of 9.3 – 10.6 days (SFO kinetics, multi-compartment modelling, chi^2 error ≤ 15.0 %, n = 3) with a formation fraction from the parent in a range of 0.66 – 0.98. Following normalization to 20 °C and pF 2 (conducted by the RMS), a geometric mean $DegT_{50}$ of 8.9 days was derived. Note: For modelling purposes higher tier DT_{50} values from field studies (refer to B.8.3.1) are available. The formation fraction from the parent was set to 1.0 (100 %) for conservative reasons.

The minor soil metabolite **BH 517-TSO2** (maximum occurrence 9.5 % of AR) degraded with a $DegT_{50}$ in a range of 5.8 – 29.6 days (SFO kinetics in most cases, multi-compartment modelling or applied as parent, chi^2 error ≤ 35.1 %, n = 9). In one soil (metabolite applied as parent) best fit was archived by FOMC kinetics. In this case a conservative $DegT_{50}$ obtained by FOMC-$DegT_{90}$ divided by 3.32 was used. Following multi compartment-modelling (n = 3) a formation fraction (from BH 517-TSO) in the range of 0.193 – 0.262 (arithmetic mean 0.217) could b derived. After normalization to 20 °C and pF2 a geometric mean $DegT_{50}$ of 10.6 days

could be stated. Note: For modelling purposes higher tier DT_{50} values from field studies (refer to B.8.3.1) are available.

The minor soil metabolite **BH 517-T2SO** (maximum occurrence 8.1 % of AR) was found to degrade with a $DegT_{50}$ of 17.9, 19.6 and 292 days (SFO kinetics, chi^2 error < 16.0 %). The longest $DegT_{50}$ value derives from multi-compartment modelling and is considered not fully valid (p of t-test > 0.1). Nevertheless, the value was included to derive a conservative final kinetic endpoint for modelling. The two shorter $DegT_{50}$ values were obtained by SFO kinetics starting at the maximum occurrence of the metabolite, which can be considered a conservative approach. Multi-compartment modelling of these two soils did not result in valid degradation rates at all. The formation fraction from the precursor BH 517-TSO was calculated to be in a range of 0.139 – 0.214 with an arithmetic mean value of 0.165. For modelling purposes the average formation fraction was used together with the geometric mean $DegT_{50}$ of 41.8 days when normalized to 20 °C and pF 2.

For the minor soil metabolite **BH 517-T2SO2** (maximum occurrence 9.9 % of AR, observed in one soil only) no valid degradation rates could be archived by multi-compartment modelling. Therefore, a separate study was conducted with this metabolite resulting in $DegT_{50}$ values of 21.2, 25.0 and 118 days. Normalized to 20 °C and pF 2 a geometric mean $DegT_{50}$ of 35.2 days could be derived. The formation fraction of this metabolite, which may be formed from BH 517-TSO2 and BH 517-T2SO, was set to 0.33 from both precursors for conservative reasons. This value represents the arithmetic mean of 0.0, 0.0 and 1.0, whereby the zero values are considered representative for the two soils where the metabolite was not found whereas the value of 1 represents a conservative assumption for the study, where BH 517-T2SO2 was found.

Table 121: Summary on aerobic soil degradation rate experiments conducted with cycloxydim or metabolites.

Study No.	Test compound	Application rate [mg kg^{-1}]	Application rate[a] [g ha^{-1}]	Moisture conditions	Temperature [°C]	Reverence
1	Cycloxydim	10	7500	40 % of MWHC	22	Huber (1987)
2	Cycloxydim	10	7500	40 % of MWHC	22	Huber (1988)
3	Cycloxydim	0.8	600	40 % of MWHC	20	Bayer (2000)
4	BH 517-TSO2	0.16	120	40 % of MWHC	20	Richter (2006)
5	BH 517-T2SO2	0.13	100	40 % of MWHC	20	Simmonds and Early (2005)

[a] Assuming 5 cm soil depth and 1.5 g cm^{-3} soil density

Table 122: Summary on aerobic laboratory and normalized (20 °C, pF2) degradation rates of cycloxydim and metabolites.

Compound	Study No.	Soil texture	pH (Matrix)	Organic C [%]	Clay [%]	Non normalized		Normalized (20 °C, pF 2)	Formation fraction [0 .. 1]	Chi^2 error [%]	Kinetics
						$DegT_{50}$ [d]	$DegT_{90}$ [d]	$DegT_{50}$ [d]			
Cycloxydim	1	Loamy sand	6.1 (uk)	3.5	8	0.4[a]	1.3[a]	0.4[a]	na	2.8[a]	SFO (FOMC $DegT_{90}$ / 3.32)
	2	Loamy sand	6.7 (uk)	0.52	8	2.6[a]	8.6[a]	nc[b]	na	2.8[a]	SFO
	2	Loam	7.2 (uk)	1.54	22	< 1[e]	na	nc[b]	na	na	SFO (only 3 values available)
	3	Sandy loam	7.2 (Ca)	1.63	10	< 1[f]	na	<1	na	na	SFO (MCM) (only 2 values available)
	3	Sandy loam	6.5 (Ca)	1.24	8	< 1[f]	na	< 1	na	na	SFO (MCM) (only 1 value available)
	3	Loamy sand	5.4 (Ca)	1.96	4	0.2	0.5	0.2	na	5.8	SFO (MCM)
	Geometric mean					**nc**	**nc**	**1.0[c]**	**-**	**-**	
BH 517-	3	Sandy	7.2	1.63	10	10.6	35.2	9.5	0.904	9.2	SFO (MCM)

Compound	Study No.	Soil texture	pH (Matrix)	Organic C [%]	Clay [%]	Non normalized		Normalized (20 °C, pF 2)	Formation fraction [0 .. 1]	Chi^2 error [%]	Kinetics
						$DegT_{50}$ [d]	**$DegT_{90}$ [d]**	**$DegT_{50}$ [d]**			
TSO		loam	(Ca)								
	3	Sandy loam	6.5 (Ca)	1.24	8	10.1	33.5	8.0	0.979	7.0	SFO (MCM)
	3	Loamy sand	5.4 (Ca)	1.96	4	9.3	30.9	9.3	0.660	15.0	SFO (MCM)
	Geometric mean					**10.0**	**33.2**	**8.9**	**-**	**-**	**-**
	Arithmetic mean					**-**	**-**	**-**	**0.848**	**-**	**-**
BH 517-TSO2	3	Sandy loam	7.2 (Ca)	1.63	10	12.6	41.8	11.3	0.262	12.4	SFO (MCM)
	3	Sandy loam	6.5 (Ca)	1.24	8	10.8	35.9	8.5	0.193	32.9	SFO (MCM)
	3	Loamy sand	5.4 (Ca)	1.96	4	8.8	29.2	8.8	0.197	35.1	SFO (MCM)
	4	Sand	5.9 (Ca)	0.82	3	25.2	83.7	25.2	na	6.0[a]	SFO
	4	Loamy sand	5.4 (Ca)	2.47	6	5.8	19.3	5.8	na	9.4[a]	SFO
	4	Loam	7.1 (Ca)	2.84	18	29.6[d]	98.3	22.4[a]	na	7.6[a]	SFO (FOMC $DegT_{90}$ / 3.32)
	4	Sandy loam	7.1 (Ca)	1.73	10	7.4	24.5	6.8	na	9.7[a]	SFO
	4	Sandy loam	7.2 (Ca)	2.72	12	13.3	44.2	11.2	na	5.8[a]	SFO
	4	Loamy sand	6.1 (Ca)	0.60	6	8.6	28.6	8.2	na	8.8[a]	SFO
	Geometric mean					**11.8**	**39.1**	**10.6**	**-**	**-**	**-**
	Arithmetic mean					**-**	**-**	**-**	**0.218**	**-**	**-**
BH 517-T2SO	3	Sandy loam	7.2 (Ca)	1.63	10	19.6	65.1	14.2	0.139	12.5	SFO (starting from max. occurrence)

Compound	Study No.	Soil texture	pH (Matrix)	Organic C [%]	Clay [%]	Non normalized		Normalized (20 °C, pF 2)	Formation fraction [0 .. 1]	Chi^2 error [%]	Kinetics
						$DegT_{50}$ [d]	**$DegT_{90}$ [d]**	**$DegT_{50}$ [d]**			
	3	Sandy loam	6.5 (Ca)	1.24	8	17.9	59.4	17.6	0.214	10.1	SFO (starting from max. occurrence)
	3	Loamy sand	5.4 (Ca)	1.96	4	292	969	292	0.141	16.0	SFO (MCM)
	Geometric mean					**46.8**	**155**	**41.8**	**-**	**-**	**-**
	Arithmetic mean					**-**	**-**	**-**	**0.165**	**-**	**-**
BH 517-T2SO2	3	Loamy sand	5.4 (Ca)	1.96	4	na	na	na	1.00 / 0.934	na	SFO (MCM)
	5	Sandy loam	7.3 (Ca)	1.5	13	21.2	70.4	19.1	na	4.8	SFO
	5	Loam	7.2 (Ca)	2.6	16	25.0	83.0	19.4	na	3.0	SFO
	5	Loamy sand	5.3 (Ca)	1.3	8	118	392	118	na	2.9	SFO
	Geometric mean					**39.7**	**132**	**35.2**	**-**	**-**	**-**
	Arithmetic mean					**-**	**-**	**-**	**na**	**-**	**-**

MCM: multi-compartment modelling
na: not applicable
[a] Added by the RMS
[b] No study moisture available
[c] Pragmatic endpoint for numerical reasons
[d] FOMC best fit kinetics (RMS assessment)
[e] addresses point 4(5) in the reporting table of DAR
[f] addresses point 4(6) in the reporting table of DAR

Dossier submitter's summary on field studies

Field dissipation trials were conducted on four sites in the UK with cycloxydim (Kellner, 1996). The formulation BAS 517 01 H containing 200 g L^{-1} cycloxydim was applied once at 0.42 to 0.48 kg as ha^{-1} to plots (2 x 15 metres and 3 x 10 metres, respectively) cropped with salad onions. The study confirms the fast dissipation of cycloxydim in soil. However, owing to several analytical shortcomings the study is not considered valid by the RMS.

New field dissipation trials were conducted with formulated cycloxydim and metabolite BH-517-TSO2 at six representative sites in the EU following the CTB check list (Bayer 2009, Bayer and Richter, 2009). In order to exclude any impact of irradiation, the field trials were covered with a small layer of sand after application. The field dissipation trials were sampled up to 90 cm soil depth (depending on the deepest layer with residues below the LOQ). Residues of BH 517-TSO and BH 517-TSO2 were summed up and subjected to rate-normalization in accordance with pertinent FOCUS guidance in order to derive higher tier degradation rates for these two metabolites.

Cycloxydim was extremely fast dissipating; no residues could be measured by 0 DAT sampling above the LOQ.

Owing to the instantaneous transformation of the parent cycloxydim, residues of metabolite BH 517-TSO were highest at 0 DAT sampling rapidly declining thereafter (dissipation about 3 – 4 times faster than observed in the lab). No residues of BH 517-TSO were found above the LOQ below 40 cm of soil depth. Since soil photolysis can be ruled out (sand cover), volatilisation is considered negligible and no significant residues were lost by leaching, dissipation of BH 517-TSO in the field trials can be considered equivalent to (microbial) degradation. Following rate-normalization to 20 °C (using a Q_{10} value of 2.58) and pF 2 (in accordance to pertinent FOCUS guidance), the DT_{50} of BH 517-TSO was in a range of 0.4 – 3.8 days with a geometric mean of 1.7 days. SFO kinetics was considered the best fit in case of 5 of the 6 field trials, HS kinetics in one trial. In case of HS kinetics the DT_{50} of the second and slower phase was used for conservative reasons. The normalized geometric mean field DT_{50} of BH 517-TSO is considered appropriate as higher tier endpoint for the degradation rate of BH 517-TSO for modelling purposes.

In the study with formulated BH 517-TSO2 applied, a rapid dissipation/degradation of the compound could be observed also. Similar to BH 517-TSO, no residues were observed below a soil depth of 30 cm above the LOQ, indicating that the dissipation rate can be considered equivalent to (microbial) degradation. Following normalization to 20 °C (using a Q_{10} value of 2.58) and pF 2 (in accordance to pertinent FOCUS guidance), the DT_{50} of BH 517-TSO2 was in a range of 1.5 – 4.6 days with a geometric mean of 2.5 days. SFO kinetics was considered the best fit in case of 3 trials and HS kinetics in the other 3 trials. In case of HS kinetics the DT_{50} of the second and slower phase was used for conservative reasons. The normalized geometric field DT_{50} of BH 517-TSO2 is considered appropriate as higher tier endpoint for modelling purposes.

Table 123: Summary on rate normalized (for soil temperature and moisture) DT50 values of BH 517-TSO and BH 517-TSO2 in field dissipation trials.

Compound	Field trial	Trial number	Norm. $DegT_{50}$ [days]	Chi^2 error [%]	p ≤ 0.05	Kinetics
BH 517-TSO	Clifford Chambers, UK	L0703 27	3.83	13.3	Yes	SFO
	Middelfart, DK	L0703 28	0.35	4.5	Yes	SFO
	Fehrbellin, DE (East)	L0703 29	1.62	8.3	Yes	SFO (second phase of HS)[a]
	Goch-Nierswalde, DE (West)	L0703 30	1.15	9.6	Yes	SFO
	Latour Bas Elne, FR (South)	L0703 31	2.58	7.2	Yes	SFO
	Utrera, SP	L0703 32	3.33	10.6	Yes	SFO
	Geometric mean		**1.67**	**-**	**-**	**-**
BH 517-TSO2	Clifford Chambers, UK	L0703 33	4.64	22.8	Yes	SFO (second phase of HS)[a]
	Middelfart, DK	L0703 34	2.68	6.4	Yes	SFO (second phase of HS)[a]
	Fehrbellin, DE (East)	L0703 35	1.71	17.2	Yes	SFO (second phase of HS)[a]
	Goch-Nierswalde, DE (West)	L0703 36	1.50	10.0	Yes	SFO
	Latour Bas Elne, FR (South)	L0703 37	2.45	5.1	Yes	SFO
	Utrera, SP	L0703 38	2.89	12.3	Yes	SFO
	Geometric mean		**2.47**	**-**	**-**	**-**

[a] Conservative assessment

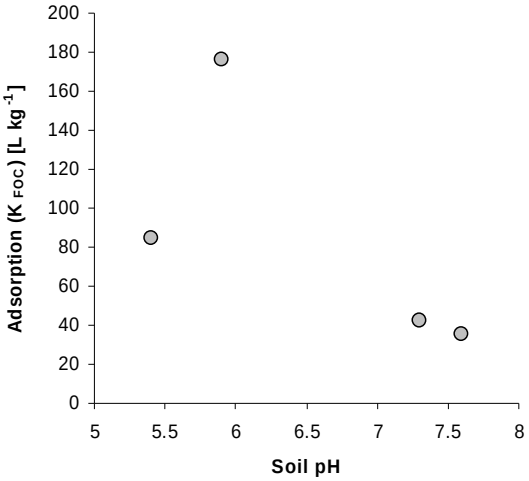
5.2.1 Adsorption/Desorption information provided by the dossier submitter

Adsorption, desorption and mobility in soil

The adsorption behaviour of **cycloxydim** was studied in four soils (pH 6.0 – 7.3, % OC 0.31 – 2.76) using the batch equilibrium method. The K_d values were in the range of 0.03 – 0.72 L kg^{-1} and showed that cycloxydim was weakly adsorbed to soil. The K_{OC} values were calculated to be in the range of 5 to 183 L kg^{-1} with an arithmetic mean of 59 L kg^{-1}, indicating very high to moderate mobility. At pH > 7 no or weak adsorption was measured. Since no 1/n value is available for cycloxydim a PRAPeR 32 agreed default value of 1.0 was used assuming linear adsorption.

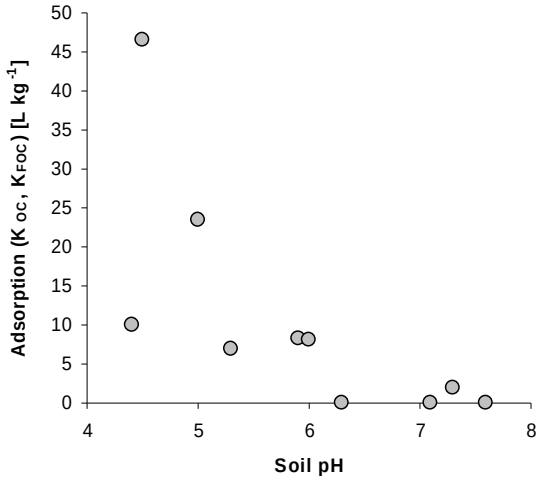
K_{FOC} values of **BH 517-TSO** were determined in six soils (pH 5.4 – 7.6, % OC 0.74 – 3.1). Adsorption values were in the range of 22 – 176 L kg^{-1} with a Freundlich exponents (1/n) in a range of 0.56 – 0.99, whereby soil Lufa 2.2 shows insufficient quality of the adsorption

isotherm. Therefore, this K_{FOC} value (22 mg L^{-1}) was not considered for further assessment. For soil Bruch Ost (sandy loam, pH 7.0, % OC 3.1) no measurable adsorption to soil was detected at all. The remaining four valid values indicate a high to moderate mobility of BH 517-TSO in soil. No clear correlation between adsorption to soil and pH value could be determined, but a tendency for stronger adsorption to soils with lower pH values can be deduced from the available data set. Therefore, it was considered appropriate to split the data set of BH 517-TSO for modelling into soils pH ≥ 6 (with an arithmetic mean K_{FOC} and 1/n value of 38.8 L kg^{-1} and 0.98, respectively) and into soils with a pH < 6 (with a arithmetic mean K_{FOC} and 1/n value of 130 L kg^{-1} and 0.71, respectively).



Adsorption of BH 517-TSO in relation to soil pH.

The adsorption behaviour of the metabolite **BH 517-TSO2** was tested in 10 different soils in two separate studies. In the first study with five soils (pH 5.3 – 7.6, % OC 0.42 – 2.72) only for two soils with pH values < 6 Freundlich adsorption isotherms could be established. The determined K_{FOC} values for these two soils were 6.9 and 8.3 L kg^{-1}. For the soil with a pH value of 7.3 only at the lowest test concentration (0.004 µg L^{-1}) a significant adsorption could be measured. The determined K_{OC} value was 2 L kg^{-1}. Therefore, a second adsorption study was conducted to test the influence of pH on the sorption behaviour of BH 517-TSO2. In that study, 5 acidic soils (pH 4.4 – 6.3, % OC 0.58 – 1.85) were tested. It was shown that acidic soils have a capability to at least weakly adsorb BH 517-TSO2. In all soils with pH > 6 no adsorption was determined. In soils with pH values < 6 weakly adsorption with K_{OC} values in a range of 8.1 – 46.5 L kg^{-1} was detected, but no clear correlation between pH value and adsorption in this range of pH could be shown. If no reliable 1/n value was available the value was set to PRAPeR agreed default value of 1.0. Furthermore, an additional study (Richter, 2006) was conducted on six soils (pH 5.4 – 7.3, % OC 0.6 – 2.84) to investigate the degradation of BH 517-TSO2 and it was tried to identify a long-term sorption effect. It was shown that sorption did not increase with incubation time, except in two soils (loamy sand, pH 5.4 and 6.1). As it was not possible to show a significant adsorption to soil particles for soils with pH values ≥ 6, a K_{FOC} value of 0 L kg^{-1} and a PRAPeR agreed default value of 1.0 for 1/n was assumed for soils with pH ≥ 6 for groundwater exposure assessment. For soils < pH 6, an arithmetic mean K_{FOC} of 17.2 L kg^{-1} and a 1/n value of 0.97 are considered appropriate.



Adsorption of BH 517-TSO2 in relation to soil pH.

Adsorption of BH 517-TSO2 in relation to soil pH.

K_{FOC} values of **BH 517-T2SO** were determined in 10 soils (pH 5.4 – 7.5, % OC 0.35 – 3.1). In one soil (sand, pH 5.4, % OC 0.74) no significant adsorption could be observed. The remaining valid K_{FOC} values indicate a high to moderate mobility (K_{FOC} values in a range of 88 – 216 L kg^{-1} with 1/n values in a range of 0.77 – 0.93), except in one soil (silt loam, pH 5.4, % OC 0.35), where a much higher adsorption was determined (6585 L kg^{-1}). This high K_{FOC} value was assumed as an outlier and not considered further. No soil properties were found to affect the sorption of BH 517-T2SO. Therefore, the arithmetic mean K_{FOC} value of 143 L kg^{-1} and arithmetic mean 1/n value of 0.87 are considered appropriate adsorption endpoints for modelling.

The sorption behaviour of metabolite **BH 517-T2SO2** was determined in five soils (pH 5.3 – 7.6, % OC 0.42 – 2.72). The K_{FOC} values observed were in a range of 72 – 308 L kg^{-1} with a 1/n value in the range of 0.85 – 0.89 indicating a high to medium mobility in soil. No soil properties were found to affect the sorption of BH 517-T2SO2. Therefore, the arithmetic mean K_{FOC} value of 159 L kg^{-1} and arithmetic mean 1/n value of 0.88 are considered appropriate adsorption endpoints for modelling.

Table 124: Summary on adsorption of cycloxydim and metabolites in soil.

Compound	Soil texture	pH (matrix)	OC [%]	K_d [L kg^{-1}]	K_{OC} [L kg^{-1}]	K_F [L kg^{-1}]	K_{FOC} [L kg^{-1}]	1/n
Cycloxydim	Sand	6.0 (uk)	0.31	0.57	183	-	-	1.00[a]
	Sandy loam	6.0 (uk)	0.84	0.19	22	-	-	1.00[a]
	Loamy sand	6.2 (uk)	2.76	0.73	26	-	-	1.00[a]
	Loam	7.3 (uk)	0.58	0.03	5	-	-	1.00[a]
	Arithmetic mean			**-**	**59**	**-**	**-**	**1.00**
BH 517-TSO	Sand	5.4 (uk)	0.74	nc	nc	0.63	85	0.73
	Sandy loam	5.9 (uk)	0.95	nc	nc	1.67	176	0.68
	Loamy sand	6.0 (uk)	2.66	nc	nc	-	-	-
	Sandy	7.0 (uk)	3.1	nc	nc	-	-	-

Compound	Soil texture	pH (matrix)	OC [%]	K_d [L kg^{-1}]	K_{OC} [L kg^{-1}]	K_F [L kg^{-1}]	K_{FOC} [L kg^{-1}]	1/n
	loam							
	Sandy loam	7.3 (Ca)	1.1	nc	nc	0.47	42.3	0.97
	Sandy loam	7.6 (Ca)	1.1	nc	nc	0.39	35.2	0.99
	Arithmetic mean (soil pH ≤ 6)			**-**	**-**	**-**	**130**	**0.71**
	Arithmetic mean (soil pH > 6)			**-**	**-**	**-**	**38.8**	**0.98**
BH 517-TSO2	Loamy sand	4.4 (Ca)	0.58	0.06	10.0	-	-	1.00[a]
	Sand	4.5 (Ca)	1.33	0.62	46.5	-	-	1.00[a]
	Sandy loam	5.0 (Ca)	1.44	0.34	23.5	-	-	1.00[a]
	Loamy sand	5.3 (Ca)	2.24	-	-	0.15	6.9	0.96
	Sand	5.9 (Ca)	0.42	-	-	0.04	8.3	0.83
	Silt loam	6.0 (Ca)	1.70	0.14	8.1	-	-	1.00[a]
	Silt loam	6.3 (Ca)	1.85	nsa	nsa	-	-	-
	Sandy loam	7.1 (Ca)	1.61	nsa	nsa	-	-	-
	Sandy loam	7.3 (Ca)	2.72	0.001	2	-	-	1.00[a]
	Sandy loam	7.6 (Ca)	1.51	nsa	nsa	-	-	-
	Arithmetic mean (soil pH ≤ 6)			**-**	**-**	**-**	**17.2[b]**	**0.97**
	Arithmetic mean (soil pH > 6)			**-**	**-**	**-**	**0.0**	**1.00**
BH 517-T2SO	Sand	5.4 (uk)	0.74	-	-	nsa	-	-
	Loamy sand	6.0 (uk)	2.66	-	-	2.34	88	0.86
	Sandy loam	5.9 (uk)	0.95	-	-	1.66	175	0.82
	Sandy loam	7.0 (uk)	3.1	-	-	5.94	191	0.77
	Loamy sand	5.8 (uk)	0.5	-	-	0.57	114	0.88
	Sandy loam	5.8 (uk)	1.8	-	-	1.60	89	0.93
	Sandy loam	6.5 (uk)	1.0	-	-	2.16	216	0.90
	Sandy loam	7.5 (uk)	1.8	-	-	2.33	129	0.89
	Loamy sand	6.7 (uk)	0.35	-	-	0.51	145	0.89
	Silt loam	5.4 (uk)	0.35	-	-	23.0	6585[c]	0.93[c]

Compound	Soil texture	pH (matrix)	OC [%]	K_d [L kg^{-1}]	K_{OC} [L kg^{-1}]	K_F [L kg^{-1}]	K_{FOC} [L kg^{-1}]	1/n
	Arithmetic mean			**-**	**-**	**-**	**143**	**0.87**
BH 517-T2SO2	Sand	5.9 (Ca)	0.42	-	-	1.29	308	0.89
	Sandy loam	7.1 (Ca)	1.61	-	-	2.66	165	0.88
	Loamy sand	5.3 (Ca)	2.24	-	-	2.01	90	0.88
	Sandy loam	7.3 (Ca)	2.72	-	-	1.95	72	0.85
	Sandy loam	7.6 (Ca)	1.51	-	-	2.42	160	0.89
	Arithmetic mean			**-**	**-**	**-**	**159**	**0.88**

nsa denotes no significant adsorption
[a] Set to PRAPeR agreed default value of 1.0 if no reliable 1/n value available
[b] Arithmetic mean value of K_{oc} values (with 1/n value set to 1.0) and valid K_{FOC} values (with available 1/n values) (n=6)
[c] Excluded as an outlier

5.2.2 Volatilisation information provided by the dossier submitter

Considering the low vapour pressure (1.0 x 10^{-5} Pa at 20 °C) and Henry's law constant (6.1 x 10^{-8} kPa m^3/mol) cycloxydim has a very low volatilization potential. The photochemical oxidative half-life was estimated by a model calculation according to Atkinson to be 2.1 hours (12 hours day - 1.5 x 10^6 OH-radicals/cm^3) indicating a quick degradation of cycloxydim in the troposphere.

5.2.3 The RAC assessment of environmental distribution and comparison with criteria

Degradation

According to the information supplied in the dossier cycloxydim does not fulfil the criteria for rapidly degradable substances.Cycloxydim was hydrolytically rather stable at neutral and alkaline conditions (in two hydrolysis studies, DT50 at pH 7 =172 and 264 days, DT50 at pH 9 =206 and 958 days).

It is rapidly photolytically degraded; however, the main product BH 517-T1SO, detected at concentrations >60% of AR in both solutions (pH 7 and pH 9) is considered stable.

There is no available data with regard to the ready biodegradability.

In water/sediment studies the substance was degraded with a DT_{50} whole system (arithmetic geomean between two system) =20.8 d. Therefore the degradation cannot be considered as rapid, since *the substance is not ultimately degraded within 28 days with half-life <16 days corresponding to a degradation rate >0.043 day^{-1}.*

There are also two metabolites which appear at concentrations >10% AR: BH 517-TSO (76.2% at 60 d) and BH 517-T1S (11% at 100 d). However, there are not data about DT_{50}, and therefore they should be considered stable. In addition, the mineralization to CO_2 was low with maximal amounts of 13.9% at day 100, which confirms the conclusion of no rapidly degradable.

Soil degradation studies were also supplied in the dossier, and although according to the laboratory studies, cycloxydim is rapidly degradable with a DT_{50} (12ºC) <1.9 d, its main metabolite BH 517-TSO (90.4% at 1 d) shows a DT_{50} (12ºC) >16 days. Although field studies

show a faster degradation than laboratory studies, cycloxydim should not be considered rapidly degradable because of the evidences of the other tests.

In conclusion, cycloxydim should be considered not readily / not rapidly degradable.

Summary of the maximum occurence, degradation and availiability of ecotoxicological data for cycloxydim and all its identified degradation products.

Compound	Photolisys						Hydrolisys						Water-sediment study		Soil study		Aquatic toxicity data available
	Max. Occurence % (time)			Half-life time (max. values) (days at 12ºC)			Max. Occurence (time)			Half-life time (max. values) (days at 12ºC)			Max. Occurence (time)	DT 50 (days)	Max. Occurence (time)	DT50 (days at 12º C)	
	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9					
BH 517-TSO	31.6 (1 d)	53.1 (1 d)	17.6 (7h)	2.99	3.06	5.16	10.7 (1h)	15.6 (32 d)	19.3 (21 d)	stable	stable	stable	76.2 (60 d)	nd	90.4 (1 d)	16.88	Y
BH 517-T1SO	68.8 (6 d)	64.2 (15d)	68.1 (9d)	stable	stable	stable	-	-	-	-	-	-	-	-	-	-	Y
BH 517-T1S	-	-	27.9 (1 d)	-	-	5.40	-	-	-	-	-	-	10.8 (100 d)	nd	-	-	N
BH 517-T2S	12.4 (1 d)	-	-	0.56	-	-	50.9 (14 d) 78.1 (30 d)	-	-	stable	-	-	-	-	-	-	Y
BH 517-T2SO	18.3 (1 d)	15.7 (3 d)		8.76	4.68	-	-	-	-	-	-	-	-	-	-	-	N
BH 517-TSO2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

BH 517-TGSO	26.4 (9 d)	20.6 (15 d)	18.0 (15 d)	8.62	stable	stable	-	-	-	-	-	-	-	-	-	-	Y
BH 517-T2SO2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N

Other metabolites also appear in the different degradation tests; however, the percentage of occurrence was lower than 10%.

Note: Aquatic toxicity studies for metabolites BH 517-TSO, BH 517-T1SO, BH 517-T2S and BH 517-TGSO are available but are missing for BH 517-T1S and BH 517-T2SO. Thus a reliable classification regarding the hazard to the aquatic environment for all degradation products is not possible.

Adsortion/desorption

The adsorption and desorption of cycloxydim and its metabolites were studied in different soils and the results are:

Compound	**Koc [L.kg^{-1}]**
Cycloxydim	59
BH 517-TSO (soil pH ≤ 6)	130
BH 517-TSO (soil pH ≥ 6)	38.8
BH 517-TSO2 (soil pH ≤ 6)	17.2
BH 517-T2SO (soil pH ≥ 6)	0.0
BH 517-T2SO	143
BH 517-T2SO2	159

Parent compound and its metabolites show a low adsorption.

5.2.4 Distribution modelling

No study available.

5.3 Aquatic Bioaccumulation

Table 125 : Summary of relevant dossier submitter information on aquatic bioaccumulation provided by the dossier submitter.

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water Guideline: OECD 107 flask method (HPLC)	At 25 °C (99.4% w/w) log P_{OW} = 3.09 at pH 5 = 1.36 at pH 7 = - 0.42 at pH 9		Redeker J., (1988a)

5.3.1 Bioaccumulation estimation

No estimations available.

5.3.2 Measured bioaccumulation data

No study available. No study triggered, because of a logPow <3.

5.3.3 Summary and discussion of aquatic bioaccumulation

Physical-chemical properties important for evaluation of aquatic hazards for the purpose of classification				
	Test guideline / design	pH	GLP (y/n)	Reliability
Water solubility: 900 mg/L	EEC/A6 Flask method	7	y	y
At 25 °C (99.4% w/w) log P_{OW} = 3.09 at pH 5 **= 1.36 at pH 7** = - 0.42 at pH 9	OECD 107 flask method (HPLC)	5 7 9	y	y
Comments: **Cycloxydim is soluble in water. The log K_{ow} is < 4, indicating low potential for bioaccumulation,**				

5.3.4 The RAC assessment of aquatic bioaccumulation and comparison with criteria

No measured BCF is available. The log K_{ow} (=1.36 at 25ºC and pH =7) shows a low potential for bioaccumulation.

5.4 Aquatic toxicity

Table 126: Summary of relevant information on aquatic toxicity provided by the dossier submitter.

Method	Test organism	test condition	time	endpoint	test conc.	NOEC [mg/L]	EC_{50}/LC_{50} [mg/L]	Reference
cycloxydim-Na (BAS 517 H sodium salt)								
EPA 72-1	*Oncorhynchus mykiss* Rainbow trout	static	96 hr	mortality	n	8.1	20	Munk & Gelbke (1984a)
EPA 72-1	*Lepomis macrochirus* Bluegill sunfish	static	96 hr	mortality	n	100	100	Munk & Gelbke (1984b)
OECD 204	*Oncorhynchus mykiss* Rainbow trout	flow through	28 d	sublethal effects/ growth	n	1.5	6.4	Munk (1990)
cycloxydim (BAS 517 H)								
OECD 202	*Daphnia magna* Waterflea	static	48 hr	immobility	m	70.8	70.8	Dohmen (2000)
OECD 202	*Daphnia magna* Waterflea	semi static	21 d	reproduction	n	2.5	25	Jatzek (1989)
OECD 201	*Pseudokirchn. subcapitata* Green alga	static	96 hr	biomass growth rate	m	2.4 8.5	4.9 84.9	Kubitza (1998)
OECD 201, ASTM E1218-90	*Anabaena flos-aquae* Blue alga	static	96 hr	biomass growth rate	m	.8 1.9	8.2 74.9	Kubitza (2003)
ASTM E 1415-91 EPA 850.4400	*Lemna gibba* Duckweed	static renewal	7 d	biomass growth rate	n	.64 7.4	1.7 100	Dohmen (1999)

Test conc.: Test concentration based on mean measured (m), initial measured (im) or nominal (n) concentration

5.4.1 Fish information provided by the dossier submitter

5.4.1.1 Short-term toxicity to fish

Reference: Munk R. & Gelbke H.-P. (1984a): Report of the study of the acute toxicity – BAS 517H/Reg.No. 172 999 - rainbow trout (*Salmo Gairdneri* RICH.). Report no: 1984/0401

Guideline: EPA 72-1
GLP: Yes
Test item: Cycloxydim-Na (BH 517-H sodium salt), purity: 94.8 %, batch no: N 64

Material and methods:
Test species: Rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri)*
Number of organisms, weight, length: 10 fish per treatment, 1.0 g (0.8 – 1.2 g), 4.7 cm (4.5 – 4.9 cm)
Type of test and duration: static test, 96 hours
Applied concentrations: 0 (control), 46.4, 68.1, 100, 147, 215, 316, 464 and 681 mg/L (nominal); 0 (control), 48.2, 71.2, 101, 147.6, 215.4, 318, 466.8 and 689.7 mg/L (mean measured)
Test conditions:
Water quality: Reconstituted freshwater, hardness: 2.5 mmol/L
Temperature: 12 – 13 °C
pH: 8.0 – 8.4 (0 h), 7.8 – 7.9 (96 h)
Oxygen content: > 60 %, 7.5 – 8.6 mg O_2/L
Light regime: 16 hours light / 8 hours darkness
Test parameters: Mortality and sublethal effects were assessed after 1, 4, 24, 48, 72 and 96 hours; for chemical analysis of the test substance (HPLC) samples were taken at 0, 24, 48, 72 and 96 hours
Statistics: LC_{50} by Probit analysis, NOEC was derived directly from the results

Findings:
Chemical analysis: Mean measured values: 100.2 – 104.6 % of nominal
Mortality: None in control, after 96 hours no mortality was observed at concentrations up to 147 mg/L, at 215 mg/L five fish (50 %) were dead, 100 % mortality was noted at 316 mg/L and higher concentrations
Behavioural effects: After 96hours no effects were observed up to 68.1 mg/L, at 100 mg/L and higher concentrations effects like gasping, tumbling and convulsions were observed.

Conclusion: LC_{50} (96 h): 220 mg/L, NOEC: 68.1 mg/L based on nominal concentrations

Reference: Munk R. & Gelbke H.-P. (1984b): Report on the study of the acute toxicity – BAS 517 H (Reg.No. 172 999) - Bluegill (*Lepomis macrochirus* RAF.). Report no: 1984/0402

Guideline: EPA 72-1
GLP: Yes
Test item: Cycloxydim-Na (BH 517-H sodium salt), purity: 94.8 %, batch no: N 64

Material and methods:
Test species: Bluegill sunfish (*Lepomis macrochirus)*
Number of organisms, weight, length: 1 x10 fish per control and 50 mg/L treatment and 3 x10 per 100 mg/L treatment, 1.2 g (0.5 – 2.9 g), 4.4 cm (3.5 – 5.9 cm)
Type of test and duration: Static test, 96 hours
Applied concentrations: 0 (control), 50 and 100 mg/L (nominal); 0 (control), 49.5 and 99 mg/L (mean measured)
Test conditions:
Water quality: Reconstituted freshwater, hardness: 2.5 mmol/L
Temperature: 22 – 23 °C
pH: 8.8 – 8.4 (0 h), 7.8 – 7.9 (96 h)
Oxygen content: > 60 %, 7.5 – 8.6 mg O_2/L
Light regime: 16 hours light / 8 hours darkness

Test parameters: Mortality and sublethal effects were assessed after 1, 4, 24, 48, 72 and 96 hours; for chemical analysis of the test substance (HPLC) samples were taken after 1 hour and 96 hours
Statistics: LC_{50} by Probit analysis, NOEC was derived directly from the results
Findings:
Chemical analysis: Mean measured values: 99 % of nominal
Mortality: None in control, no mortality at 50 mg/L and 100 mg/L
Behavioural effects: None

Conclusion: LC_{50} (96 h): > 100 mg/L, NOEC: ≥ 100 mg/L based on nominal concentrations

5.4.1.2 Long-term toxicity to fish

Reference: Munk R. (1990): Sublethal toxic effects on rainbow trout (*Salmo gairdneri* RICH.) of Reg.No. 172 999-Na-Salz (Cycloxydim) (threshold level of the lethal and other effects, NOEC, at least 14 days). Report no: 1990/0394

Guideline: OECD 204
GLP: Yes
Test item: Cycloxydim-Na (BH 517-H sodium salt), purity: 93.2 %, batch no: N 175, P.24

Material and methods:
Test species: Rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri)*
Number of organisms, weight, length: 20 fish per treatment, 2.1 g (2.0 – 2.2 g), 6.0 cm (5.6 – 6.5 cm)
Type of test and duration: Flow through, 28 days
Applied concentrations: 0 (control), 1, 10, 21.5, 46.4 and 100 mg/L (nominal)
Test conditions:
Water quality: Drinking water, hardness: 2.4 mmol/L
Temperature: 14 – 15 °C
pH: 7.8 – 8.1
Oxygen content: 9.9 – 10.8 mg O_2/L
Light regime: 16 hours light / 8 hours darkness
Test parameters: Mortality and sublethal effects were assessed daily, the body weight and the length were measured at the beginning and the end of the study; for chemical analysis of the test substance samples were taken weekly from all test concentrations
Statistics: NOEC for body weight and length by ANOVA and Dunnett test
Findings:
Chemical analysis: Generally the mean measured concentrations were in the range of 97.8 – 117.2 % of nominal for the 10, 21.5, 46.4 and 100 mg/L treatment groups, however the recovery for the lowest concentration group (1.0 mg/L) was poor with a mean value of 61 % of nominal. However this was not relevant for the determination of the endpoints.
Mortality: One fish died in the control after 2 days, in all treatment groups after 28 days no mortalities were noted
Behavioural effects: After 96 hours no effects up to 21.5 mg/L, at 46.4 mg/L and 100 mg/L effects like apathy, aggressiveness and reduced feed consumption were observed
Body weight and length: significant effects at 46.4 mg/L and 100 mg/L

Conclusion:
NOEC (sublethal effects, body weight and length): 21.5 mg/L, LOEC: 46.4 mg/L based on nominal concentrations

5.4.2 Aquatic invertebrates information provided by the dossier submitter

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference: Dohmen G.P. (2000): Effects of BAS 517 H on the immobility of *Daphnia magna* STRAUS in a 48 hour static, acute toxicity test. Report no: 2000/1011452
Guideline: OECD 202
GLP: Yes
Test item: BAS 517 H, purity 98.7 %, batch no: 01311-180

Material and methods:
Test species: Waterflea (*Daphnia magna*)
Number of organisms, age: 4 replicates each with 5 daphnids per treatment, < 24 hours
Type of test and duration: Static test, 48 hours
Applied concentrations: 0 (control and solvent control), 10, 18, 32, 56 and 100 mg/L (nominal); 6.8, 12.4, 21.1, 38.8 and 70.8 mg/L (mean measured)
Test conditions:
Water quality: "M4" Elendt medium, hardness: 2.53 mmol//L
pH: 8.11 – 8.32 (0 h), 8.13 – 8.21 (48 h)
Temperature: 22.8 – 25.0°C (0 h), 20.5 – 20.6 °C (48 h)
Oxygen content: 8.1 – 8.8 mg O_2/L
Light regime: 16 hours light / 8 hours darkness
Test parameters: Immobility was assessed after 24 and 48 hours; for chemical analysis of the test substance (HPLC) samples were taken at test initiation (0 h) and termination (48 h)
Statistics: none

Findings:
Chemical analysis: Mean measured values were 66 – 71 % of nominal after 48 hours
Immobility: After 48 hours no effects in the control and all treatment groups

Conclusion: EC_{50} (48 h): > 70.8 mg/L, NOEC: ≥ 70.8 mg/L based on mean measured concentrations

Comment: At the beginning the temperature in all test units was higher than recommend in OECD guideline. However, this had no adverse effects on the results of the test. Study considered acceptable.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:. Jatzek H.-J. (1989): Determination of the longterm effects of Cycloxydim techn. BAS 517 H on the parthenogenetic reproduction rate of the waterflea *Daphnia magna* STRAUS. Report no: 1989/0559

Guideline: OECD 202
GLP: Yes
Test item: Cycloxydim (BAS 517 H), batch no: N 175, purity: > 90%.

Material and methods:
Test species: Waterflea (*Daphnia magna*)
Number of organisms: 10 replicates each with 1 adult daphnid per treatment
Type of test and duration: Semi static (renewal every 2-3 days), 21 d
Applied concentrations: 0 (control), 3.906, 7.81, 15.6, 31.3, 62.5, 125, 250 and 500 mg/L (nominal)
Test conditions:
Water quality: Artificial fresh water, hardness: 2.7 ± 0.5 mmol//L
Temperature: 18.9 – 21.9 °C
pH: 8.09 – 8.23 (0 d), 8.07 – 8.19 (21 d)

Oxygen content: 7.07 – 8.3 mg O_2/L
Light regime: 16 hours light / 8 hours darkness
Test parameters: Mortality of adults was assessed on days 0, 2, 5, 7, 9, 12, 14, 16, 19 and 21, reproduction (number of offspring) was assessed on days 7, 9, 12, 14, 16, 19 and 21, for chemical analysis of the test substance *s*amples were taken at treatment levels of 3.906, 62.5 and 500 mg/L at day 0, 9 and 19 (new solutions) and day 2, 12, 21 (old solutions)
Statistics: NOEC: Duncan test

Findings:
Chemical analysis: The measured values ranged from 86.2 – 114.5% of the nominal concentrations

Table 127: Effects of Cycloxydim on *Daphnia magna* reproduction and parent mortality after 21d

Endpoint	**Nominal test concentrations (mg/L)**								
	0	**3.906**	**7.81**	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**
Offspring/parent	124.3	111.3	127.0	107.9	116.2	112.3	21.4*	0*	0*
% mortality adults	0	0	0	0	0	0	0	80	100

*significantly different when compared to the control (Duncan test, α = 0.05)

Conclusion: 21 d NOEC (reproduction): 62.5 mg/L, based on nominal concentrations

5.4.3 Algae and aquatic plants information provided by the dossier submitter

Reference: Kubitza J. (1998): Effect of BAS 517 H on the growth of the green alga *Pseudokirchneriella subcapitata.* Report no: 1998/11218
Guideline: OECD 201
GLP: Yes
Test item: BAS 517 H, purity 98.7 %, batch no: 01311-180

Material and methods:
Test species: Green alga (*Pseudokirchneriella subcapitata*)
Number of organisms: 3 x 10^3 cells/mL, 5 replicates at each concentration, 10 replicates for the solvent control (acetone) and 5 replicates for the standard control
Type of test and duration: Static test, 96 hours
Applied concentrations: 0 (control, solvent control), 3.13, 6.25, 12.5, 25, 50 and 100 mg/L (nominal); 0 (control), 2.4, 5.0, 10.4, 21.6, 42.1 and 84.9 mg/L (mean measured)
Test conditions:
Water quality: Standard algal medium according OECD 201
Temperature: 22 ± 1°C
pH: 8 (start of the test), 7.73 – 8.16 (after 96 hours)
Light regime: Continuous illumination, universal white-type fluorescent lamps (~ 8000 lux)
Test parameters: Cell concentration was measured by spectrophotometer after 48, 72 and 96 hours; for chemical analysis of the test substance (HPLC, UV detection) *s*amples were taken at test initiation (0 h) and termination (96 h)
Statistics: EC_{50}/EC_{10} by probit analysis
Findings:
Chemical analysis: Mean measured values were 78.1 – 86.1 % of nominal after 96 hours
Morphological effects: None
Growth rate and biomass:

Table 128: Effects of cycloxydim (BAS 517 H) on the green alga *P. subcapitata*

BAS 517 H [mg/L] mean measured	**Biomass (AUC) % Inhibition after 96 h**	**Growth rate % Inhibition in 96 h**
2.4	0.9	-0.3
5.0	5.3	0.7
10.4	5.8	0.8
21.6	14.1	2.6
42.1	35.4	6.4
84.9	87.9	32.9
EC_{50} (0-96 h)	44.9 mg/L (31.5 – 47.1 mg/L)	> 84.9 mg/L
EC_{10} (0-96 h)	12.4 mg/L (3.7 – 20.9 mg/L)	38.5 mg/L (31.5 – 47.1 mg/L)

Conclusion:
E_rC_{50} (0-96 h): > 84.9 mg/L, E_rC_{10} (0-96 h): 38.5 mg/L;
E_bC_{50} (0-96 h): 44.9 mg/L, E_bC_{10} (0-96 h): 12.4 mg/L,
based on mean measured concentrations

Reference: Kubitza J. (2003): Effect of BAS 517 H (Cycloxydim) on the growth of the blue-green alga *Anabaena flos-aquae*. Report no: 2003/1004104

Guideline: OECD 201 and ASTM E1218-90
GLP: Yes
Test item: BAS 517 H, purity 92.9 %, batch no: WH16884

Material and methods:
Test species: Blue-Green alga (*Anabaena flos-aquae*)
Number of organisms: 1 x 10^4 cells/mL, 5 replicates at each concentration, 10 replicates for the control
Type of test and duration: Static test, 96 hours
Applied concentrations: 0 (control, solvent control), 10, 18, 32, 56 and 100 mg/L (nominal); 0 (control), 8.8, 16.4, 28.5, 43.6 and 74.9 mg/L (mean measured)
Test conditions:
Water quality: AAP-medium according to ASTM-method
Temperature: 24 ± 1°C
pH: 7.52 – 7.92 (after 96 hours)
Light regime: Continuous illumination, universal white-type fluorescent lamps (~ 2300 lux)
Test parameters: Cell concentration was measured by spectrophotometer (445 nm, 5 cm glass cuvettes) after 0, 48, 72 and 96 hours; for chemical analysis of the test substance (HPLC, UV detection) samples were taken at test initiation (0 h) and termination (96 h)
Statistics: EC_{50}/EC_{10} by Probit analysis

Findings:
Chemical analysis: Mean measured values were 76.2 – 96.8 % of nominal after 96 hours
Morphological effects: None
Growth rate and biomass:

Table 129: Effects of cycloxydim (BAS 517 H) on blue-green alga *A. flos-aquae*

BAS 517 H [mg/L] mean measured	**Biomass (AUC) % Inhibition after 96 h**	**Growth rate % Inhibition in 96 h**
8.8	3.0	0.4
16.4	28.7	10.0
28.5	53.0	15.7
43.6	61.4	21.4
74.9	57.9	23.4
EC_{50} (0-96 h)	38.2 mg/L (36.3 – 40.1 mg/L)	> 74.9 mg/L
EC_{10} (0-96 h)	7.8 mg/L (7.1 – 8.7 mg/L)	21.9 mg/L (19.8 – 24.1 mg/L)

Conclusion:
E_rC_{50} (0-96 h): > 74.9 mg/L, E_rC_{10} (0-96 h): 21.9 mg/L;
E_bC_{50} (0-96 h): 38.2 mg/L, E_bC_{10} (0-96 h): 7.8 mg/L,
based on mean measured concentrations

Reference: Dohmen G.P. (1999): Effects of BAS 517 H on the aquatic plant *Lemna gibba*. Report no. 1999/10180

Guideline: ASTM E 1415-91, EPA 850.4400
GLP: Yes
Test item: BAS 517 H, purity 98.7 %, batch no: 01311-180

Material and methods:
Test species: Higher aquatic plant (*Lemna gibba,* G3)
Number of plants: 3 plants with 12 fronds (1 plant with 3 fronds, 1 plant with 4 fronds and 1 plant with 5 fronds) at each beaker 3 replicates at each concentration, 6 replicates for the control
Type of test and duration: Semi static test, 7 days
Applied concentrations: 0 (control, solvent control), 1, 2.15, 4.64, 10, 21.5, 46.4 and 100 mg/L (nominal); 0, 0.825, 1.76, 3.93, 8.78, 18.6, 45.95 and 91.45 mg/L (mean measured)
Test conditions:
Water quality: 20X-AAP-medium according to ASTM-method
Temperature: 25.3 – 25.4°C
pH: 7.47 – 7.57 (fresh solutions) and 8.55 – 9.06 (aged solutions)
Light regime: Continuous illumination, fluorescent lamps (~ 5.8 klux)
Test parameters: Frond production (frond number and growth rate) and appearance on days 3. 5 and 7; for chemical analysis of the test substance (HPLC, UV detection) samples were taken from fresh (days 0 and 3) and aged solutions (days 3 and 5)
Statistics: NOEC by Bonferroni/Williams test, EC50 by probit analysis

Findings:
Chemical analysis: Mean measured values were 81.6 – 99 % of nominal
Morphological effects: Discoloration was noted in the two highest test concentrations.
Growth rate and biomass:

Table 130: Effects of cycloxydim (BAS 517 H) on the growth of *Lemna gibba*

BAS 517 H [mg/L] nominal	**Frond number (biomass) % Inhibition**	**Growth rate % Inhibition**
1	1.72	0.58
2.15	0.52	0.20
4.64	5.69	1.98
10	11.03	3.93
21.5	14.31	5.24
46.4	36.03	14.65
100	59.83	29.29
EC_{50}	81.7 mg/L (61.12 – 109.3 mg/L)	> 100 mg/L
EC_{10}	9.5 mg/L (7.2 – 12.5 mg/L)	27.4 mg/L (20.2 – 37.1 mg/L)

NOEC (biomass) = 4.54 mg/L
LOEC (biomass) = 10.0 mg/L

Conclusion:
E_rC_{50} (7 d): > 100 mg/L, E_rC_{10} (7 d): 27.4 mg/L;
E_bC_{50} (7 d): 81.7 mg/L, E_bC_{10} (7 d): 9.5 mg/L, NOEC (biomass): 4.64 mg/L based on nominal concentrations

5.4.4 Information on other aquatic organisms (including sediment) provided by the dossier submitter

No studies/information available.

Summary and discussion: Acute (short-term) aquatic toxicity

Data element: Acute (short-term) aquatic toxicity Generally expressed in terms of LC_{50} or EC_{50} (mg/L)				
	$L(E)C_{50}$ [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC_{50}):				
Oncorhynchus mykiss	220	EPA 72-1	y	y
Lepomis macrochirus	> 100	EPA 72-1	y	y
Crustacea (48 hr EC_{50}):				
Daphnia magna	> 70.8	OECD 202(1984)	y	y
Algae/aquatic plants (72 or 96 hr E_rC_{50}):				
Pseudokirchneriella subcapitata	>84.9	OECD 201	y	y
Anabaena flos-aquae	>74.9	ASTM E 1415-91 EPA 850.4400	y	y
Lemna gibba	> 100	ASTM E 1415-91 EPA 850.4400	y	y
Conclusion: **Cycloxydim is of low acute toxicity to aquatic organisms because all relevant endpoints (EC/LC50) are in the range from > 70.8 to > 100 mg/L.**				

Summary and discussion: Chronic (long-term) aquatic toxicity

Data element: Chronic (long-term) aquatic toxicity Generally expressed in terms of NOEC (mg/L)				
	NOEC [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (21 d NOEC):				
Oncorhynchus mykiss	21.5	OECD 204 (1984)	y	y
Crustacea (21 d NOEC):				
Daphnia magna	62.5	OECD 202 (1984)	y	y
Algae/aquatic plants (/ErC_{10}):				
Pseudokirchneriella subcapitata	38.5	OECD 201	y	y
Anabaena flos-aquae	21.9	ASTM E 1415-91 EPA 850.4400	y	y
Lemna gibba	27.4	ASTM E 1415-91 EPA 850.4400	y	y
Conclusion: **Cycloxydim is of low chronic toxicity to aquatic organism because all relevant chronic endpoints (NOEC/EC_{10}) are > 10 mg/L.**				

5.4.5 The RAC assessment of aquatic toxicity and comparison with criteria

Toxicity.

Cycloxydim shows a low toxicity, both acute and chronic, for the three trophic levels (fish, Daphnia and algae). According to these tests the most sensitive species in the acute studies is *Daphnia Magna* with an EC_{50} >70.8 mg/L.

In chronic studies on invertebrates, algae and aquatic plants, the endpoints (NOEC/EC10s) are >10 mg/L. There is not a suitable chronic test for fish because the available prolonged toxicity study following the OECD 204 guideline cannot be considered a chronic test[1]. However, LC50

[1] Technical Guidance Document on Risk Assessment. Part II. Pag. 186.

for fish is >100 mg/L and therefore, applying the surrogate approach, fish data warrant no classification for long term hazards.

Aquatic toxicity of degradation products

Acute (short-term) aquatic toxicity studies for metabolites E/Z BH 517-TSO, E/Z BH 517-T1SO, E/Z BH 517-T2S and E/Z BH 517-TGSO are available and are described in the following table:

Test organism	**test condition**	**time**	**endpoint**	**test conc.**	**EC_{50}/LC_{50} [mg/L]**	**Reference**
metabolite BH 517-TSO						
Oncorhynchus mykiss Rainbow trout	static	96 hr	mortality	n	> 100	Munk 1997
Daphnia magna Waterflea	static	48 hr	immobility	n	> 100	Dohmen 1997a
Pseudokirchn. subcapitata Green alga	static	72 hr	biomass growth rate	n	> 100 > 100	Dohmen 1997b
metabolite BH 517-T1SO						
Daphnia magna Waterflea	static	48 hr	immobility	n	> 100	Dohmen 1998b
Pseudokirchn. subcapitata Green alga	static	72 hr	biomass growth rate	n	> 100	Dohmen 1998a
metabolite BH 517-TGSO						
Daphnia magna Waterflea	static	48 hr	immobility	m	16.6	Funk 2005a
Pseudokirchn. subcapitata Green alga	static	72 hr	biomass growth rate	n	67.0 > 100	Hoffmann 2005a
metabolite BH 517-T2S						
Oncorhynchus mykiss Rainbow trout	static	96 hr	mortality	m	10.4	Zok 2005
Daphnia magna Waterflea	static	48 hr	immobility	n	44.7	Funk 2005b
Pseudokirchn. subcapitata Green alga	static	72 hr	biomass growth rate	n	11.4 67.3	Hoffmann 2005b

This information is missing for BH 517-T1S and BH 517-T2SO. Thus a reliable classification regarding the hazardous to aquatic environment for all degradation products is not

possible. Nevertheless, according to the information supplied in the monograph prepared in the context of inclusion of cycloxydim in Annex I of the Council Directive 91/414/EEC, it can be considered that the toxicity of metabolites is low and therefore, the classification of the parent compound is not influenced.

At this regard, the conclusion on the peer review of the pesticide risk assessment of the active substance cycloxydim (EFSA Journal 2010; 8(7):1669)) states: *"…the risk to aquatic organisms from the metabolites BH 517-TSO, BH 517-T1SO, BH 517-TGSO and BH 517-T2S was assessed as low, based on the available aquatic toxicity data. The risk to aquatic organisms from the metabolites BH 517-TSO2, BH 517-T2SO and BH 517-T2SO2 was assessed as low, assuming ten times higher toxicity of the respective metabolites, compared to the parent substance. No toxicity data were available for the sediment metabolite BH 517-T1S. The risk was however assessed as low, assuming that it is 100 times more toxic to Chironoms sp. than the tested limit concentration for BH 517-T1SO for Daphnia and algae…"*

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

5.5.1 Dossier submitter's proposal

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

Hazard symbols:	None
Indication of danger:	None
Risk phrases:	None
Safety phrases:	None

Conclusions of environmental classification according to Regulation EC 1272/2008

No information is available to conclude the ready biodegradability of Cycloxydim. Cycloxydim was hydrolytically rather stable at neutral and alkaline conditions (DT50 at pH 7 = 172 and 264 days, DT50 at pH 9 = 206 and 958 days) in two hydrolysis studies.

Cycloxydim was rapidly photolytically degraded but the main product BH 517-T1SO, detected with > 60 % of AR in both solutions (pH 7 and pH 9) is considered stable. The maximal amounts of volatiles, whereas the radioactivity was found only in NaOH-traps, were 0.2 % (pH 7) and 0.7 % (pH 9) after 15 days.

The decision that cycloxydim **does not meet the criteria for rapid degradation** is based on the water/sediment-study with a $DT50_{\text{whole system}}$ geomean = 20.8 d (pH 8.8 – 8.5).

No experimental data on bioconcentration factor is available but Cycloxydim has a low potential of bioaccumulation in aquatic system because of a measured logPow value of 1.36 at pH 7 (25°C).

Cycloxydim is of low acute toxicity to aquatic organisms (fish, daphnids, algae and aquatic higher plants: EC/LC50 > 70.9 to > 100 mg/L) and relevant chronic endpoints (NOEC/EC_{10s}) are > 10 mg/L.
Thus, for cycloxydim **no classification and labelling** is proposed regarding environmental hazards.

5.5.2 The RAC proposal

The RAC supports the Dossier submitter's proposal of no classification under DSD and CLP (2º ATP) regarding environmental hazards, according to the following assessment.

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

Cycloxydim does not meet the criteria for ready degradation. This conclusion is based on the water/sediment-study with a $DT_{50\ \text{whole system}}$ geomean =20.8 d (pH 8.8 – 8.5).

No experimental data on the bioconcentration factor is available. However since the measured log Kow value is 1.36 (at pH 7 and 25°C) cycloxydim has a low potential of bioaccumulation in aquatic system.

Cycloxydim shows low acute and chronic toxicity to aquatic organisms (fish, Daphnia, algae and aquatic higher plants) because all relevant acute endpoints (EC/LC_{50}) are >70.8 mg/L and the available chronic endpoints for invertebrates, algae and aquatic plants (NOEC/EC_{10}) are >10 mg/L.

Thus, no classification is proposed regarding environmental hazards.

Conclusions of environmental classification according to Regulation EC 1272/2008

Cycloxydim does not meet the criteria for rapid degradation. This conclusion is based on the water/sediment-study with a $DT_{50\ whole\ system\ geomean}$ = 20.8 d (pH 8.8 – 8.5).

No experimental data on bioconcentration factor is available but cycloxydim has a low potential of bioaccumulation in aquatic system because of a measured logPow value of 1.36 at pH 7 (25°C).

Acute Aquatic Hazard

The relevant acute endpoints (EC/LC_{50}) of cycloxydim are higher than 1 mg/L (>70.8 mg/L), therefore no classification for acute aquatic hazard is proposed.

Chronic Aquatic Hazard

Although cycloxydim is considered a not rapidly degradable substance, the available chronic endpoints (NOEC/ EC_{10}) are higher than 10 mg/L.

A suitable chronic test for fish has not been submitted but the LC50 (fish) is >100 mg/L and therefore, applying the surrogate approach, fish data warrant no classification for long term hazard.

According to these data no classification for long term aquatic hazards is proposed.

Note: Aquatic toxicity studies for metabolites BH 517-TSO, BH 517-T1SO, BH 517-T2S and BH 517-TGSO are available but are missing for BH 517-T1S and BH 517-T2SO. Thus a reliable classification regarding the hazard to aquatic environment for all degradation products is not possible.

Summary table: RAC comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Endpoint	**Classification Criteria**		**Evidence for Cycloxydim**
	CLP (2nd ATP)	**DSD**	
Degradation	The substance cannot be degraded (biotically and/or abiotically) in aquatic environment to a level of >70% within 28 days (Corresponding to a degradation half-life, t½ < ln 2/0.043 = 16 days [Guidance on C&L, ECHA 2010]).	The substance cannot be degraded (biotically and/or abiotically) in aquatic environment to a level of >70% within 28 days.	Water/Sediment simulation study (cycloxydim): $DT_{50\ whole\ system,\ geomean}$ = 20.8 d (pH 8.8 – 8.5) Aquatic toxicity studies for metabolites E/Z BH 517-TSO, E/Z BH 517-T1SO, E/Z BH 517-T2S and E/Z BH 517-TGSO are available but are missing for E/Z BH 517-T1S and E/Z BH 517-T2SO. Thus a reliable classification regarding the hazardous to aquatic environment for all degradation products

			is not possible. ***Conclusion:*** **Not readily and not rapidly degradable.**
Bioaccumulation	No experimental data on bioconcentration factor is available; Log K_{ow} is < 4.	No experimental data on bioconcentration factor is available; Log K_{ow} is < 3.	Log K_{ow}=1.36 at pH 7, indicating low potential for bioaccumulation. ***Conclusion*****: not bioaccumulative.**
Acute aquatic toxicity	Aquatic Acute 1: L(E)C_{50} ≤ 1 mg/L. Fish, 96h LC_{50} ≤ 1 mg/L *Daphnia*, 48h EC_{50}≤ 1 mg/L Algae, 72h E_rC_{50} ≤ 1 mg/L	**R50**: L(E)C_{50} ≤ 1 mg/L **R51/R53:** 1 mg/l < LC_{50}< 10 mg/l + Not readily degradable and/or BCF >100 (or if absent, LogP_{ow} ≥ 3). **R52/R53*:** 10 mg/l < LC_{50}< 100 mg/l + Not readily degradable and/or BCF >100 (or if absent, LogP_{ow} ≥ 3). *no classify if: - convincing scientific evidence is available to demostrate that the substance can be rapidly degraded or - NOEC (fish or Daphnia) >1mg/L	Cycloxydim is of low acute toxicity to aquatic organisms because all relevant endpoints (L(E)C_{50}) are in the range from **> 70.8 to > 100 mg/L**.
Chronic aquatic toxicity	*<u>For non- rapidly degradable substances with chronic data.</u>* Aquatic Chronic 1: NOECx ≤ 0.1 mg/L Aquatic Chronic 2:		Not rapidly degradable; Log K_{ow} = 1.36 at pH 7; Cycloxydim is of low chronic toxicity to aquatic organism because the available chronic endpoints

	NOECx: > 0.1 to ≤ 1mg/L		(NOEC/ EC_{10}s) are **> 10 mg/L,** and although a suitable chronic test for fish has not been submitted, the LC50 (fish) is > 100 mg/L and therefore, applying the surrogate approach, fish data also warrant low toxicity.

6 OTHER INFORMATION

7 REFERENCES

7.1 Physico-chemical properties

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Anonymous	1997	MT 181 solubility in organic solvents CIPAC, Collaborative Internat. Pestic. Anal. Council Ltd.; Harpenden AL5 2HG, Hertfordshire; United Kingdom 1998/1002594 No published	N	CIPAC
Bitterlich S.	2007	Evaluation of physical and chemical properties according to Directive 94/37/EC (67/548/EC Annex V) BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2007/1013310 Yes unpublished	Y	BASF
Class T.	2008	Cycloxydim (BAS 517 H): Determination of solubility in water at pH 4, pH 7 and pH 9 PTRL Europe GmbH; Ulm; Germany Fed.Rep. 2008/1021518 Yes unpublished	Y	BASF
Daum A.	2006	Report Amendment to Determination of the appearance, the melting point and thermal conversions of Reg.No. 172 999 (PAI) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2006/1019582 No unpublished	Y	BASF
Daum A.	1998	Determination of the solubility of BAS 517 .. H (Reg.No. 172 999) pure active ingredient (PAI) in organic solvents at 20°C BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1998/10949 Yes unpublished	Y	BASF

Daum A.	1997	Determination of the octanol/water-partition coefficient of Reg.No. 239 930 by HPLC BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1997/11453 Yes unpublished	Y	BASF
Daum A.	2000	Determination of the partition coefficient (n-octanol/water) of Reg.No. 211 725 (BH 517-TSO) at 20°C by flask shaking method BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1013186 Yes unpublished	Y	BASF
Gerlach H.	2003	Safety data sheet - Cycloxydim Techn.Ber. 100% in Solvesso BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2003/1004151 No, not subject to GLP regulations unpublished	Y	BASF
Goetz N. von	2000	Aqueous photolysis of Cycloxydim (BAS 517 H) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1000143 Yes unpublished	Y	BASF
Hassink J.	2009	Aqueous hydrolysis of BAS 517 H BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1090871 Yes unpublished	Y	BASF
Kaestel R.	1997a	Physical properties report for 172 999 BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1997/10238 Yes unpublished	Y	BASF
Kaestel R.	1997b	Physical properties report for Cycloxydim BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1997/10385 Yes unpublished	Y	BASF
Kaestel R.	1997c	Physical and chemical properties report for BAS 517 23 H BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1997/10654 Yes	Y	BASF

		unpublished		
Kroehl T.	2007	UV/VIS spectrum in acidic medium of Cycloxydim PAI (Reg.No. 172 999, BAS 517 H) BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2007/1017028 Yes unpublished	Y	BASF
Kroehl T.	2008	UV/VIS spectrum in basic medium of Cycloxydim PAI (Reg.No. 172 999, BAS 517 H) BASF SE; Limburgerhof; Germany Fed.Rep. 2008/1009861 Yes unpublished	Y	BASF
Keller E.	1985	Hydrolysis of 14C-BAS 517 H at pH 3, 5, 7 and 9 BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1985/0446 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished	N	BASF
Loeffler U.	1997a	Safety characteristics of the active ingredient Cycloxydim BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1997/10446 Yes unpublished	Y	BASF
Loeffler U.	1997b	Safety characteristics of the crop protection product BAS 517 23 H BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 1997/10655 Yes unpublished	Y	BASF
Loeffler U.	2000	Safety characteristics according to directive 92/69/EEC, annex A9-A17 BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2000/1013215 Yes unpublished	Y	BASF
Löhr	2010	Fire propagation rate (UN) 10/2386 2010/1155866 No unpublished	Y	BASF
Ohnsorge U.	2000	Henry's law constant for Cycloxydim	Y	BASF

		BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1013164 No, not subject to GLP regulations unpublished		
Pawliczek J.B.	1988	Determination of the solubility of Cycloxydim in water at various pH values BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1988/11669 Yes, study was conducted prior to the implementation of GLP certificates unpublished	N	BASF
Petersen-Thiery M.	2006	Statement concerning proceeding of Cycloxydim TK BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. 2006/1019535 No unpublished	Y	BASF
Redeker J.	1988a	Partition coefficient of Cycloxydim in the system n-octanol/water BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1988/10545 Yes, study was conducted prior to the implementation of GLP certificates unpublished	N	BASF
Redeker J.	1988b	Determination of the dissociation constant of Cycloxydim in water BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1988/10557 Yes, study was conducted prior to the implementation of GLP certificates unpublished	N	BASF
Sarafin R.	1991a	Photochemical oxidative degradation of Cycloxydim (Atkinson) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1991/10319 No, not subject to GLP regulations unpublished	N	BASF
Tuerk W.	1996a	Determination of the solubility of Reg.No. 172 999 in water at 20°C by flask method and by HPLC BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1996/10935 Yes unpublished	Y	BASF
Tuerk W.	1996b	Spectra of Reg.No. 172 999 (PAI)	Y	BASF

		BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1996/10960 Yes unpublished		
Tuerk W.	1996c	Determination of the appearance, the melting point and thermal conversions of Reg.No. 172 999 (PAI) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1996/10961 Yes unpublished	Y	BASF

7.2 Human health hazard assessment

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Beimborn D.B., Leibold E.	2003	14C-BAS 517 H – Study of the dermal absorption in rats BASF RegDoc# 2003/1004043 GLP unpublished	Y	BASF
Boer W. C. den, Hoorn A. J. W.	1985a	**4** Mutagenicity evaluation of BAS 517 .. H (Na-salt) in the CHO HGPRT forward mutation assay Litton Bionetics, PE Veenendaal, The Netherlands BASF RegDoc# 1985/404 GLP unpublished	N	BASF
Boer W. C. den	1985b	Mutagenicity evaluation of BAS 517 .. H (Na-salt) in the mouse lymphoma forward mutation assay Litton Bionetics, PE Veenendaal, The Netherlands BASF RegDoc# 1985/347 GLP unpublished	N	BASF
Cifone M. A., Brusick D. J.	1985	Evaluation of BAS 517 H, 84/312, sodium salt in the *in vitro* rat primary hepatocyte unscheduled DNA synthesis assay Litton Bionetics, Inc., Kensington, United States of America BASF RegDoc# 1985/357 GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Cifone M. A., Myhr C. B.	1985	Evaluation of ZNT-Nr.84/312, Reg. Nr. 172 999 in the *in vitro* rat primary hepatocyte unscheduled DNA synthesis assay Litton Bionetics, Inc., Kensington, United States of America BASF RegDoc# 1985/358 GLP unpublished	N	BASF
Engelhardt G., Gelbke H.-P.	1983	Report on the study of Reg. No. 172 999 (ZNT test substance No.: 83/292) in the AMES test (Standard plate test with Salmonella typhimurium) BASF RegDoc# 1984/075 no GLP unpublished	N	BASF
Engelhardt G., Gelbke H.-P.	1985a	Cytogenetic investigations in NMRI mice after a single oral administration of Reg. No. 172 999, Na salt BASF RegDoc# 1985/378 GLP unpublished	N	BASF
Engelhardt G., Gelbke H.-P.	1985b	Report on the study of Reg. No. 172 999 Na salt (ZNT test substance No.: 84/312) in the AMES test (standard plate test with Salmonella typhimurium) BASF RegDoc# 1985/385 GLP unpublished	N	BASF
Gamer, O., Fabian, E., Landsiedel, R.	2007	14C-BAS 517 H in BAS 517 24 H – Study of penetration through rat and human skin in vitro BASF RegDoc# 2007/1027030 GLP unpublished	Y	BASF
Hawkins D.R. ; Kirkpatrick D. ; Finn C.M.; Till C.P.; Dean G.M.; Biggs S.R.; Whitby B.R.	1986	The biokinetics and metabolism of ^{14}C-Cycloxydim in the rat Huntingdon Research Centre, Huntingdon, United Kingdom RegDoc# HRC/BSF 412/413/86543 non-GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Hellwig J., Deckardt K., Freisberg K.O., Hildebrand B.	1985a	Report on the study of the toxicity of Reg. No. 172 999, Na salt in Beagles after 4-week administration in the diet (Range-finding study) BASF RegDoc# 1985/336 GLP unpublished	N	BASF
Hellwig J., Deckardt K., Freisberg K.O., Hildebrand B.	1985b	Report on the study of the toxicity of Reg. No. 172 999, Na-salt in Beagles after 3-month administration in the diet (Range-finding study) BASF RegDoc# 1986/019 GLP unpublished	N	BASF
Hellwig J., Hildebrand B.	1987a	Report on the study of the prenatal toxicity of Reg. No. 172 999 NA salt in rats after oral administration (gavage) BASF RegDoc# 1987/0176 GLP unpublished	N	BASF
Hellwig J., Hildebrand B.	1987b	Report on the study of the pre-, peri-, postnatal toxicity of Reg. No. 172 999 Na-salt in rats after oral administration (gavage) BASF RegDoc# 1987/0177 GLP unpublished	N	BASF
Hellwig J., Hildebrand B.	1987c	Report on the study of the prenatal toxicity of Reg. No. 172 999 Na salt in rats after oral administration (gavage) with special attention to maternal toxicity BASF RegDoc# 1987/0178 GLP unpublished	N	BASF
Hellwig J., Hildebrand B.	1988	Study of the toxicity of Reg. No. 172 999 - Na salt in purebred Beagle dogs. Administration over 12 months via the diet BASF RegDoc# 1988/0063 GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Hellwig J., Freisber K.O., Hildebrand B.	1988	Reproduction study with Reg. No. 172 999 Na-salt in rats. Continuous administration with the drinking water over 2 generations (2 litters in the first and 1 litter in the second generation) BASF RegDoc# 1988/0057 GLP unpublished	N	BASF
Jaeckh R., Gelbke H.-P.	1986	Report on a point mutation test carried out on CHO cells (HGPRT locus) with the test substance BAS 517 . H-Na-salt (Reg. No. 172 999) BASF RegDoc# 1986/036 no GLP unpublished	N	BASF
Kieczka H., Kirsch P.	1984a	Reg. No. 172 999: Report on the study of acute oral toxicity in the rat BASF RegDoc# 1984/216 non-GLP unpublished	N	BASF
Kieczka H., Kirsch P.	1984b	Report on the acute dermal toxicity of Reg. No. 172 999 in the rat based on OECD and EPA (FIFRA) BASF RegDoc# 1984/230 GLP unpublished	N	BASF
Kieczka H., Kirsch P.	1984c	Report on the acute dermal irritation / corrosivity of Reg. No. 172 999 to the intact dorsal skin of the white rabbit based on OECD and EPA (FIFRA) BASF RegDoc# 1984/231 GLP unpublished	N	BASF
Kieczka H., Kirsch P.	1984d	Report on the acute irritation of Reg. No. 172 999 to the eye of the white rabbit based on OECD and EPA (FIFRA) BASF RegDoc# 1984/232 GLP unpublished	N	BASF
Kieczka H., Kirsch P.	1985a	Report on the sensitizing effect of Reg. No. 172 999 in the guinea pig - Maximization test BASF RegDoc# 1985/317 GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Kieczka H., Kirsch P.	1985b	Reg. No. 172 999: Report on the study of acute oral toxicity on the mouse based on OECD BASF RegDoc# 1985/325 GLP unpublished	N	BASF
Klimisch H.-J., Gelbke H.-P. and Freisberg K.O.	1985	Acute inhalation toxicity LC_{50} 4 hours (rat) - Liquid aerosol study of Reg. No. 172 999 tested as Sodium-salt BASF RegDoc# 1985/364 GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Kuehborth B., Deckardt K., Freisberg K.O., Hildebrand B.	1985	Report on the study of the toxicity of Reg. No. 172 999 Na salt in rats after 3-month administration via the drinking water and a 6-week observation period BASF RegDoc# 1985/397 GLP unpublished	N	BASF
Kuehborth B., Deckardt K., Freisberg K.O., Hildebrand B.	1986a	Report on the study of the toxicity of Reg. Nr. 172 999 Na-salt project No. 51S0151/8407 in mice after 4-weeks administration via the drinking water (1. Range-finding study) BASF RegDoc# 1986/059 GLP unpublished	N	BASF
Kuehborth B., Deckardt K., Freisberg K.O., Hildebrand B.	1986b	Report on the study of the toxicity of Reg. No. 172 999 Na-salt project No. 51S0151/8426 in mice after 4-weeks administration via the drinking water (2. range-finding study) BASF RegDoc# 1986/060 GLP unpublished	N	BASF
Kuehborth B., Deckardt K., Freisberg K.O., Hildebrand B.	1986c	Report on the study of the toxicity of Reg. No. 172 999 Na-salt in rats after 28-days administration via the drinking water (range finding study) BASFRegDoc# 1986/089 GLP unpublished	N	BASF
Kuehborth B., Deckardt K., Freisber K.O., Schilling K., Hildebrand B.	1988a	Study on the toxicity of Reg. No. 172 999 - Na salt in rats. Administration via the drinking water over 24 months BASF RegDoc# 1988/0125 GLP unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Kuehborth B., Deckardt K., Freisber K.O., Hildebrand B.	1988b	Study on the toxicity of Reg. No. 172 999 - Na salt in mice. Administration in the drinking water over 24 months BASF RegDoc# 1988/0127 GLP unpublished	N	BASF
Kuehborth B., Deckardt K., Freisber K.O., Schilling K., Hildebrand B.	1988c	Study on the toxicity of Reg. No. 172 999 - Na salt in rats. Administration in the drinking water over 18 months BASF RegDoc# 1988/0068 GLP unpublished	N	BASF
Mellert W., Deckardt K., Gembardt C., Ravenzwaay B.	2001a	Reg. No. 172 999 (Cycloxydim) - Repeated dose dermal toxicity study in Wistar rats. Administration for 4 weeks BASF RegDoc# 2001/1010709 GLP unpublished	Y	BASF
Merkle J., Hildebrand B.	1985	Study to determine the prenatal toxicity of Reg. No. 172 999 - Na salt in rabbits after oral administration (stomach tube) BASF RegDoc# 1985/384 GLP unpublished	N	BASF
Makris SL, Solomon HM, Clark R, et al.	2009	Terminology of developemtal abnormalities in commom laboratory mammals (version 2) Reproductive Toxicology 28:371-434	N	
Neubert D.	1987	Effect of Cycloxydim on embryonic development in vitro FU Berlin BASF RegDoc# 1987/0464 GLP unpublished	N	BASF

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Solecki R, Bürgin H, Buschmann J, et al.	2001	Harmonisation of rat fetal skeletal terminology and classification. Report of the third workshop on the terminology in developmental toxicology. Berlin 14-16 September 2000. Reproductive Toxicology 15:713-721	N	
Taalman R. D.	1985a	Mutagenicity evaluation of BAS 517 .. H (Na-salt) in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in Chinese hamster ovary (CHO) cells Litton Bionetics, PE Veenendaal, The Netherlands BASF RegDoc# 1985/219 GLP unpublished	N	BASF
Taalman R. D.	1985b	Mutagenicity evaluation of BAS 517 .. H (acid) in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies Chinese hamster ovary cells Litton Bionetics, PE Veenendaal, The Netherlands BASF RegDoc# 1985/220 GLP unpublished	N	BASF
Taalman R. D.	1987	Clastogenic evaluation of BAS 517 .. H (Na-salt) in the Chinese hamster bone marrow cytogenetic assay Hazleton Biotechnologies Venendaal, The Netherlands BASF RegDoc# 1987/069 GLP unpublished	N	BASF

7.3 Environmental hazard assessment

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Bayer, H.	2009	Field soil dissipation study of BAS 517 H (Cycloxydim) in the formulation BAS 517 24 H on bare soil at six different locations in Europe, 2007 BASF SE; Limburgerhof, Germany Fed. Rep. 2008/1091225 GLP unpublished	Y	BASF
Bayer, H., Richter, T.	2009	Field soil dissipation study of BH 517-TSO2 (metabolite of BAS 517 H, Cycloxydim) in the formulation EXP 182565 H on bare soil at six different locations in Europe, 2007 BASF SE, Limburgerhof, Germany Fed. Rep. 2008/1091226 GLP unpublished	Y	BASF
Dohmen G.P. (Annex point/ reference number: II A 8.2.4)	1997a	Effect of BH 517-TSO on *Daphnia magna* STRAUS in a static acute toxicity test Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 1997/10233 GLP/GEP: Yes unpublished	Y	BASF
Dohmen G.P (Annex point/ reference number: II A 8.2.6)	1997b	Effect of BH 517-TSO on the growth of the green alga *Pseudokirchneriella subcapitata.* Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 1997/10234 GLP/GEP: Yes unpublished	Y	BASF
Dohmen G.P (Annex point/ reference number: II A 8.2.6)	1998a	Effect of BH 625-7 on the growth of the green alga *Pseudokirchneriella subcapitata* Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 1998/10010 GLP/GEP: Yes unpublished	Y	BASF
Dohmen G.P (Annex point/ reference number: II A 8.2.4)	1998b	Effect of BH 625-7 on *Daphnia magna* STRAUS in a 48 hours acute toxicity test. Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep Report No: 1998/10011 GLP/GEP: Yes unpublished	Y	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Dohmen G.P.	1999	Effects of BAS 517 H on the aquatic plant *Lemna gibba* Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 1999/10180 GLP/GEP: Yes unpublished	Y	BASF
Dohmen G.P.	2000	Effects of BAS 517 H on the immobility of *Daphnia magna* STRAUS in a 48 hour static, acute toxicity test Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 2000/1011452 GLP/GEP: Yes unpublished	Y	BASF
Ebert D.	2000	Degradation of BAS 517 H (Cycloxydim) in water/sediment-systems under aerobic conditions BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1000138 Yes unpublished	Y	BASF
Funk, M. (Annex point/ reference number: II A 8.2.4)	2005a	Effects of Reg. No. 232625 (BH 517-TGSO) on the Immobility of *Daphnia magna* STRAUSS in a 48 Hours Static, Acute Toxicity Test. Generated by: BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmen-tal Analytics, 67114 Limburgerhof, Germany Report No: 2004/1027459 GLP/GEP: Yes unpublished	Y	BASF
Funk, M. (Annex point/ reference number: II A 8.2.4)	2005b	Effects of Reg. No. 230854 (BH 517-T2S) on the Immobility of *Daphnia magna* STRAUSS in a 48 Hours Static, Acute Toxicity Test. Generated by: BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, 67114 Limburgerhof, Germany Report No: 2004/1027460 GLP/GEP: Yes unpublished	Y	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Goetz N. von	2000a	Aqueous photolysis of Cycloxydim (BAS 517 H) BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2000/1000143 Yes unpublished	Y	BASF
Hassink, J.	2008	Metabolism of BAS 517 H in soil under aerobic conditions – Verification of unknown polar degradation products BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2008/1005565 GLP unpublished	Y	BASF
Hassink, J.	2009	Aqueous hydrolysis of BAS 517 H BASF SE, Limburgerhof, Germany Fed. Rep. 2008/1090871 GLP Unpublished	Y	BASF
Hoffmann, F. (Annex point/ reference number: II A 8.2.6)	2005a	Effects of Reg. No. 232625 (BH 517-TGSO) on the Growth of the Green Alga *Pseudokirchneriella subcapitata*. Generated by: BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, 67114 Limburgerhof, Germany Report No: 2004/1027272 GLP/GEP: Yes unpublished	Y	BASF
Hoffmann, F. (Annex point/ reference number: II A 8.2.6)	2005b	Effects of Reg. No. 230854 (BH 517-T2S) on the Growth of the Green Alga *Pseudokirchneriella subcapitata*. Generated by: BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, 67114 Limburgerhof, Germany Report No: 2004/1020900 GLP/GEP: Yes unpublished	Y	BASF
Jatzek H.-J.	1989	Determination of the longterm effects of Cycloxydim techn. BAS 517 H on the parthenogenetic reproduction rate of the waterflea *Daphnia magna* STRAUS Generated by: BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. Report No: 1989/0559 GLP/GEP: Yes Unpublished	N	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Jene B.	2006c	Predicted environmental concentrations of BAS 517 H - Cycloxydim and metabolites in surface water and sediment on a European level according to FOCUS BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 2006/1037685 No, not subject to GLP regulations unpublished	Y	BASF
Keller E.	1985b	Hydrolysis of 1^{4}C-BAS 517 H at pH 3, 5, 7 and 9 BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1985/0446 No, studies were conducted prior to the implementation of GLP but are scientifically valid unpublished	N	BASF
Kroehl, T.	2009a	Vapour pressure of Reg. No. 182565 (Metabolite BH 517-TSO2 of Cycloxydim) BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2009/1030263 GLP Unpublished	Y	BASF
Kroehl, T.	2009b	Vapour pressure of Reg. No. 211725 (Metabolite BH 517-TSO of Cycloxydim) BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2009/1030265 GLP Unpublished	Y	BASF
Kubitza J.	1998	Effect of BAS 517 H on the growth of the green alga *Pseudokirchneriella subcapitata* Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 1998/11218 GLP unpublished	Y	BASF
Kubitza J.	2003	Effect of BAS 517 H (Cycloxydim) on the growth of the blue-green alga *Anabaena flos-aquae*. Generated by: BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. Report No: 2003/1004104 GLP/GEP: Yes unpublished	Y	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Leite, R	2009	Investigation of the storage stability of BH 517-TSO (metabolite of Cycloxydim – BAS 517 H) in soil samples under usual storage conditions BASF SA; Guaratingueta; Brazil DocID 2009/1013407 GLP Unpublished	Y	BASF
Munk R.	1997	Reg.No. 211 725 (BH 517-TSO) - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792) in a static system (96 hours) Generated by: BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. Report No: 1997/10583 GLP/GEP: Yes unpublished	Y	BASF
Munk R. & Gelbke H.-P.	1984a	Report of the study of the acute toxicity-BAS 517H/Reg.No. 172 999 - rainbow trout (*Salmo Gairdneri* RICH.) Generated by: BASF AG; Ludwigshafen/Rhein; Germany Fed. Rep. Report No: 1984/0401 GLP/GEP: Yes unpublished	N	BASF
Munk R. & Gelbke H.-P.	1984b	Report on the study of the acute toxicity - BAS 517 H (Reg.No. 172 999) - Bluegill (*Lepomis macrochirus* RAF.) Generated by: BASF AG; Ludwigshafen/Rhein; Germany Fed. Rep. Report No: 1984/0402 GLP/GEP: Yes unpublished	N	BASF
Platz, K	2009a	Estimation of normalized DegT50 values for BH 517-TSO and BH 517-TSO2, metabolites of BAS 517 H – Cycloxydim, from terrestrial field dissipation studies BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2009/1017876 GLP not applicable Unpublished	Y	BASF

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Platz, K.	2009c	Addendum to report 2000/1000141: "Aerobic Degradation of Cycloxydim (BAS 517 H) in 3 Different Soils". Update of the kinetic evaluation following the recommendations of FOCUS kinetics BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2009/1017881 GLP not applicable unpublished	Y	BASF
Platz, K.	2009b	Addendum to report 2005/1010532: "Aerobic Rate of Degradation of [14C]-BH 517-T2SO2 (Metabolite of BAS 517 H, Cycloxydim) in Soil (DT50/DT90)". Estimation of DegT50 values of BH 517-T2SO2 according to the recommendations of FOCUS kinetics BASF SE, Limburgerhof, Germany Fed. Rep. DocID 2009/1017879 GLP not applicable unpublished	Y	BASF
Redeker J.	1988a	Partition coefficient of Cycloxydim in the system n-octanol/water BASF AG, Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 1988/10545 according to GLP*): study was performed before 25/07/1993, no GLP certificate available	N	BASF
Zok, S. (Annex point/ reference number: II A 8.2.1)	2005	Reg. No. 230854 (Metabolite of BAS 517 H, Cycloxydim) – Acute Toxicity Study on Rainbow Trout (*Oncorhynchus mykiss*) in a Static System over 96 hours. Generated by: Experimental Toxicology and Ecology BASF AG, 67056 Ludwigshafen, Germany Report No: 2005/1005744 GLP/GEP: Yes unpublished	Y	BASF

8 ANNEXES