Committee for Risk Assessment

RAC

Annex 1

Background document

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

tembotrione

EC number: N/A

CAS number: 335104-84-2

CLH-O-0000002527-72-03/A2

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 June 2013

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Tembotrione

EC Number:

CAS Number: **335104-84-2**

Index Number: **-**

Contact details for dossier submitter:

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Version number: **3** **Date:** **26.04.2012**

CONTENTS

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Tembotrione***
EC number:	
CAS number:	***335104-84-2***
Annex VI Index number:	**-**
Degree of purity:	***940 g/kg***
Impurities:	***Toluene max. 10 g/kg***

1.2 Harmonised classification and labelling proposal

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 entry	No entry	
Current proposal for consideration by RAC	Skin Sens. 1, H317 STOT RE 2, H373 Aquatic Acute 1; H400 M=10 Aquatic Chronic 1; H410 M=10	Xi; R43 Xn; R48/22 N; R50/R53 **SCLs**	
		Classification	Concentration [in %]
		N, R50/53	≥ 2.5
		N, R51/53	≥ 0.25 - < 2.5
		R52/53	≥ 0.025 - < 0.25
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)			

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

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Conclusive but not sufficient for classification
2.2.	Flammable gases	-	-	-	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	-	-	-	Conclusive but not sufficient for classification
2.4.	Oxidising gases	-	-	-	Conclusive but not sufficient for classification
2.5.	Gases under pressure	-	-	-	Conclusive but not sufficient for classification
2.6.	Flammable liquids	-	-	-	Conclusive but not sufficient for classification
2.7.	Flammable solids	-	-	-	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-	-	-	Data lacking
2.9.	Pyrophoric liquids	-	-	-	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Data lacking
2.11.	Self-heating substances and mixtures	-	-	-	Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	-	-	-	Conclusive but not sufficient for classification
2.14.	Oxidising solids	-	-	-	Conclusive but not sufficient for classification
2.15.	Organic peroxides	-	-	-	Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-	-	-	Data lacking

3.1.	Acute toxicity - oral	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - dermal	-	-	-	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Data lacking
3.4.	Skin sensitisation	H317	-	-	-
3.5.	Germ cell mutagenicity	**-**	**-**	**-**	**Conclusive but not sufficient for classification**
3.6.	Carcinogenicity	**-**	**-**	**-**	**Conclusive but not sufficient for classification**
3.7.	Reproductive toxicity	**-**	**-**	**-**	**Conclusive but not sufficient for classification**
3.8.	Specific target organ toxicity –single exposure	-	-	-	**Conclusive but not sufficient for classification**
3.9.	Specific target organ toxicity – repeated exposure	**H373**	**-**	**-**	**-**
3.10.	Aspiration hazard	**-**	**-**	**-**	**Data lacking**
4.1.	**Hazardous to the aquatic environment**	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	M =10 M = 10	**-**	**-**
5.1.	Hazardous to the ozone layer	- No Data available	-	-	Data lacking

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

Labelling:

Signal word: Warning

Pictogram: GHS09

Hazard statements:

H317	May cause an allergic skin reaction
H373	May cause damage to organs through prolonged or repeated exposure (if swallowed)
H400	Very toxic to aquatic life
H410	Very toxic to aquatic life with long lasting effects

Precautionary statements:

(resulting from hazard statements according to Annex I of Regulation (EC) No. 1272/2008 without any further selection)

P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
P321	Specific treatment (see … on this label).
P363	Wash contaminated clothing before reuse.
P260	Do not breathe dust/fume/gas/mist/vapours/spray.
P314	Get medical advice/attention if you feel unwell.
P273	Avoid release to the environment.
P391	Collect spillage.
P501	Dispose of contents/container to …

Proposed notes assigned to an entry: -

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs		Current classification [1)]	Reason for no classification [2)]
Explosiveness	-	-		-	Conclusive but not sufficient for classification
Oxidising properties	-	-		-	Conclusive but not sufficient for classification
Flammability	-	-		-	Conclusive but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	-	-		-	-
Thermal stability	-	-		-	Conclusive but not sufficient for classification
Acute toxicity	-	-		-	Conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	-	-		-	Conclusive but not sufficient for classification
Repeated dose toxicity	R48/22	-		-	-
Irritation / Corrosion	-	-		-	Conclusive but not sufficient for classification
Sensitisation	R43	-		-	-
Carcinogenicity	-	-		-	**Conclusive but not sufficient for classification**
Mutagenicity – Genetic toxicity	-	-		-	**Conclusive but not sufficient for classification**
Toxicity to reproduction – fertility	-	-		-	**Conclusive but not sufficient for classification**
Toxicity to reproduction – development	-	-		-	**Conclusive but not sufficient for classification**
Toxicity to reproduction – breastfed babies. Effects on or via lactation	-	-		-	Conclusive but not sufficient for classification
	N; R50/53	Classification	Concentration [in %]		
		N, R50/53	≥ 2.5		
		N, R51/53	≥ 0.25 - < 2.5		
		R52/53	≥ 0.025 - < 0.25		

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

Labelling:

Indication of danger:

Xn Harmful

Xi Irritant

N Dangerous for the Environment

R-phrases:

R43	May cause sensitisation by skin contact
R48/22	Harmful: Danger of serious damage to health by prolonged exposure if swallowed
R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

SCLs

Classification	Concentration* [in %]
N, R50/53	≥ 2.5
N, R51/53	≥ 0.25 - < 2.5
R52/53	≥ 0.025 - < 0.25

S-phrases:

S24	Avoid contact with skin.
S36/37	Wear suitable protective clothing and gloves.
S46	If swallowed, seek medical advice immediately and show this container or label
S56	Dispose of this material and its container to hazardous or special waste collection point.
S57	Use appropriate container to avoid environmental contamination.
S60	This material and its container must be disposed of as hazardous waste.
S61	Avoid release to the environment. Refer to special instructions/safety data sheets.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Tembotrione is a new triketone herbicide. The compound was applied for Annex I listing as new active substance under Council Directive 91/414/EEC, with Austria as rapporteur Member State (RMS). The draft assessment report (DAR), which is based on one full data package submitted by one company, was submitted to EFSA (European Food Safety Authority) in February 2007. In April 2009, an addendum was written and the DAR was revised according to the comments received during the commenting period and additional information provided by the notifier. The new active substance tembotrione was discussed in the PRAPeR (pesticide risk assessment peer review) expert meetings of round 14 (April – May 2009). Thereafter, a second revision of the DAR was written taking into account the discussions and results of the PRAPeR. The hazard assessment of the active substance tembotrione was finalised in the PRAPeR meeting, whereas some aspects of the risk assessment (assessment of reference values) remained open. In December 2009 a second addendum was written evaluating further studies submitted by the notifier. This second addendum is not peer reviewed yet.

Tembotrione is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). In accordance with Article 36(2) of the CLP Regulation, tembotrione should now be considered for harmonised classification and labelling. This proposal considers all physico-chemical properties and all mammalian toxicology and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based on the information presented in the evaluation of tembotrione under Directive 91/414/EEC.

2.2 Short summary of the scientific justification for the CLH proposal

Regarding its physico-chemical properties no classification is proposed for tembotrione.

Regarding human health effects, the following classification and labelling is proposed for tembotrione: In a guinea pig skin sensitisation study (Magnusson & Kligman method) tembotrione showed positive results. According to Regulation (EC) No. 1272/2008 tembotrione belongs to Skin sensitisation Category 1 and requires classification with **H317 "May cause an allergic skin reaction"**. Furthermore, based on the findings of mortality in pregnant rabbits at the dose level of 100 mg/kg bw/d in a developmental toxicity study, tembotrione requires classification with **H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)"** (STOT RE Category 2).

Regarding environment (considering 2nd ATP criteria) following classification will be proposed:

DSD: N, R50/53 (DSD)

SCLs

Classification	Concentration [in %]
N, R50/53	≥ 2.5
N, R51/53	≥ 0.25 - < 2.5
R52/53	≥ 0.025 - < 0.25

CLP: Aquatic Acute 1, H400, M=10; Aquatic Chronic 1, H410, M=10

Aquatic Acute classification is based on:

- LC50 value for *Americamysis bahia* is 0.1 mg/L (0.01 < L(E)C50 ≤ 0.1), resulting in N, R50 (DSD) and Aquatic Acute 1, H400, M =10 (CLP)
  Americamysis bahia LC50 (96 h) = 0.1 mg/L

Aquatic chronic classification is based on:

- Tembotrione is is not considered as ready biodegradable/rapid degradable. Therefore R53 (DSD) classification is proposed.
- Based on the non rapid degradability (see above) and on the chronic toxicity of *Lemna gibba* NOEC(7 d) = 0.0024 mg/L (0,001 < NOEC ≤ 0,01) a classification with Aquatic Chronic 1, H410 M=10 (CLP) is proposed.

2.3 Current harmonised classification and labelling

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

The substance is not listed in Annex VI of the CLP Regulation.

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

The substance is not listed in Annex VI of the CLP Regulation.

2.4 Current self-classification and labelling

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

No current self-classification based on the CLP Regulation criteria.

2.4.2 Current self-classification and labelling based on DSD criteria

Proposed classification and labelling by notifier

Hazard symbols:		Black St. Andrew's Cross on orange square Dead fish, dead tree
Indication of danger:	Xi	Irritant
	N	Dangerous for the environment
	R43	May cause sensitisation by skin contact
Risk phrases:	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effect in the aquatic environment.
Safety phrases:	S24	Avoid contact with skin
	S37	Wear suitable gloves
	S60	This material and its container must be disposed as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions /Safety data sheet

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Tembotrione is used as a pesticide. For pesticides there is no need for justification (cf. Article 36(3) CLP Regulation).

general comment
The RAC has evaluated only those hazard classes: a) for which a classification was proposed by the dossier submitter (DS), b) for which comments were received during the public consultation and data was made available or, c) those which were specifically requested by the RAC. Any other hazard classes related to this substance should be considered as **'not evaluated'** and their exclusion should not be taken to mean 'not classified'.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

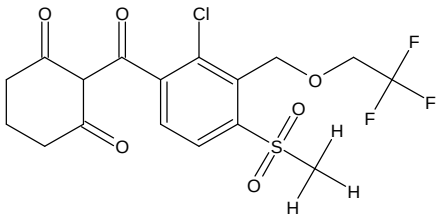
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	
EC name:	
CAS number (EC inventory):	-
CAS number:	335104-84-2
CAS name:	1,3-Cyclohexanedione, 2-[2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl]-
IUPAC name:	2-{2-chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl} cyclohexane-1,3-dione
CLP Annex VI Index number:	-
Molecular formula:	$C_{17}H_{16}ClF_3O_6S$
Molecular weight range:	440.82

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Tembotrione	940 g/kg		

Current Annex VI entry of Tembotrione:

The substance is not listed in Annex VI of the CLP Regulation.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Toluene	1.1 g/kg	0.6 – 1.4 g/kg	Toluene can be present up to 1% (10 g/kg) in tembotrione according to the proposed reference specification

Current Annex VI entry of **toluene (index number 601-021-00-3, EC No: 203-625-9, CAS No 108-88-3)**:

Classification based on Directive 67/548/EEC:

Class of Danger: F: Highly flammable

Xn: Harmful

R-Phrases: R11: Highly flammable

R38: Irritating to skin

R48/22: Danger of serious damage to health by prolonged exposure

Repr. Cat.3, R63: Possible risk of harm to the unborn child

R65: Harmful: may cause lung damage if swallowed

R67: Vapours may cause drowsiness and dizziness

Classification based on CLP Criteria:

Signal Word	Danger
Classification:	Flam. Liq. 2
	Repr. 2
	Asp. Tox. 1
	STOT RE 2 *
	Skin Irrit. 2
	STOT SE 3
H-statements:	H225: Highly flammable liquid and vapour
	H361d: Suspected of damaging the unborn child
	H304: May be fatal if swallowed and enters airways
	H373: May cause damage to organs through prolonged or repeated exposure
	H315: Causes skin irritation
	H336: May cause drowsiness or dizziness

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks

Current Annex VI entry:

1.2.1 Composition of test material

Physico-chemical properties: see table 9 (purity of tested technical material is in the range from 94.7% to 98.9%)

Human health hazard assessment: purity of tested technical material is in the range from 94.0% to 95.4%

Environmental hazard assessment: purity of tested technical material is in the range from 94.0% to 97.4%

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Study	Method	Material	Results	Conclusion/Comment	Reference
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	EEC/A1 OECD 102 DSC and capillary tube in a metal block GLP	pure substance 989 g/kg	The melting point is 123 °C.	Acceptable	M-248060-01-1 Smeykal H. 2005
B.2.1.2 Boiling point (IIA 2.1.2)	EEC/A2 OECD 103 DSC GLP	pure substance 989 g/kg	No boiling point at atmospheric pressure; decomposition started around 150 °C.	Acceptable	M-248060-01-1 Smeykal H. 2005
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	OECD 113 DSC GLP	pure substance 989 g/kg	Exothermic decomposition in the range of 140 - 220 °C. Thermal stability checked with DSC	Acceptable	M-248060-01-1 Smeykal H. 2005
B.2.1.4 Relative density (IIA 2.2)	EEC/A3 OECD 109 pycnometer -air comparison GLP	pure substance 989 g/kg	D_4^{20} = 1.56	Acceptable	M-229996-01-1 Mühlberger B., Lemke G., 2004

Study	Method	Material	Results	Conclusion/Comment	Reference
B.2.1.5 Vapour pressure (IIA 2.3.1)	EEC/A4 OECD 104 vapour pressure balance GLP	pure substance 989 g/kg	$1.1*10^{-10}$ hPa at 20 °C $2.9*10^{-10}$ hPa at 25 °C $2.6*10^{-8}$ hPa at 50 °C	Acceptable The vapour pressure is measured in the recommended range for the effusion method (balance) between 95 °C and 117 °C and then extrapolated. This approach is acceptable according to method EEC/A.4 if the vapour pressure is < 10^{-5} Pa at ambient temperature. Furthermore the measured value must meet the requirement of the methods and not the value which has been extrapolated.	M-078780-01-1 Franke J., 2002
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	calculation	–	$1.71*10^{-10}$ $Pa.m^3.mol^{-1}$ at 20 °C: values used for calculation: vapour pressure at 20 °C : $1.1*10^{-10}$ hPa water solubility at 20 °C : 28.3 g/L	Acceptable	M-108444-01-1 Mühlberger B., 2003a
B.2.1.7 Appearance: physical state (IIA 2.4.1)	OPPTS 830.6302 830.6303	pure substance 989 g/kg	beige powder	Acceptable	M-223199-01-1 Mühlberger B., Strunk B., 2003
	OPPTS 830.6302 830.6303	tech. substance 947 g/kg	beige powder	Acceptable	M-255563-01-1 Bogdoll B., Strunk B., 2005
B.2.1.9 Appearance: odour (IIA 2.4.2)	OPPTS 830.6304	pure substance 989 g/kg	no characteristic odour	Acceptable	M-223199-01-1 Mühlberger B., Strunk B., 2003
	OPPTS 830.6304	tech. substance 947 g/kg	no characteristic odour	Acceptable	M-255563-01-1 Bogdoll B., Strunk B., 2005
B.2.1.10	GLP	pure	UV/VIS		M-220680-01-1

Study	Method	Material	Results			Conclusion/Comment	Reference
Spectra of the active substance (IIA 2.5.1)		substance 989 g/kg	Solution 10.009 mg a.s./L	Wave-length [nm]	ε [L/mol x cm]	Acceptable	Mühlberger B., Wiche A., 2003
			neutral (methanol)	203 232 284 291	31021 14224 13800 12960		
			acidic (methanol/1 N HCl (90/10)	205 231 283 291	31015 15370 14303 12937		
			basic (methanol / 1 N NaOH (90/10)	217 258 291	17015 22080 13415		
			IR-, NMR- and MS-spectra were found to be in agreement with the proposed chemical structure of tembotrione.				
B.2.1.10.1 Optical purity						Not relevant since the active substance is not a resolved optical isomer	
B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)			Impurity toluene is considered relevant by Section toxicology			Spectra are not required for identification since a MS-spectrum is provided within the analytical method AM10305FP1 for the determination of this impurity. See volume 4.	
B.2.1.12 Solubility in water (IIA 2.6)	EEC/A6 OECD 105 flask method GLP	pure substance 989 g/kg	all at 20 °C 0.22 g/L pH 4 (buffered solution 0.1 mol/L) 28.30 g/L pH 7 * (in reality: pH: 6.2 buffer 0.3 mol/L) 29.69 g/L pH 9 * (in reality: pH: 6.1 buffer 0.3 mol/L) * Even a buffer with a concentration of 0.3 mol/L was not sufficient. A further increase of the buffer concentration did not show the desired pH value.			Acceptable	M-078747-01-1 Mühlberger B., 2002a

Study	Method	Material	Results	Conclusion/Comment	Reference
B.2.1.13 Solubility in organic solvents (IIA 2.7)	EEC/A6 OECD 105 flask method GLP	pure substance 989 g/kg	The solubility in different solvents at 20 °C was determined to be: Ethanol: 8.2 g/L n-Hexane: 47.6 mg/L Toluene: 75.7 g/L Dichloromethane: > 600 g/L Acetone: 300 to 600 g/L Ethyl acetate: 180.2 g/L DMSO: > 600 g/L	Although the test should be performed with the technical substance, it is not expected that a decreased purity will influence the result significantly. The result is acceptable	M-078761-01-1 Mühlberger B., 2002b
B.2.1.14 Partition coefficient *n*-octanol/water (IIA 2.8.1) Effect of pH (4-10) on the n-octanol/water partition co-efficient (IIA 2.8.2)	EEC/A8 OECD 107 & 117 Flask-shaking with HPLC method GLP	pure substance 989 g/kg	pH = 2 logPow = 2.16 (Pow = 144.9) at 23 °C pH = 7 logPow = - 1.09 (Pow = 0.0807) at 24 °C pH = 9 logPow = - 1.37 (Pow = 0.0430) at 23 °C	Acceptable	M-108278-01-1 Mühlberger B., 2003b
B.2.1.15 Hydrolysis rate (IIA 2.9.1)	OECD 111 USEPA 161-1 OPPTS 835.2110 GLP	[cyclohexyl-U-^{14}C]-AE 0172747 (> 99 %)	The rate of hydrolysis has been determined in a sterile aqueous buffer solutions at pH 4, 7 and 9 and at 50°C and 25°C. The test substance was stable to hydrolysis under the conditions of the test.	Acceptable For details see B.8.4 Fate and behaviour	M-107923-01-1 Fliege R., 2003
B.2.1.16 Direct phototrans-formation (IIA 2.9.2)	EEC 95/36 SETAC Europe Section 10 USEPA 162-1 GLP	[cyclohexyl-U-^{14}C]-AE 0172747 (> 99 %, HPLC) and [phenyl-U-^{14}C]-AE 0172747 (> 99 %, HPLC)	The photo-degradation has been studied in sterile aqueous buffer solution at pH 7 at 25°C under artificial sunlight for a total of 10 days. The test substance was moderately photolysed with an experimental half-life of 56.3 days under the conditions of the test. A minor metabolite (glutaric acid, M4) was found at 7% at the end of the irradiation period.	Acceptable For details see B.8.4 Fate and behaviour	M-063564-01-1 Hellpointner, E., 2004
B.2.1.17 Quantum yield (IIA 2.9.3)	EEC 94/37 and 95/36 German UBA ECETOC (1992)	pure substance 989 g/kg	Mean Quantum Yield was determined to $\Phi = 4.93 \times 10^{-5}$.	Acceptable For details see B.8.4 Fate and behaviour	M-105933-01-1 Hellpointner E., 2003

Study	Method	Material	Results	Conclusion/Comment	Reference
B.2.1.18 Lifetime in the top layer of aqueous systems (calculated and real) (IIA 2.9.4)	EEC 94/37 and 95/36 German UBA ECETOC (1992) GLP	pure substance 989 g/kg	Calculated half-life expressed as summer days: 269 days (Athens/Greece) Real photolytic half-lives in the environment according to software GCSOLAR (days): Degree latitude (°N): 30 40 50 60 Spring 10 11 13 17 Summer 9 9 10 11 Fall 15 19 31 60 Winter 20 34 72 234	Acceptable For details see B.8.4 Fate and behaviour	M-063564-01-1 Hellpointner, E., 2004 M-105933-01-1 Hellpointner E., 2003
B.2.1.19 Dissociation constant (pKa) (IIA 2.9.5)	OECD 112 spectrophotometric titration GLP	pure substance 989 g/kg	pKa = 3.18	Acceptable	M-254999-01-1 Mühlberger B., Eyrich U., 2005
B.2.1.20 Stability in air, photochemical oxidative degradation (IIA 2.10)	EEC 95/36 German BBA Part IV, 6-1 (1990) software AOPWIN	calculation	The half-life of AE 0172747 in air is 2.9 hours the chemical lifetime τ is 4.3 hours.	Acceptable	M-116064-01-1 Hellpointner, E., 2003
B.2.1.21 Flammability (IIA 2.11)	EEC/A10 GLP	tech. substance 956 g/kg	Preliminary Test: the test substance melted but did not ignite According to EEC A10 a full test is not required	Acceptable Not classified as highly flammable under the test conditions	M-242885-01-1 Smeykal H., 2004a
B.2.1.22 Auto-flammability (IIA 2.11.2)	EEC/A16 GLP	tech. substance 956 g/kg	No self-ignition temperature observed up to 402 °C.	Acceptable Compound is not considered as auto-flammable under the test conditions	M-242887-01-1 Smeykal H., 2004b
B.2.1.23 Flash point (IIA 2.12)			Not relevant, tembotrione is a solid with a melting point > 40 °C		

Study	Method	Material	Results	Conclusion/Comment	Reference
B.2.1.24 Explosive properties (IIA 2.13)	EEC/A14 OECD 113 GLP	tech. substance 956 g/kg	As a screening method for the determination of explosive properties a differential scanning calorimetry (DSC) under nitrogen was performed. Two DSC-measurements in closed glass crucibles showed an endothermic effect (melting) in the temperature range 105 - 135℃ and exothermal decomposition in the temperature range 140 – 270 ℃ and a small exothermal effect in the temperature range 280 - 400 ℃ with an overall energy of 212 and 213 J/g, respectively. If the decomposition energy is below 500 J/g a main test for explosive properties is not necessary (Recommendations on the Transport of Dangerous Goods / Manual of Tests and Criteria)	Acceptable Compound is not considered as explosive.	M-242886-01-1 Smeykal H., 2004c
B.2.1.25 Surface tension (IIA 2.14)	EEC/A5 OECD 115 GLP	tech. substance 947 g/kg	σ = 64.2 mN/m The solution was prepared as 90 % of the saturation concentration at 20 ℃. (Saturated solutions in distilled water have been filtered and diluted to 90% of the saturation concentration)	Acceptable Tembotrione is not regarded as surface active.	M-255557-01-1 Bogdoll B., Lemke G., 2005
B.2.1.26 Oxidizing properties (IIA 2.15)	EC A17 GLP	tech. substance 947 g/kg	Not an oxidising substance. The maximum overall burning rate is 1.02 mm/s for the reference mixture (55 % $Ba(NO_3)_2$ and cellulose). This is higher than the max. burning rate of the reference mixture (10 % test substance and cellulose) which is 0.87 mm/s.	The test item has no oxidising properties under the conditions of the test.	M-255234-01-1 Smeykal H., 2005b
B.2.1.2.27 pH (IIA 2.16)				This is not an EC data requirement	
Storage stability (IIA 2.17.1)				This is not an EC data requirement	

Study	Method	Material	Results	Conclusion/Comment	Reference
Stability (temperature, metals) (IIA 2.17.2)				This is not an EC data requirement	
Other/special studies (IIA 2.18)				No other/special studies	

According to Directive 91/414/EEC, granulometry is not required for active substances. Thus, no study considering this end-point has been provided

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Tembotrione is used as herbicide against grasses and broad leaved weeds.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification required.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Studies analyzing the absorption, distribution, metabolism, and excretion (ADME) in the rat have been performed with two different radiolabels, Cyclohexyl-^{14}C-radiolabel and Phenyl-^{14}C-radiolabel. Recovery of the cyclohexyl- and the phenyl-^{14}C-radiolabel was very good in all studies with total recoveries ranging from 95.9% to 102.7%. In male rats, the majority of the radioactivity was excreted via the faeces, and in female rats, the major excretion route was via urine.

Absorption: Tembotrione is well and rapidly absorbed after oral administration to rats. In the low dose groups (5 mg/kg bw), maximum blood and plasma concentrations were reached between 0.5 and 1 hour for the phenyl- and between 2.42 to 3.05 hours for the cyclohexyl-^{14}C-radiolabel. For the high dose (1000 mg/kg bw), maximum concentration was reached between 2.5 and 5 hours for the phenyl- and 6.55 and 8.83 hours for the cyclohexyl-^{14}C-radiolabel.
Based on the sum of radioactivity recovered in tissues and excreted via urine (plus cage wash) and expired air, the minimum absorption rates range between 23-37% for males and 77-87% for females of the low dose group (5 mg/kg bw). For the high dose (1000 mg/kg bw), these rates are in the magnitude of 53% and 74% for males and females, respectively.
Taking into consideration also recoveries obtained in bile, evaluation of oral absorption levels of tembotrione yield 93.9% for the males and 93.8% for the females. Noteworthy, in male rats the hepatobiliary concentration was twice compared to females (61.5% vs. 31.1% in males and females, respectively).

Distribution: Following oral administration to rats, radioactivity is widely distributed into organs and tissues with highest concentrations measured for the 0 – 3 h time period in all investigated samples, whereas 7 hours after dosing the levels had already declined in all analysed tissues.
For the low doses (5 mg/kg bw), the highest levels of radioactivity were detected in the liver followed by the kidney. In all other organs, levels of residues were very low (below 0.06 µg equiv./g). The distribution was similar at the high dose (1000 mg/kg bw) with the exception that the highest concentrations were measured in skin/fur followed by liver and kidney. There were no differences between the sexes.
After repeated applications, tissue distribution to the various organs and body compartments was similar to single application. No significant differences between male and female animals were observed. Mean increases in radioactivity after 14 days application over the single oral dose were 5.2-fold for males and 5.1-fold for females.

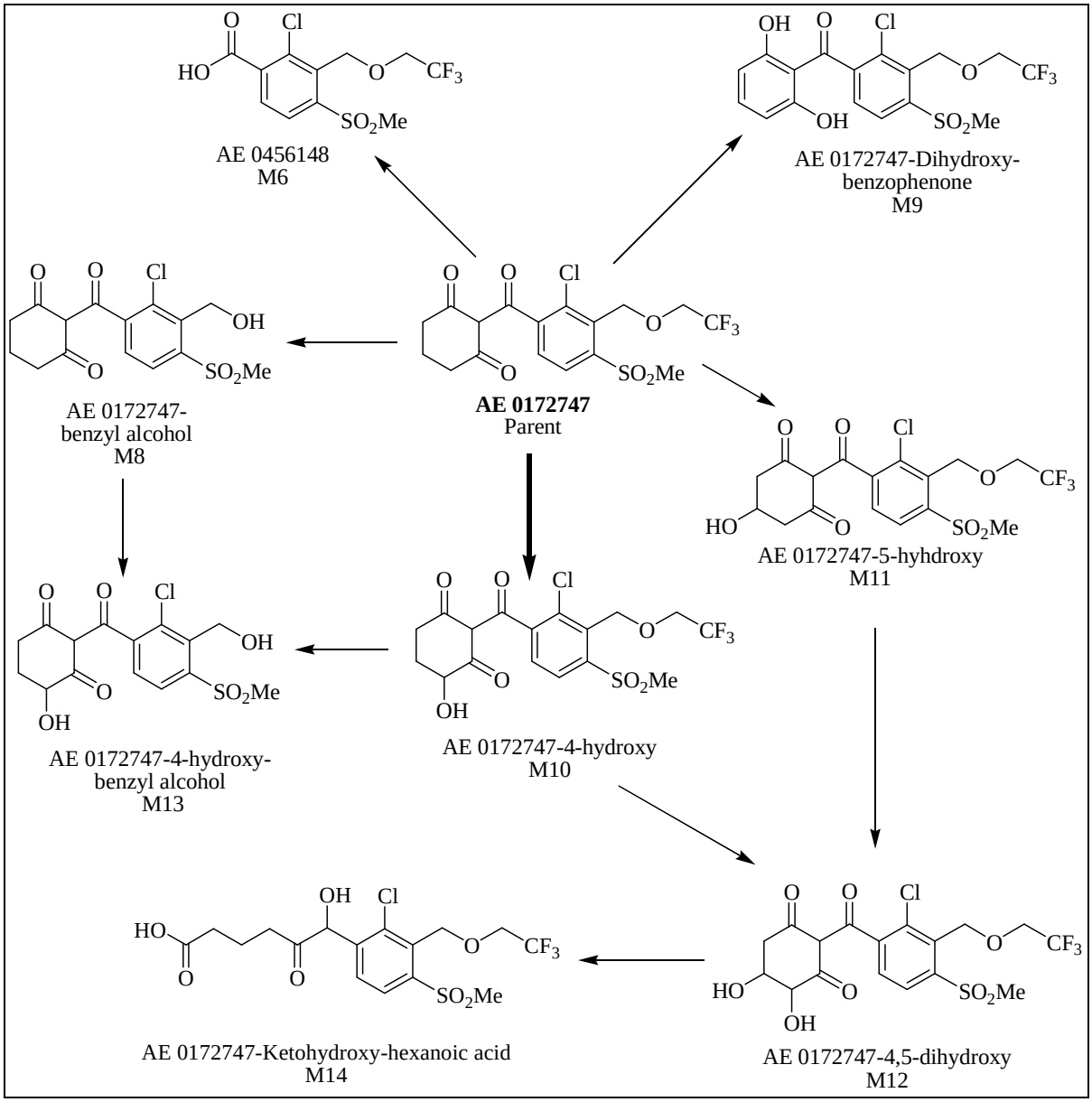
Excretion: In rats radiolabelled tembotrione was almost completely excreted (> 93% of the administered dose within 96 hours) following single oral dose with the majority excreted within the first 24 hours. Animals dosed for 14 consecutive days excreted > 90% within 24 hours after administration. Regarding the major routes of elimination, clear sex diffenerences could be

observed: In the single low dose groups (5 mg/kg bw), the major route of elimination in males was via the faeces (20-35% radioactivity found in urine vs. 59-73% in faeces), whereas in females radioactivity was preferentially excreted via urine (74-84% in urine vs. 15-22% in faeces). Accordingly, biliary excretion in male rats was twice compared to females (61.5% vs. 31.1% in males and females, respectively).
The repeated dosing groups showed that daily dosing over a period of 14 days did not significantly alter the sex-specific differences regarding excretion (males: 34% urine vs. 56% faeces; females: 82% urine vs. 14% faeces). In contrast, high dose administration to rats (1000 mg/kg bw) provoked a clear shift in males towards excretion via urine (males: 53% urine vs. 49% faeces; females: 74% urine vs. 28% faeces). Radioactivity measured in expired air was negligible or even not detectable.

Metabolism: Following oral dosing tembotrione was extensively metabolised in the rat although with some sex-related differences.
After single administration of 5 mg/kg bw, in male rats the main excreted compound in faeces and urine was M10, followed by M11, M12, and the parent compound. In females, the major excreted compound was the parent molecule, followed by M10 and M11. All other metabolites were present in much lower amounts. Comparing rat metabolism of tembotrione after single application and after repeated dosing (administration of 5 mg/kg bw for 14 days), the results demonstrate that there are no significant differences in the metabolite profiles after repeated application in both genders.
In the single high dose group (1000 mg/kg bw), where a decrease of sex-specific differences in the major routes of excretion has been observed, the major excreted component was the parent compound in both genders, followed by M10, M11, M6, M8, and M12.

In the rat, the metabolite profiles obtained with the phenyl- and the cyclohexyl-^{14}C-radiolabel were similar. Tembotrione was mainly converted via oxidative mechanisms with the formation of hydroxyl groups on either or both rings of the molecule. In the low dose group, the only cleavage product that was obtained was AE0456148 (M6) which was present at 2.9% in the male and 0.77% in the female rats. The major excreted compound was M10 in male rats (48-53%) and parent compound in female rats (46-60% administered radioactivity).
The following metabolic pathway for tembotrione was proposed for the rat:

Metabolic pathway of tembotrione (AE 0172747) following oral administration in rats



Dermal absorption: Two studies were submitted on the dermal absorption of a concentrated and a diluted preparation *in vivo* in rats and *in vitro* to compare the absorption characteristics of human and rat skin. In the *in vivo* study 18,585% of the concentrate and 11,24% of the dilution of the applied dose were considered to be absorbable (Odin – Feurtet M.; 2005). In the *in vitro* study dermal absorption in rat skin was 9,3 times higher for the undiluted formulation and 2,4 times higher for the diluted formulation than in human skin. (Artus – Jacenko L.; 2005). For human skin *in vivo* absorption rates of 1.99% of the applied dose for the concentrate and 4.68% for the diluted formulation can be calculated. In order to simplify exposure calculations, these values have been rounded to 2% and 5% dermal absorption for the concentrate and the dilution, respectively.

Additional study: Metabolism of tembotrione was also investigated in the mouse (repeated oral administration, Spiegel K and Koester J, 2005). 3000 ppm non-radiolabelled substance was administered for 28 days, followed by a single application of Phenyl-^{14}C-radiolabeled substance. The animals were sacrificed 3 hours after application and radioactivity was recovered from plasma, liver, bile, kidney, and urine. The overall metabolic profile was similar to the rat, but no gender

specific differences in the excretion routes were observed. The metabolite M6 was not detected in mice and a glucuronidated form of metabolite M9 was found in urine, kidney, liver and bile of mice.

4.1.2 Human information

Not available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption	Rapid and almost completely to > 93% (based on urinary and biliary excretion) after single oral low dose (rat study)
Distribution	Widely distributed (highest residues found in liver and kidneys)
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Almost completely excreted (> 93%) within 96 hours; in males 20-35% of radioactivity found in urine vs. 59-73% in faeces; in females radioactivity was preferentially excreted via urine (74-84% vs. 15-22% in faeces)
Metabolism in animals	Extensively metabolised mainly via oxidative mechanisms with the formation of hydroxyl groups on either or both rings of the molecule; some sex-related quantitative differences in metabolite profile

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity in rats	LD_{50} > 2000 mg/kg bw	-	Eigenberg D., 2003a
Acute dermal toxicity in rats	LD_{50} > 2000 mg/kg bw	-	*Eigenberg D., 2003b*
Acute inhalation toxicity in rats (4 hours, nose only)	LC_{50} > 4.58 mg/L	-	*Wesson C., 2003*

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

AE 0172747 was administered via gavage to fastened female Wistar rats at doses of 630 (1 animal) and 2000 mg/kg (3 animals) to evaluate the acute oral toxicity of tembotrione. AE 0172747 was formulated as a suspension in 0.5% aqueous carboxymethyl-cellulose and administered at a volume of 10 mL/kg. Animals were monitored for detailed clinical observations within 30 minutes after dosing and up to 4 hours after treatment and then checked daily during the 14-day follow-up period after dosing. All animals were weighed on the day of dosing and on days 7 and 14 after treatment. Animals were sacrificed and were necropsied and examined for gross pathological changes. No deaths and no clinical symptoms were observed during the observation period. Yellow staining of the urogenital area was found in one rat dosed with 2000 mg/kg bw. Body weights of the rats were not affected. Also no gross abnormalities at necropsy were found.

The oral LD_{50} of tembotrione in female rats was > 2000 mg/kg therefore it does not require labeling.

4.2.1.2 Acute toxicity: inhalation

In an acute inhalation toxicity study, ten Sprague-Dawley rats (5/sex) were exposed to a dust atmosphere of tembotrione for 4 hours using a nose only exposure system, followed by a fourteen day observation period. Each rat was individually held in a tapered, polycarbonate restraining tube, fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring. Only the nose of each animal was exposed to the test atmosphere. Following an appropriate equilibrium period a single group of ten rats (5/sex) was exposed to an atmosphere of the test material for a period of four hours. A target concentration of 5.0 mg/L was used for the exposure. As no deaths occurred and the mean achieved concentration was 91.6% of the target, no further levels were tested. All animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the retraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days. Individual bodyweights were recorded prior to treatment on the days of exposure and on day 7 and 14. At the end of the fourteen day observation period, the animals were killed by intravenous overdose of sodium pentobarbitione. All animals were subjected to a full external and internal examination, and any macroscopic abnormalities. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity. No mortality occurred up to 4.58 mg/L, the maximum technically achievable concentration. The mean mass median aerodynamic diameter was 2,34 µm and the inhalable fraction (%< 4µm) 77,6%. The clinical signs included hunched posture, wet fur, pilo-erection and red/brown staining around the eyes and were seen observed for short periods after removal from the chamber. They were considered to be associated with the restraint procedure, with the exception of the increased respiratory rate. One day after exposure, all animals appeared normal and no clinical signs were noted during the 14-day observation period. Bodyweight developed normally and no gross necropsies could be identified.

The acute inhalation LC50 of tembotrione in rats for combined sexes was found to be > 4.58 mg/L. Tembotrione does not warrant classification as being toxic or harmful on the basis of its acute inhalation toxicity.

4.2.1.3 Acute toxicity: dermal

In an acute dermal toxicity study, groups of young adult Wistar rats, 5/sex were exposed by the dermal route to tembotrione. Approximately 24 hours before dosing the fur was removed from the dorsal and lateral areas of the trunk of the rats. The test material was mixed with deionised water and applied in a dose of 2000 mg/kg bw to 10% of the body surface area. The gauze containing the moistened substance was secured with a hypoallergenic tape and further with a Vetrap bandage which was fixed with another tape. After an exposure period of 24 hours, the occlusion was removed and residual test material was gently removed with moistened dry paper towels to remove the test substance. Animals were observed for clinical signs and mortality several times on the day of dosing and subsequently at least once daily for an observation period of at least 14 days. Individual body weights were recorded on the day of dosing and on days 7 and 14. On day 14, all animals were sacrificed and were necropsied and examined for gross pathological changes. One female rat died on day 1, it was not considered substance related, but as a result of asphyxiation caused by the tightness of the wrapping. All other animals survived. The following clinical signs were seen: red discharge and dark red staining around the eye (1 male), lesions on the right side of the mouth (1 male), red discharge from the nose (1 male and 2 females), red discharge and dark red staining around the nose (1 female). The signs mainly occurred during the first two days of the study and disappeared thereafter. Body weight was not affected and no gross abnormalities at necropsy were found.

The dermal LD_{50} of tembotrione for male and female rats was > 2000 mg/kg therefore it does not require labelling.

4.2.1.4 Acute toxicity: other routes

No information available.

4.2.2 Human information

Not available.

4.2.3 Summary and discussion of acute toxicity

All studies presented in this section were conducted in 2002 and complied with the EU and OECD testing guidelines and Good Laboratory Practice (GLP). Tembotrione is of very low acute toxicity in the rat by the oral, dermal and inhalation route. No classification is required for acute toxicity.

4.2.4 Comparison with criteria

All estimated LD_{50} values are above the criteria for classification and labelling (both DSD and CLP).

4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

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

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

There was no evidence of any specific, non-lethal target organ toxicity arising from a single exposure to tembotrione. Clinical signs of toxicity were observed after single exposure to tembotrione by the dermal route and by inhalation but were considered to be non-specific signs of general acute toxicity. No classification as STOT SE is proposed.

4.3.2 Comparison with criteria

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

4.3.3 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Primary skin irritation in rabbits	Non skin irritating	-	*Rees P., 2003a*

4.4.1.1 Non-human information

Three male New Zealand White rabbits each received a single semi-occlusive dermal application. On the day before dosing, hairs were removed with clippers from the dorso-lumbar region of each rabbit. The treatment site was 'wetted' with 0.5 mL of filtered water and approximately 0.5 g of the test substance was applied under a 2-ply 25 mm x 25 mm porous gauze pad to intact skin sites of three animals. The control site was treated with vehicle only. At the end of the exposure period the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with lukewarm water (30-40°C) to remove any residual test substance. Examination of the treated skin was made on Day 1 (i.e. approximately 60 minutes after removal of the dressings) and on Days 2, 3 and 4 (equivalent to approximately 24, 48 and 72 hours after exposure). As indicated by the notifier a primary irritation index (PII) was calculated from the erythema and oedema scores according to the formula as described in Technical Report No. 66 "Skin irritation and Corrosion: Reference chemicals data bank" (March 1995) ECETOC, Brussels. There were no signs of toxicity or illness in any rabbit during the observation period. In addition, no dermal irritation was observed in any animal throughout the duration of the study.

The Primary Irritation Index (PII) was calculated to be 0.0 and therefore tembotrione does not require labelling.

4.4.1.2 Human information

Not available.

4.4.1.3 Summary and discussion of skin irritation

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

4.4.1.4 Comparison with criteria

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

4.4.1.5 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No.1272/2008: no classification proposed

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation study in rabbits	Mild eye irritation; does not warrant classification	-	*Rees P., 2003b*

4.4.2.1 Non-human information

The study was designed to assess the eye irritation potential of tembotrione following a single instillation of a volume of 0.1 mL of the test substance (weighing approximately 100 mg) into the eye of three male rabbits. Animals were then observed for 8 days. The duration of the exposure period was limited to 30 seconds by irrigation of the treated eye at the appropriate time after instillation. The behaviour of each rabbit was observed immediately following instillation of the test substance to allow assessment of the initial pain response. The animals were checked at least twice during the first hour after dosing and at regular intervals throughout the day to ensure no severe injury passed unnoticed. In the main study ocular reactions to treatment were assessed 1, 24, 48 and 72 hours and seven days after treatment. The untreated eye was used as a comparison with the treated eye during assessment of ocular lesions. Mean values were calculated using all scores recorded 24, 48 and 72 hours after treatment. There was no sign of toxicity or illness in any rabbit during the main study observation period. Injection of the conjunctival blood vessels was apparent throughout the first 24 hours after instillation in all animals, persisting up to the 72-hour observation in two animals. Additionally, very slight chemosis with very slight discharge was evident in two animals one hour after instillation. The mean scores for eye irritation were 0.78 for conjunctival redness and 0.0 for cornea opacity, iris lesion and chemosis. Instillation of the test substance gave rise to a slight initial pain response.

Tembotrione caused only mild transient ocular irritancy but does not require labelling in according

to the Commission Directive 2001/59/EEC.

Table 14: Eye irritation scores

	Cornea			Iris			Conjunctival redness			Conjunctival chemosis		
Animal number	4300	4301	4327	4300	4301	4327	4300	4301	4327	4300	4301	4327
Time of observation												
1 hour	0	0	0	0	0	0	1	1	1	1	0	1
24 hours	0	0	0	0	0	0	1	1	1	0	0	0
48 hours	0	0	0	0	0	0	1	1	0	0	0	0
72 hours	0	0	0	0	0	0	1	1	0	0	0	0
Mean scores 24-72 hours	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1**	**1**	**0.3**	**0.0**	**0.0**	**0.0**

4.4.2.2 Human information

Not available.

4.4.2.3 Summary and discussion of eye irritation

Tembotrione caused only mild transient ocular irritancy and does not require classification and labelling in accordance to the Commission Directive 67/548/EEC and Regulation (EC) No. 1272/2008.

4.4.2.4 Comparison with criteria

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

4.4.2.5 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

4.4.3 Respiratory tract irritation

Table 15: Summary table of relevant respiratory tract irritation studies

Method	Results	Remarks	Reference
Acute inhalation toxicity in rats (4 hours, nose only)	LC_{50} > 4.58 mg/L	-	*Wesson C., 2003*

4.4.3.1 Non-human information

No signs of respiratory tract irritation were reported in an acute inhalation study in rats.

The clinical signs included hunched posture, wet fur, pilo-erection and red/brown staining around the eyes and were seen observed for short periods after removal from the chamber. They were considered to be associated with the restraint procedure, with the exception of the increased respiratory rate. One day after exposure, all animals appeared normal and no clinical signs were noted during the 14-day observation period. Bodyweight developed normally and no gross necropsies could be identified.

4.4.3.2 Human information

Not available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No signs of respiratory tract irritation were reported in an acute inhalation study in rats. Taking into account the absence of any skin irritation effects and only very mild eye irritation by tembotrione, no classification is proposed for respiratory tract irritation under either Directive 67/548/EEC or CLP Regulation.

4.4.3.4 Comparison with criteria

No irritating effects on respiratory tract were observed in acute inhalation study with tembotrione (according to both DSD and CLP).

4.4.3.5 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

4.5 Corrosivity

Tembotrione did not show any corrosive properties in rabbit skin and eye irritation studies. (see chapter *4.4 Irritation*).

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 16: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea pig skin sensitisation study (Magnusson & Kligman method)	Sensitising to the skin	-	Coleman, D.G.; 2003

4.6.1.1 Non-human information

Reference:	**AE 0172747 - Guinea-pig skin sensitization study (Magnusson & Kligman method)**
Author(s), year:	Coleman, D.G.; 2003
Report/Doc	AES 116-023862; MO-04-000664 / M-106505-01-1
Guideline(s):	OECD 404 (1997)
GLP:	Yes
Deviations:	None
Validity:	Yes

Material and methods:

Test Material:	AE 0172747 (tembotrione)
Lot/Batch:	PFI 0215 (OP 2250027)
Purity:	94.0%
Stability of test compound:	Not reported
Positive control:	Hexyl cinnamic aldehyde (HCA)

Test animals:

Species:	Guinea pig (male)
Strain:	Dunkin/Hartley
Age:	4 - 5 weeks approximately
Weight at dosing:	Males: 336 - 392 g
Source:	D. Hall, Newchurch, Staffordshire, UK

In a dermal sensitisation study, tembotrione in Alembicol D was tested using young adult male guinea pigs of the strain Dunkin/Hartkey. Ten test and five control animals were used in this study. The treatment regime involved induction of sensitisation by intradermal injection on day 1, induction of sensitisation by topical administration on day 8 and challenge by topical administration on day 22. The doses for the induction and challenge treatments were selected on the basis of the results of the dose range-finding study. In the main study, the dorsal region and the flanks of the guinea pigs were shorn one day prior the intradermal induction. Three injections were made on the left and the right side of the spinal column for each animal.

All animals received at the first injection site complete Freund's adjuvant diluted with water for irrigation (50:50), at the second injection site 2.5% tembotrione formulated in Alembicol D for the treated animals or vehicle alone for the control animals, and at the third injection site 2.5% tembotrione formulated in equal parts of Alembicol D and complete Freund's adjuvant for treated animals or 1:1 mixture of Alembicol D and complete Freund's adjuvant for control group animals.

The preliminary investigations indicated that the maximum practical concentration of the test substance for topical application (100% w/v) did not produce skin irritation. Therefore, six days following the injections (day 7) the same 4 x 6 cm scapular area was clipped and shaved free of hair and the site was pre-treated by gentle rubbing with 0.5 mL per site of 10% w/w sodium lauryl sulphate in petrolatum. The topical induction was performed one week after the intradermal induction (on day 8). Hypoallergenic patches were placed between and on the injection sites, covered by a length of impermeable plastic adhesive tape and held securely in place using an elastic adhesive tape (Elastoplast). In the treated group the patches contained 0.4 mL of 100% tembotrione in Alembicol D and in the control group; the patches contained 0.4 mL of Alembicol D. At the end of the 48 hour exposure period, the remaining test item was removed with sterile physiological

saline solution.

The control and test animals were challenged topically two weeks after the topical induction application (day 22) using tembotrione, 100% w/v and 50% w/v in Alembicol D. One day prior to dosing hair was removed by clipping and then shaving from an area on the left flank of each guinea-pig to expose naïve area of skin. A 2 x 2 cm patch was saturated with approximately 0.2 mL of tembotrione, 100% w/v in Alembicol D and applied to an anterior site on the flank. Tembotrione, 50% w/v in Alembicol D was applied in a similar manner to the posterior site. The patches were sealed to the flank for 24 hours under strips of "Blenderm" secured with an elastic adhesive tape. The challenge sites were evaluated approximately 24 and 48 hours after removal of the patches.

Findings:

No signs of morbidity or toxicity were observed. Bodyweight increases were similar in test and control groups.

Following the intradermal injections, necrosis was recorded at sites of Freund's Complete Adjuvant application in test and control animals. No erythema was seen in test animals receiving tembotrione, 2.5% w/v in Alembicol D and no erythema was observed in control animals receiving Alembicol D.

Following topical application, neither erythema nor oedema were observed in test animals following topical application with tembotrione, 100% w/v in Alembicol D.

Following the challenge application with tembotrione, dermal reactions were observed in eight test animals compared to none in controls; therefore these eight test animals gave clear positive responses. The bandage came adrift during the challenge application period for the remaining two test animals; therefore no score was recorded for these two animals.

Table 17: Dermal reactions observed after the challenge application with tembotrione

Guinea-pig Number	E = Erythema O = Oedema	Score				Results
		24 Hours		48 Hours		
		A	P	A	P	
110	E O	2 0	1 0	2 1	2 1	+
111	E O	0 0	1 0	0 0	2 1	+
112	E O	1 0	1 0	2 0	2 0	+
113	E O	1 0	2 0	2 0	2 0	+
114	E O	0 0	0 0	2 0	2 0	+
115	E O	#	#	#	#	#
116	E O	#	#	#	#	#
117	E O	1 0	1 0	1 0	2 0	+
118	E O	1 0	1 0	2 1	2 1	+
119	E O	1 0	1 0	2 1	2 1	+

Scoring: Positive (+), Negative (-), Inconclusive (±) and no score because patch came adrift (#)
A Anterior site, exposed to AE 0172747, 100% w/v in Alembicol D
P Posterior site, exposed to AE 0172747, 50% w/v in Alembicol D

Conclusion:

Overall tembotrione (formulated in Alembicol) was considered to cause skin sensitisation in guinea pigs. As more than 30% of the test animals gave positive responses, according to the Commission Directive 67/548/EEC, tembotrione requires classification and labelling with the risk phrase R43 "May cause sensitisation by skin contact".

4.6.1.2 Human information

Not available.

4.6.1.3 Summary and discussion of skin sensitisation

In a guinea pig skin sensitisation study (Magnusson & Kligman method) eight test animals showed dermal reactions following the challenge application with tembotrione, whereas no dermal reactions were observed in the control group. Therefore these eight test animals gave clear positive responses. For the remaining two test animals no score was recorded because the bandage came adrift during the challenge application period. Overall tembotrione (formulated in Alembicol) was considered to cause skin sensitisation in guinea pigs.

4.6.1.4 Comparison with criteria

The criteria for classification as skin sensitiser based on an adjuvant type guinea pig test method are the same in Directive 67/548/EEC as in the CLP-Regulation: A response of at least 30% of the animals is considered positive. Since more than 30% of the test animals gave positive responses, according to the Commission Directive 67/548/EEC tembotrione requires classification and labelling with the **Xi; R43 "May cause sensitisation by skin contact"**. According to Regulation (EC) No. 1272/2008 tembotrione belongs to Skin sensitisation Category 1 and requires classification and labelling with **H317 "May cause an allergic skin reaction"**.
Considering the 2nd ATP to CLP [Commission Regulation (EU) No 286/2011, March 10th 2011] a substance has to be classified into sub categorie 1b if the percentage of responding animals is either ≥ 30 to < 60 after > 0,1 to ≤ 1 % intradermal induction dose or ≥ 30 after > 1% intradermal induction dose. As a consequence of this new criteria for classification tembotrione has to be assigned to sub category 1b as the the percentage of responding animals is more than 30% after intradermal injection of 2.5% tembotrione formulated in Alembicol D.

4.6.1.5 Conclusions on classification and labelling

Directive 67/548/EEC: Xi; R43

Regulation (EC) No. 1272/2008: Skin Sens.1b, H317

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal The CLH report refers to one Magnusson and Kligman Maximisation test on skin sensitisation in Guinea-pigs (Coleman, 2003) performed according to OECD guideline 406. Because of technical problems (bandage lost from 2 animals), eight animals were used for the final challenge. All eight responded positively to the challenge with 50 or 100% tembotrione (in 1:1 Alembicol D and complete Freund's adjuvant), whereas none of the five controls reacted to the vehicle. As more than 30% of the animals reacted to a concentration of 50 or 100%

tembotrione, the dossier submitter concluded that tembotrione is a skin sensitiser and proposed classification with Skin Sens. 1B; H317 and R43.

Comments received during public consultation
The CLH report variously proposed both for Skin Sens.1 (without specifying 1A or 1B) and Skin Sens. 1B. Four comments were received, all from MS, and they agreed with classifying tembotrione as a skin sensitiser, albeit two agreed with category 1 and two with category 1B.

Assessment and comparison with the classification criteria
The RAC considered that the above study showed a sensitisation potential of tembotrione. After 24 hours, the average erythema score was 1 (discrete or patchy). The effects became more severe with time, with grade 2 (moderate and confluent) erythema in all animals 48 h after the challenge with 50% tembotrione (and an average score of 1.6 with 100% tembotrione). As the intra-dermal induction dose was >1% (i.e. 2.5%), the data support classification in sub-category 1B. However, with such a high level of responders (100%) after intradermal induction with 2.5%, there is a possibility that at a slightly lower intradermal induction concentration of 1% there will still be a considerable level of responders, potentially over 60% (which would support classification in category 1A). Lower intradermal induction concentrations than 2.5% have however not been tested, so in principle the data are insufficient for sub-categorisation.
The RAC thus concluded that classification of tembotrione with Skin Sens. 1; H317 (R43 according to DSD) was warranted.

4.6.2 Respiratory sensitisation

No data available.

4.7 Repeated dose toxicity

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Mouse oral, 28-days	0, 500, 2000, 7000 ppm/diet (equivalent to 0, 81.1, 332 and 1219 mg/kg bw/d in males and 0, 94.5, 396 and 1550 mg/kg bw/d in females) NOAEL = ♂ 81.1 mg/kg bw/d NOAEL = ♀ 94.5 mg/kg bw/d Effects at LOAEL: <u>Both sexes:</u> - increased liver weight	Dose finding study, inhouse method used, not GLP	Kennel, P.; 2002
Mouse oral, 90 days	0, 35, 350, 3500, 7000 ppm/diet (equivalent to 0, 5.9, 64, 631 and 1317 mg/kg bw/d in males and 0, 7.3, 76, 782, and 1833 mg/kg bw/d in females) NOAEL = ♂ 64 mg/kg bw/d NOAEL = ♀ 76 mg/kg bw/d Effects at LOAEL: <u>Both sexes:</u> - changes in clinical chemistry (↑ ALT, ↑urea) - liver histopathology - gastric erosive / ulcerative lesions <u>Females:</u> - ↑ corpora lutea	-	Steiblen, G.; 2003
Rat oral, 90-days	0, 1.25, 75, 1500, 7000 ppm/diet (equivalent to 0, 0.07, 4.45, 86.4 and 413 mg/kg bw/d in males and 0, 0.08, 5.59, 107.2 and 465 mg/kg bw/d in females) NOAEL = ♂ 0.07 mg/kg bw/d NOAEL = ♀ 0.08 mg/kg bw/d Effects at LOAEL: <u>Both sexes:</u> - corneal opacities and neovascularisation of the cornea <u>Males:</u> - plasma cholesterol ↑ - increased liver wt - hepatocellular hypertrophy	-	Steiblen, G.; 2002
Rat oral, 90-days	0, 6, 20, 40 ppm/diet (equivalent to 0, 0.30, 0.98 and 2.2 mg/kg bw/d in males and 0, 0.35, 1.18 and 2.68 mg/kg bw/d in females) LOAEL = ♂ 0.30 mg/kg bw/d LOAEL = ♀ 0.35 mg/kg bw/d Effects at LOAEL: <u>Males:</u> - corneal opacities - increased liver wt - hepatocellular hypertrophy	-	Kennel, P.; 2005a
Dog oral, 90-days	0, 125, 750, 4500/2250 ppm/diet (equivalent to 0, 4.5, 26.7 [♂]/28.5 [♀] and 124	The dose of	Kennel, P.; 2004

	[♂]/111 [♀] mg/kg bw/d) NOAEL = ♂ 26.7 mg/kg bw/d NOAEL = ♀ 28.5 mg/kg bw/d Effects at LOAEL: <u>Both sexes:</u> - clinical signs of neurotoxicity - changes in blood parameters (↓ MCV, ↓ MCH, RBC morph. changes) - histological alterations (digestion chambers in myelin, hepatoc. swelling, pigment deposition in hepatocytes and Kupffer cells) <u>Males:</u> - bilateral corneal opacities (in one ♂) - changes in clinical chemistry (↓ albumin, ↑ chloride conc.)	4500 ppm was found to be acute toxic in male dogs. Therefore the high does had to be decreased to 2250 ppm from day 30 onwards.	
Dog oral, 1-year	0, 75, 300, 1200 ppm/diet (equivalent to 0, 2.5, 9 [♂]/ 10.2 [♀] and 37.8 [♂]/41.6 [♀] mg/kg bw/d) NOAEL = ♂ 9.0 mg/kg bw/d NOAEL = ♀ 10.2 mg/kg bw/d Effects at LOAEL: <u>Females:</u> - changes in blood parameters (↓ MCV, ↓ MCH, erythrocytosis, platelets↑) - increased AP-activity - increased liver weight - hepatocellular hypertrophy - pigments in thyroid cells <u>Males:</u> - changes in blood parameters (↓ MCV, ↓ MCH, platelets↑) - increased liver weight (no dose – relationship) - digestion chambers in myelin	-	Kennel, P.; 2005b
Rat dermal, 28-days	250, 500 and 1000 mg/kg bw/d **LOAEL** = 250 mg/kg bw/d Effects at LOAEL: <u>Females:</u> - changes in blood parameters - histological alterations in pancreas and thyroid <u>Males:</u> - decreased AP-activity - histological alterations in pancreas and thyroid	-	Kroetlinger, F.; 2005
Rat dermal, 28-days	50, 250 and 1000 mg/kg bw/d **LOAEL** = 50 mg/kg bw/d Effects at LOAEL: <u>Males:</u> - increased rel. liver weight - histological alterations in pancreas and thyroid - increased albumin	-	Kroetlinger, F., Schladt, L.; 2005
Rabbit developmental toxicity	0, 1, 10, 100 mg/kg bw/day (gavage) NOAEL (maternal) = 1 mg/kg bw/day LOAEL (maternal) = 10 mg/kg bw/day Effects at LOAEL:	See chapter 4.7.1.6 (other relevant information)	Wason, S., 2003b

	- reduced food consumption - no or few faeces		

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

A series of studies was carried out to investigate the effects following repeated exposure of orally administered tembotrione in mice (one 28-day and one 90-day study), rats (two 90-day studies) and dogs (one 90-day and one 1-year study).

Mouse:

Tembotrione was administered continuously via the diet to groups of C57BL/6 J mice (10/sex/group) for 28 days at concentrations of 0, 500, 2000 and 7000 ppm (Kennel, P.; 2002). In all treated groups and for both sexes, abnormal color of urine was noted after one week of treatment and up to the end of the study. In both sexes, most adverse effects were confined to the top dose only and consisted of significantly reduced body weight and body weight changes throughout the study. The liver was the target organ and the following effects were identified: changes in clinico-chemical parameters (lower alkaline phosphatase activity in both sexes and higher alanine aminotransferase activity in males), significantly increased liver weights and histological alterations in the liver. The NOAEL was determined as 500 ppm (81.1 mg/kg/day in males and 94.5 mg/kg/day in females respectively) based on increased liver weights in the mid dose group of 2000 ppm.

In a 90-day dietary toxicity study (Steiblen, G.; 2003), C57BL6 mice were given tembotrione in the diet at concentrations of 0, 35, 350, 3500 and 7000 ppm. Body weight gain was reduced in males at the top dose group during the first two weeks of the study. The liver was the target organ at 3500 ppm and above: changes in clinical chemical parameters were accompanied by increased liver weights and also associated with hepatocellular hypertrophy in both sexes, together with a midzonal focal/ multifocal hepatocellular single cell necrosis in males. The study NOAEL was 350 ppm (corresponding to 64 mg/kg/day for males and 76 mg/kg/day for females).

For details see DAR.

Rat:

In the first 90-day study (Steiblen, G.; 2002), tembotrione was given to rats in the diet at concentrations of 0, 1.25, 75, 1500 and 7000 ppm. Major target organs were eyes and liver. The NOAEL of tembotrione in this study was set at 1.25 ppm (equivalent to 0.07 mg/kg bw/day for males and 0.08 mg/kg/day for females). The LOAEL was set at 75 ppm (equivalent to 4.45 and 5.59 mg/kg bw/d in males and females, respectively) based on the following findings: corneal opacities (superficial and snow flake shape), neovascularisation of the cornea (not fully reversible), increased plasma cholesterol levels (+22% in male rats), increased urinary ketone levels, increased liver weights (> 20%) and diffuse centrilobular hepatocellular hypertrophy. In addition, at the very high dose level of 7000 ppm histopathological alterations in the testes were observed.

In the second 90-day study (Kennel, P.; 2005a), tembotrione was administered continuously in the diet to Wistar rats at dose levels of 0, 6, 20 and 40 ppm. The principle target organs were the same

as in the first study. At 20 ppm (equivalent to 0.98 and 1.18 mg/kg bw/d in males and females, respectively) and above snow flake-like corneal opacity of the left eye was observed in one male. In addition at necropsy statistically significant increased liver weights (≥ 17%, in male rats), increased prominent hepatological lobulation (in males) as well as diffuse centrilobular hepatocellular hypertrophy (80% incidence) were found at dose-levels of 20 ppm and above. At 6 ppm (equivalent to 0.30 and 0.35 mg/kg bw/d in males and females, respectively) liver weights were statistically significant increased (≥ 16%) in male rats, associated with centrilobular hepatocellular hypertrophy. However, it has to be considered that effects on liver weight and histological alterations in this organ observed were not accompanied by other biomarkers of hepatotoxicity but are more likely a result of tyronsinaemia which is of limited relevance for humans. Nevertheless, the liver findings at 6 ppm were considered adverse by the experts at the PRAPeR (Pesticide Risk Assessment Peer Review) meeting in May 2009 and it was criticized that tyrosin levels have never been measured in the relevant dose group of 6ppm. Therefore, no NOAEL could be derived from this study. A further study was therefore submitted by the notifier to show that low levels of tembotrione effect blood tyrosin levels, predominantly in male rats. (see 4.12.1.3 *Specific investigations: other studies*).

Dogs:

In a 90-day dietary toxicity study (Kennel, P.; 2004) tembotrione was administered to beagle dogs at doses of 0, 125, 750 and 4500 ppm. The dose of 4500 ppm was found to be acute toxic: one male was sacrificed prematurely due to severe toxicity. Therefore the high dose was decreased to 2250 ppm from day 30 onwards. Major targets were eyes, blood, liver and peripheral nerves.

The following findings were observed at the top dose: clinical signs (uncoordinated movements, reduced motor activity and abnormal posture, hopping, placing, wheelbarrowing and locomotion), corneal opacities (snow flake) of both eyes in one male, effects in various erythrocyte parameters (decreased mean corpuscular volume and mean corpuscular haemoglobin, hypochromonia, microcytosis, anisochromia, anicytosis), increased liver weights and histopathological changes in the liver (hepatocellular cloudy swelling, multifocal golden brown pigments in hepatocytes) and also in nerves (increased number of digestion chambers in myelin). Therefore, 750 ppm (equal to 26.7 and 28.5 mg/kg/day in males and females, respectively) was regarded as NOAEL.

In a one-year study (Kennel, P.; 2005b), dietary administration of tembotrione to male and female beagle dogs at 0, 75, 300 and 1200 ppm for 52 weeks did neither induce treatment-related mortalities, clinical signs, ophthalmological effects, nor effects on body weight and food consumption parameters. Major targets were blood and livers. Erythrocytosis and enhanced alkaline phosphatase acticity were seen at all dose level, but statistical significance was only reached at the highest dose level. Also increased liver weights were found at all dose levels (predominantly in males). However, histological alterations (hepatocellular hypertrophy) were evident only at the top dose (predominantly in females). Therefore the NOAEL in this study was set at 300 ppm (equivalent to 9.0 and10.2 mg/kg bw/d in males and females, respectively).

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

Two 28-day dermal toxicity studies were performed in the rat. Groups of 10 male and 10 female rats received tembotrione at dose levels of 0, 250, 500 and of 1000 mg/kg body (first study) or 0,

50, 250 or 1000 mg/kg bw/ day (second study) by dermal application.

In the first study (Kroetlinger, F.; 2005) no treatment-related clinical signs were observed in treated animals and survival was not affected. No skin reddening and no effects on the mean skinfold thickness were observed at 1000 mg/kg bw/d and below in both sexes. Microscopically, no alterations were detected either in the treated or in the untreated skin regions. There were no effects on body weight, on food and water consumption. The major targets were blood, liver, pancreas and thyroid. At 250 mg/kg bw/d and above alkaline phosphatase activity was decreased and liver weights were increased in male rats. In females reticulocyte counts were increased dose-dependently. An overall NOAEL was not determined due to the effects observed at all dose levels consisting of changes in haematological and clinical chemical parameters, increased liver weights and histological alterations in the liver (condensed cytoplasm and peripheral hypertrophy change), pancreas (slight to moderate degenerative changes/increase of apoptotic bodies of the exocrine acinar tissue accompanied by inflammatory infiltrates) and thyroid (colloidal alteration and follicular cell hypertrophy). The lowest dose of 250 mg/kg bw/d can only be considered a LOAEL.

In the second study (Kroetlinger, F., Schladt, L.; 2005) no effects were found on survival, body weight, skin reddening or thickness in treated animals. Nevertheless, due to the effects observed in clinical chemistry parameters (increased albumin and protein concentrations and decreased levels of blood urea more prominent in males), increased relative liver weight in males and histopathological findings in pancreas (degeneration apoptosis) and thyroid (colloid alteration) no NOAEL could be set. The lowest dose tested (50 mg/kg bw/day) was considered to be a LOAEL.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

Not available.

4.7.1.6 Other relevant information

In a developmental toxicity study in rabbits (Wason, S.; 2003b), tembotrione was given by gavage at dose levels of 0, 1, 10 and 100 mg/kg bw/d. At the dose level of 100 mg/kg bw/d, the pregnant rabbits showed severe maternal toxicity and mortality. Five out of 25 pregnant females died prematurely between gestation day 15 and 22. Due to the severity of these effects (mortality), labelling with **R48/22** was considered justified by the experts at the PRAPeR expert meeting 69 (4-8 May 2009).

For reasons of completeness, the maternal findings observed in the rabbit developmental study (summarised in chapter *4.11.2 Developmental toxicity*) are reported here:

Reference:	**AE 0172747 - Developmental toxicity study in the rabbit by gavage**
Author(s), year:	Wason, S.; 2003b
Report/Doc. number:	SA02056; MO-03-011094; M-108558-01-1
Guideline(s):	OECD 414
GLP:	Yes
Deviations:	-
Validity:	Yes

Material and methods:

Test Material:	AE 0172747
Lot/Batch:	PFI 0195
Purity:	95.0%
Vehicle:	Aqueous solution of methyl cellulose 400
Test animals:	
Species:	Rabbit
Strain:	New Zealand White KBL (NZW) IOPS/SPF
Age:	18 weeks old
Weight at mating:	211 – 286 g
Source:	Elevage Scientifique des Dombes (ESD), Chatillon sur Chalaronne, France

Time-mated female New Zealand White rabbits were exposed to tembotrione by gavage from gestation days (GD) 6 to 28. The doses given were 0, 1, 10 and 100 mg/kg/day in suspension in aqueous solution of 0.5% methylcellulose. Stability of the compound in suspension in the vehicle was determined before the start of the study. All concentrations were checked for all formulations.The time-mated females were allocated to groups (25 per group), the day of mating being day 0 of gestation. The volume of administration was 4 ml/kg based on the most recent body weight recorded. Maternal body weights were recorded for all the females on gestation day 0, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Food consumption was measured for all females on gestation day 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 24, 26, 28 and 29. Clinical observations were recorded daily.

On gestation day 29, surviving females were sacrificed for examination of uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the liver of each pregnant female was weighed.

The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantation sites.

Number and localisation of resorption sites (classified as early and late), number of live and dead fetuses, individual weights of live fetuses: Dead fetuses were defined as fetuses showing distinct digits visible on fore and hind-paws. All the live fetuses were and subjected to an external examination. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulphide to visualise any sites which were not apparent. Tissues and carcasses were discarded. After visceral examination of the fetuses, the head of fetuses from approximately half of each litter was be immersed in Bouin fluid and the internal structures examined after fixation. The bodies of all fetuses were dissected for soft tissue anormalies and gender determined. Then the fetuses were fixed in absolute ethanol before staining. A modification of the Staples and Schnell

staining technique was used and a subsequent skeletal examination was performed.

Findings:

Maternal observations

Clinical signs: At 100 mg/kg/day, five pregnant females died prematurely between gestation day 15 and 22. Few or no faeces were noted on one or more occasion on the days prior to death, for all five animals. Treatment-related autopsy findings consisted of dark liquid present in the uterine horns or dark contents in the intestines or pale liver in these animals.

At 10 mg/kg/day, one female aborted on gestation day 23 and was subsequently necropsied. Clinical signs consisted of few or no faeces on several occasions.

At 1 mg/kg/day, one female was sacrificed on gestation day 21 for humane reasons. Clinical signs comprised of few or no faeces on several occasions and no urine on gestation day 19.

In addition in the 100 and 10 mg/kg bw/day group, treatment-related signs consisted of no faeces or few faeces. No other treatment-related signs occurred at 1 mg/kg/day.

The pregnancy rates (percentage of pregnant females per group) were comparable in the treated and control groups.

At 100 mg/kg/day, mean maternal body weight change was statistically significantly reduced in comparison with control values from gestation day 6 to 14. For the interval gestation day 6-18, the body weight change was lower (-43%) than control. At 10 and 1 mg/kg/day, body weight change was unaffected by treatment.

At 100 mg/kg/day, mean food consumption was statistically significantly reduced from gestation day 6 to 14, the reduction being more pronounced from gestation day 6 to 8, (-37 to -38%). From gestation day 14 to 18, a slight reduction in food consumption was also noted but was not statistically significant when compared to control value. At 10 mg/kg/day, food consumption from gestation day 6 to 8, was statistically significantly lower (-17%) than the control value. At 1 mg/kg/day, food consumption was unaffected by treatment.

Table 19: Maternal toxicity

Dose (mg/kg/day)	**0**	**1**	**10**	**100**
Mortality	0	1	1	5
Bodyweight (Day 14, kg)	3.64	3.68	3.67	3.55
(Day 28, kg)	3.87	3.92	3.93	3.89
Bodyweight gain (Days 6–29, kg)	0.30	0.31	0.32	0.28
Gravid uterine weight (g)	465.41	490.16	478.28	471.36
Food consumption (Day 6–8, g/kg/day)	47.7	47.8	39.4*	29.8**
Pregnancy rate (%)	96	100	100	100

Fetal examinations

See chapter 4.11 (Toxicity for reproduction)

Conclusion:

In this study, clear maternal toxicity (mortality, clinical signs) was evident at 100 mg/kg bw/d, and less marked also at 10 mg/kg bw/d. The dose level of 1 mg/kg bw/d is considered a NOAEL for maternal toxicity when administered by oral gavage to the pregnant rabbit. Due to the severity of

these effects (morality), labelling with **'R48/22'** is proposed.

4.7.1.7 Summary and discussion of repeated dose toxicity

A series of studies was carried out to investigate the effects following repeated exposure of orally administered tembotrione in mice (one 28-day and one 90-day study), rats (two 90-day studies) and dogs (one 90-day and one 1-year study). The most sensitive species was the rat (relevant oral NOAEL = 0.07 mg/kg bw/d derived from 90-day rat study), followed by the dog (relevant oral NOAEL = 9 mg/kg bw/d derived from 1-year dog study), whereas the mouse was the least sensitive species (relevant oral NOAEL = 64 mg/kg bw/d derived from 90-day mouse study).

In mice, the liver was the target organ (changes in clinico-chemical parameters, significantly increased liver weights and histological alterations). In the rat, major target organs were eyes (corneal opacities and neovascularisation of the cornea) and liver (increased liver weights and diffuse centrilobular hepatocellular hypertrophy). Furthermore, increased plasma cholesterol levels and increased urinary ketone levels were observed.
In dogs, major targets were eyes (corneal opacities), blood (decreased mean corpuscular volume and mean corpuscular haemoglobin, hypochromonia, microcytosis, anisochromia, anicytosis), liver (increased liver weights and histopathological changes) and peripheral nerves (increased number of digestion chambers).

Two 28-day dermal toxicity studies were performed in the rat. The major targets were blood (changes in haematological and clinical chemical parameters), liver (increased relative liver weight and histopathological findings), pancreas (slight to moderate degenerative changes/increase of apoptotic bodies of the exocrine acinar tissue accompanied by inflammatory infiltrates) and thyroid (colloidal alteration and follicular cell hypertrophy). No NOAEL for repeated dermal toxicity could be set in either of the two studies. The lowest dose tested (50 mg/kg bw/d) was considered to be a LOAEL.

A series of mechanistic studies has been conducted investigating the effects on and of tyrosinaemia (see chapter 4.12.1.3 Specific investigations: other studies). The main effect of systemic exposure to tembotrione in mammals is inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPDase), which is involved in the metabolism of the amino acid tyrosine. Prolonged inhibition of this enzyme results in increased plasma tyrosine levels (tyrosinaemia). The species-specificity towards the susceptibility to tyrosinaemia is linked to the different abilities of the species to metabolize tyrosine via a substitute enzymatic pathway, when the enzyme HPPDase is blocked. It was shown that human and murine cells are able to use an alternative pathway for the tyrosine catabolism when HPPDase is inhibited, which on the contrary is far less efficient in rabbits, dogs and rats. Therefore, typical tyrosine-related lesions on the eye, pancreas and thyroid as observed in rat studies are considered not to be relevant for human risk assessment. The findings of the repeated dose toxicity studies do not trigger any classification and labelling.

On the other hand, in a <u>developmental toxicity study in rabbits</u> tembotrione produced severe maternal toxicity and mortality at the dose level of 100 mg/kg bw/d: Five out of 25 pregnant females died prematurely between gestation day 15 and 22. Due to the severity of these effects, classification and labelling with **R48/22** was considered justified by the experts at the PRAPeR expert meeting 69 (4-8 May 2009).

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

In a developmental toxicity study in rabbits tembotrione produced severe maternal toxicity and mortality at the dose level of 100 mg/kg bw/d: Five out of 25 pregnant females died prematurely between gestation day 15 and 22.

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

Tembotrione requires classification with **R48/22 "Harmful: danger of serious damage to health by prolonged exposure if swallowed"** based on the finding of mortality in pregnant rabbits at the dose level of 100 mg/kg bw/d.

Criteria as specified in the Directive 67/548/EEC: "Evidence indicating that R48 should be applied: a) substance related deaths […]". Substances should be classified "when these effects are observed at levels of the order of oral, rat ≤ 50 mg/kg (bodyweight)/day […] These guide values can apply directly when severe lesions have been observed in a subchronic (90 days) toxicity test. When interpreting the results of a subacute (28 days) toxicity test these figures should be increased approximately threefold."

In the developmental toxicity study in rabbits, dosing started at gestation day 6 and mortality occurred between gestation day 15 and 22. Therefore, according to Directive 67/548/EEC, the dose level of 100 mg/kg bw/d is well within the guide range indicating that R48/22 should be applied.

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

Directive 67/548/EEC: Xn; R48/22

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

For description of findings see chapter *4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD.*

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

Tembotrione requires classification with **H373 "May cause damage to organs through prolonged or repeated exposure (if swallowed)"** based on the findings of mortality in pregnant rabbits at the dose level of 100 mg/kg bw/d.

Criteria as specified in the Regulation (EC) No. 1272/2008: For the oral route the guidance values to assist in Category 2 classification are 10 < dose ≤ 100 mg/kg bodyweight/day. These guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats.

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

Regulation (EC) No. 1272/2008: STOT RE 2, H373

RAC evaluation of specific target organ toxicity (CLP) – repeated exposure (STOT RE) and repeated dose toxicity (DSD)

Summary of the Dossier submitter's proposal

The DS based the evaluation of STOT RE on 9 studies, two in mice, four in rats, two in dogs, and one in rabbits.

In rats, specific ocular lesions were observed at very low doses (LOAEL 0.8-1.0 mg/kg/day in a 2 year oral study). However, mechanistic studies have shown this effect to be caused by a build-up of tyrosine (tyrosinaemia) following inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPD) by tembotrione. Thus, the tyrosine concentration increases *in vivo* in rats at low exposure to tembotrione. *In vitro* studies have shown tyrosine to build up in rat hepatocytes exposed to tembotrione whereas human (and mouse) hepatocytes can still metabolise tyrosine via alternative pathways. No ocular lesions are seen in mice. Experimental studies in rats have also shown that tyrosine exposure produces the same ocular lesions as tembotrione. Overall, the dossier submitter concluded that the tyrosinaemia-mechanism of action for tyrosinamea seen in rats is not relevant for humans, and that no classification for tembotrione based on the ocular lesions is warranted. Other organs, such as the liver, pancreas, and thyroid are affected at higher exposure levels in these studies, but these effects are also suggested by the DS to be related to the tyrosinaemia.

There is only one study in rabbits included in the CLH report, a developmental toxicity study with tembotrione administered by gavage during gestation days 6-28. At a dose of 100 mg/kg/day, 5 out of 25 dams died during gestation days 15-22, indicating severe toxicity at this dose. Although tyrosinaemia also occurs in rabbits, this mechanism was not thought to be involved in the deaths of the dams. Considering the short treatment period in a developmental toxicity study (23 days), the DS compared the effect level with the guidance values for 28-days studies, which state that effects seen below 300 mg/kg/day but above 30 mg/kg/day warrant classification in STOT RE category 2. The dossier submitter therefore proposed classification with STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure (if swallowed)) and Xn; R48/22 according to DSD.

Comments received during public consultation

Four comments were received, all from Member States. Three agreed with the proposal. One MS questioned the proposed classification, suggesting that further consideration should be given to the other repeated dose toxicity studies not fulfilling the classification criteria.

Assessment and comparison with the classification criteria

The CLH report describes in total 16 studies with repeated dose exposure, when including studies on carcinogenicity, neurotoxicity and reproductive toxicity. RAC has evaluated all these studies with respect to relevance for STOT RE and repeated dose toxicity. In the repeated dose toxicity studies, the triketone herbicide tembotrione has caused effects on the eyes in rats and dogs, liver (all species), pancreas (rats), the haematological system (dogs and mice), peripheral nerves (rats and dogs), and the kidney (rats).

The ocular toxicity occurred at doses relevant for classification (potentially STOT RE 1). However, mechanistic studies have been provided to suggest that the mode of action for the eye toxicity may not be relevant for humans. This mode of action builds on the observation that tembotrione is a specific inhibitor of 4-hydroxy-phenylpyruvate dioxygenase, leading to an accumulation of tyrosine, which subsequently causes eye toxicity unless it is catabolised by other metabolic pathways. The CLH report does not provide data showing the direct inhibitory

effect of tembotrione on HPPD (metabolising tyrosine) from different species, but refers to an in vitro study showing "more" degradation of tyrosine in human hepatocytes than in rat hepatocytes. Increased levels of tyrosine in rats after exposure to tembotrione are also documented.

The RAC has reviewed the literature (see in depth analyses of repeated dose toxicity), and is of the opinion that tyrosinaemia is a relevant mode of action (MoA) in humans. The triketone analogue NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) is used as a pharmaceutical drug to inhibit HPPD, and the potency of tembotrione in humans might not be that much lower than the potency of NTBC. As NTBC has been shown to greatly increase tyrosine concentrations in healthy adult volunteers treated with a single dose of 1 mg/kg/day NTBC (Lock et al, 2001), and to cause eye problems in some children treated with 1 mg/kg/day NTBC against tyrosinaemia type 1, tembotrione can be expected to have an intrinsic possibility to also cause similar problems in humans. Concerning human sensitivity in relation to the animal data, this might be intermediate to that of the very sensitive rat and the non-sensitive mouse. The RAC therefore considers the findings in the rat studies relevant to humans, but with some reservation because of the expected lower sensitivity of humans than of rats.

In rats, tembotrione-induced tyrosinaemia affected the eyes, the pancreas and the liver, with the eye as the primary target organ (NOAEL 0.04-0.1 mg/kg/day). Corneal opacities, neovascularisation and oedema of the cornea, snow flake-like corneal opacities, and keratitis were observed at doses of > about 1 mg/kg/day in a 2 year study. Some of the effects were reversible whereas others (e.g., neovascularisation) appeared irreversible. Hepatic effects were generally rather mild, but fibrosis was noted at doses of > 1 mg/kg/day. The acinar atrophy/fibrosis of the pancreas (no further data given) observed at doses of $\geq$8 mg/kg/day in the rat 2 year study (and also observed in 28 days dermal rat study), could potentially warrant classification. The guidance values for STOT RE 2 based on a 2-year study are between 1.2 and 12.5 mg/kg/day, and the ocular effects are sufficiently severe and relevant for humans to qualify for a STOT RE classification. As to the category, an effect level of about 1 mg/kg/day is obviously a borderline case, but considering that rats are likely to be more sensitive than humans, RAC considers classification in STOT RE 2 appropriate.

The haematological effects only occur in dogs at doses above the guidance values for classification. Haematological effects are also observed in the 80 weeks mouse study, at doses $\geq$ 4 mg/kg/day, potentially warranting classification. However, no information on the magnitude of these effects is given in the CLH report, and RAC can therefore not assess whether classification for haematological effects is needed.

Neurotoxicity was observed in the 90 days dog study, as determined by clinical signs and histopathological investigation of nerves, but only at and above 120 mg/kg/day, which is above the guidance values for classification. Histopathological lesions in the sciatic nerves were observed at 134 mg/kg/day in the 2 year rat study. Some clinical signs of neurotoxicity were also observed in an acute neurotoxicity study in rats at $\geq$500 mg/kg/day, but not in a 90 days neurotoxicity study in rats (top dose 160/224 mg/kg/day). A developmental neurotoxicity study in rats, with exposure from gestation day 6 until day 21 was also performed. The study is poorly reported in the CLH report. In addition to a decreased growth rate of the pups (magnitude not given) the only finding reported was a decreased acoustic startle response in the pups at 16 and 118 mg/kg/day. No information on dose-response or magnitude of effects is given. Based on the information available to RAC, no classification for neurotoxicity is warranted.

No kidney effects were reported in the studies described in the repeated dose toxicity section of the CLH report (in rats, mice, rabbit or dogs). However, in male rats of the 2 years study, an increased relative kidney weight (see table B.6.5.1-32 below under 'In depth analysis of

RAC') and histopathological findings were observed at doses ≥ 0.8 mg/kg/day (next higher doses were 8.3 and 31.7 mg/kg/day). The effects were characterised as chronic nephropathy, including tubular cell regeneration, thickened basement membranes, interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralisation, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium. The combined incidences of moderate to severe (sometimes lethal) nephropathy were 4/60, 11/60, 21/60, 23/60 and 19/60 at 0. 0.8, 8.3, and 31.7 mg/kg/da, respectively (see below 'In depth analysis of RAC'). The effects are sufficiently adverse to warrant classification, but human relevance of this chronic nephropathy has been questioned because of this effect possibly being a specific effect of old male rats. However, RAC notes that chronic nephropathy was also observed in the females, although tembotrione did not aggravate the symptoms in the females.

Although no effects on kidneys were reported in the 28 and 90 days studies in rats, increased relative kidney weights were noted in P0 and F1 males of the 2-generation study in rats. The weights were dose-dependently and statistically significantly increased, exceeding the historical control data from the lowest dose level (1.1-3.3 mg/kg/day). The incidence of dilated renal pelvis was dose-dependently increased in both male (2/30, 4/29, 15/30, and 16/30) and female F1 animals (1/30, 2/29, 4/30, and 17/30), at 0, 20, 200 and 1500 ppm, respectively with the incidence exceeding the historic control data as from the mid dose (13-31 mg/kg/day).

The CLH report provides two arguments why the kidney effects should not be considered; that they are normal findings in ageing rats, and that they are caused by an α2μ-globulin mechanism that is considered specific to male rats and of no relevance for humans. The increased kidney weights in the 2-generation study, where the males did not get very old, may indicate that this effect also can occur in younger animals. When discussing the 2-generation study, the CLH report claims that 86 and 413 mg/kg/day of tembotrione in the 90 days rat study "provoked an accumulation of hyaline droplets in the kidneys without degenerative changes in the tubules which were considered to represent an accumulation of α2μ-globulin". However, no such data are described in the reporting of the 90 days study in the CLH report.

The RAC notes that the EFSA peer review of tembotrione (EFSA Journal, 2013) did not support the conclusion that the kidney effects are not relevant for humans, that the US EPA did not disregard the kidney effects (US EPA, 2007) and that the triketone analogue sulcotrione has recently been classified as STOT RE 2 based on kidney effects starting from a dose of 0.04 mg/kg/day and appearing towards the end of the 2 year rat study. The kidney effects reported for sulcotrione were kidney cysts, kidney enlargement, pelvis dilation, and at higher doses chronic progressive nephrosis, papillary necrosis and calcification. In addition, pelvis dilation and undefined nephropathy were noted in 2-generation studies on sulcotrione.

The RAC does not consider the α2μ-globulin MoA sufficiently well proven to disregard the kidney findings. The observations of pelvis dilation in females as well indicate that α2μ-globulin is not the only MoA, if at all involved. The comparison with sulcotrione provides further support for a class-effect of these HPPD-inhibiting herbicides on the kidney. Kidney effects such as chronic nephropathy, kidney weight increase and dilated renal pelvis are reported as from doses of 0.8 mg/kg/day. The guidance values for STOT RE 2 based on a 2 years study are between 1.25 and 12.5 mg/kg/day, and the kidney effects are sufficiently severe at doses below 12.5 mg/kg/day (see below under 'In depth analysis of RAC') to qualify for a STOT RE classification. As to the category, an effect level of 0.8 mg/kg/day is obviously a borderline case between RE 1 and RE 2, but considering that rats are likely to be more sensitive than humans, RAC considers classification in STOT RE 2 appropriate.

Data relevant to the STOT RE classification was also obtained from the developmental study which was performed in rabbits'. Out of 25 dams administered 100 mg/kg/day in a

developmental toxicity study, 5 dams died prematurely between gestation day 15 and 22. The effect is unexpected considering the short exposure time (10-17 days). However, mortality was also observed in males in the 90 days dog study at 250 mg/kg/day and in male rats in the 2 year study at 134 mg/kg/day. As no other studies are available in rabbits, it has to be assumed that the rabbit mortality can be explained by a very high sensitivity. Considering the limited effects on the rabbit pups (delayed ossification), there is no reason to believe that the mortality is specific for pregnant rabbits, but rather is a general effect of tembotrione on rabbits. For a short study (28 days), the guidance value is 30-300 mg/kg/day for STOT RE 2. The rabbit mortality is clearly severe and occurs at doses below the relevant guidance value, thus warranting classification with STOT RE 2 (H373).

RAC agreed with the DS proposal that tembotrione should be classified as STOT RE 2 based on mortality seen in rabbits. In addition, RAC concludes that eye, kidney and liver toxicity in rats also warrant classification as STOT RE 2, with the hazard statement; May cause damage to the eye, kidneys and liver trough prolonged or repeated exposure. As there are no repeated dose toxicity studies in any species by the dermal or inhalation route, we cannot exclude the possibility that the substance can exert toxicity by these routes (at least in sensitive rabbits). The RAC therefore considered that the route shouldnot be given in the hazard statement. The corresponding classification according to DSD would be Xn; R48/22.

Supplemental information - In depth analyses by RAC

Human relevance

The CLH report claims that tyrosinaemia is not relevant for humans. The EFSA conclusion on the tembotrione peer review states that tyrosinaemia is "of lower relevance to humans" (EFSA, 2013). The scientific committee on plants (SCP) has assessed the relevance of the tyrosinaemia-mechanism for humans in relation to the assessment of the analogue triketone herbicide mesotrione. SCP (2002) concluded that tyrosine concentrations in humans cannot reach high enough levels to induce ocular effects even after complete inhibition of hepatic HPPD.

As there are no human data on tembotrione, human relevance of the tembotrione experimental animal toxicity data cannot be assessed if only considering tembotrione data, and the default assumption would then be to assume human relevance. However, as the mode of action (MoA) for tembotrione is known, and there isconsiderable human experience of this MoA (tyrosinaemia caused by HPPD-inhibition), the RACconsidered the information available on tyrosinaemia for other substances as supportive when assessing human relevance of the tembotrione toxicity.

Thus, the RAC noted that the use of the HPPD-inhibitor NTBC in medicine (chronic administration of in the order of 1 mg/kg/day) has led to cases of eye symptoms in children treated with NTBC against tyrosinaemia type I (Lock et al 2006, Schauwvlieghe et al 2013), questioning the alleged low relevance of tyrosinaemia in humans. These children suffer from tyrosinaemia type I, where lack of the enzyme fumarylacetoacetic acid hydrolase leads to build up of toxic tyrosine metabolites. By treating with NTBC, less of the tyrosine metabolites are formed, but on the other hand, the plasma tyrosine concentration is increased simultaneously. These children may potentially have an increased sensitivity as compared to healthy individuals, but NTBC has been shown to greatly increase tyrosine concentrations also in healthy adult volunteers treated with a single dose of 1 mg/kg/day NTBC (Lock et al, 2001).

The RAC therefore evaluated the relevance of tyrosinaemia further. Factors that have been proposed in the literature to determine sensitivity to tyrosinaemia are;

- enzyme affinity and degree of HPPD-inhibition
- other pathways for tyrosine degradation/clearance (tyrosine aminotransferase)
- specific cellular accumulation of tyrosine

- half-life of the inhibitory substance

HPPD-inhibition

Lock and co-workers studied the sensitivity to tyrosinaemia in different species using the model substance NTBC (reviewed in Lock et al 2006). While NTBC will increased the plasma concentrations of tyrosine in rats, rabbits, mice, dogs and rhesus monkeys, only two of the five species suffered from ocular toxicity (rats and dogs), with rats being particularly sensitive. The mechanism of action is assumed to be inhibition of hepatic HPPD in mammals (inhibition of HPPD is the MoA also for the herbicidal activity), and complete inhibition of HPPD by a single oral dose of 10 mg/kg has been shown in rats, rabbits, dogs and mice. The sensitivity of HPPD to inhibition by NTBC seems somewhat higher in rats than in mice, but dose-response studies to assess this difference in sensitivity in the other species are lacking. In humans, a single dose of 1 mg/kg/day NTBC to healthy adult volunteers will inhibit HPPD such that tyrosine concentrations are increased from around 100 in controls to 1100-1200 nmol tyrosine/ml plasma in the treated volunteers. Although it contributes, the degree of HPPD-inhibition is clearly not the only factor in deciding whether a species is sensitive or not.

Tyrosine clearance

The high sensitivity of rats has been proposed to be caused by lower activities of tyrosine aminotransferase (TAT) in rats than in other species. TAT will assist in degrading tyrosine, but based on male hepatic TAT-activities noted in the literature (rat - 1.7 nmol 4-hydroxy phenylpyruvate formed/min/mg protein)(Lewis and Botham 2013); rat - 13.2 (Lock 1996); rat – 19 (Lock 2000); dog - 13.5 (Lock 2006); rabbit - 3.5 (Lock 2006); human – 7.2 (Lewis and Botham 2013), a crucial role of TAT in the protection against tyrosinaemia and ocular toxicity is not obvious. However, when Lock et al compared mouse and rat clearance (Vmax/Km for TAT) they found a 5-fold higher intrinsic clearance of tyrosine by TAT in mice than in rats (Lock et al 2000). The RAC is not aware of similar data for the other species, making it difficult to assess the importance of TAT in determining the sensitivity to tyrosine-induced toxicity.

Specific cellular accumulation

Lock et al (2006) suggested that other factors must be involved in determining the sensitivity, such as uptake mechanisms for tyrosine in specific organs (e.g., the eye). In rats and mice, the concentration of tyrosine is higher in the eye than in plasma, whereas the concentrations in dogs and rabbits are fairly similar in the eye and in plasma. Whether a specific ocular uptake mechanism is operating in humans is not known.

The half-life

The tembotrione analogue mesotrione, also a triketone herbicide with HPPD-inhibition as the MoA, has been compared with the model substance NTBC in human volunteers (Hall et al, 2001). Because of a very fast excretion of mesotrione in humans ($t_{1/2}$ = 1h) versus that for NTBC ($t_{1/2}$ = 54h), mesotrione was 400-fold less potent in inducing tyrosinaemia than NTBC. The human half-life of tembotrione is not known, so animal data has to be used for comparing tembotrione with mesotrione and NTBC. In rats, 4 studies have been conducted with tembotrione, using two different radiolabels, giving elimination half-lifes in the order of 40 h after a single dose of 5 mg/kg/day. For mesotrione, there is little metabolism in the rat and the substance is rapidly excreted (Gledhill et al 2001), just as in humans. Considering the slow excretion of tembotrione, the potency of tembotrione is more likely to be similar to that of NTBC than that of mesotrione.

Although the degree of tyrosinaemia clearly affects the sensitivity of a species, the full basis for different sensitivities to tyrosinaemia-induced toxicity is not known. Accordingly, in patients, there seem to be a lack of clear relationship between plasma tyrosine concentrations and eye symptoms (Lock et al 2006), indicating other factors contributing to individual differences in sensitivity in humans. Still, it has been suggested by US FDA that plasma tyrosine concentrations should be kept <500 nmol/ml to avoid adverse effects (Lewis and

Botham 2013).

It is also noted that the effects of tyrosinaemia may differ between species, with the rabbit insensitive to ocular toxicity (after 6 weeks exposure to 10 mg/kg/day NTBC) whereas lethal effects are observed instead (Lock et al 2006).

The MoA and relevance for humans were recently assessed for mesotrione. It was concluded that HPPD inhibition and the resulting tyrosinaemia is a plausible MoA in humans, but that kinetic and dynamic differences between rats and humans will ensure that adverse effects of mesotrione will not result from the intended use of that pesticide (Lewis and Botham, 2013).

Regarding tembotrione, the RAC is of the opinion that tyrosinaemia is a relevant MoA, and that the potency of tembotrione in humans might not be that much lower than the potency of the drug NTBC. As NTBC has been shown to elevate plasma tyrosine concentrations in healthy adult volunteers, and cause eye problems in children at relatively low dose levels, tembotrione can be expected to have an intrinsic possibility to also cause similar problems in humans. Concerning human sensitivity in relation to the animal data, the human sensitivity might be intermediate to that of the very sensitive rat and the non-sensitive mouse. The RAC therefore will consider rat data, but with some reservation for expected lower sensitivity of humans than of rats.

Kidney effects

In the male kidney, the incidence of minimal to severe chronic nephropathy was statistically significantly higher at 20, 200 and 800 ppm (equivalent to 0.8, 8.3, and 31.7 mg/kg/day) (Kennel, P.; 2005d). Changes within the kidney included one or more of the following changes: tubular cell regeneration, thickened basement membranes (glomerular and tubular), interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralization, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium. Severity grades moderate or higher generally reflected a kidney with most of the above-mentioned changes, some reflecting end-stage renal disease (probable cause of death). The combined incidences in males of moderate to severe nephropathy were 4/60, 11/60, 21/60, 23/60 and 19/60.

Minimal or slight severity grades generally reflected a kidney with only a few of the above-mentioned changes. The study authors considered this effect to be treatment-related, but also noted that chronic nephropathy is a very common lesion in the aging male rat that has been described to be rodent-specific. The total incidence (minimal-severe) just about exceeds the historical control incidences, but as noted above, only considering the more serious cases better illustrates the steep increase in incidence.

The following tables summarise some kidney effects as reported in the revised Draft Assessment Report of tembotrione (2009).

Table B.6.5.1-32: Kidney weight at terminal sacrifice (% change compared to controls)

Dose level (ppm)	Control	1	20	200	800
Mean absolute kidney weight (g)	3.91 -	4.09 (+5%)	4.51 * (+15%)	4.43 (+13%)	4.63 ** (+18%)
Mean kidney to body weight ratio (%)	0.616 -	0.653 (+6%)	0.766 ** (+24%)	0.804 ** (+31%)	0.778 ** (+26%)

** $p \leq 0.01$; * $p \leq 0.05$.

After the recovery phase mean kidney weights were higher by between 15 to 25% ($p < 0.01$) in animals previously treated.

Table B.6.5.1-35: Chronic nephropathy in the kidney (carcinogenicity phase)

Dose level (ppm)	0	1	20	200	800
Incidence	38/60	46/60 NS	52/60 p ≤ 0.01	55/60 p ≤ 0.01	50/60 p ≤ 0.05
% Incidence	63%	77%	87%	92%	83%
Severity					
Minimal	27	20	9	7	4
Slight	7	15	22	25	27
Moderate	2	7	12	8	8
Marked	1	2	5	10	8
Severe	1	2	4	5	3

NS: not statistically significant

Table B.6.5.1-36: Chronic nephropathy in the kidney (males)

Dose level (ppm)	0	1	20	200	800	HCD	
Kidney						Min	Max
Chronic progressive nephropathy (%)	38/60 (63.3)	46/60 (76.7)	52/60** (86.7)	55/60** (91.7)	50/60* (83.3)	27/60 (45.0)	44/55 (80.0)

= p <0.05, ** = p < 0.01

Table B.6.5.1-37: Chronic nephropathy in the kidney (females)

Dose level (ppm)	0	2	20	2500	5000	HCD	
Kidney						Min	Max
Chronic progressive nephropathy (%)	43/60 (71.7)	40/60 (66.0)	44/60 (73.7)	43/60 (71.7)	48/60 (80.0)	20/60 (33.3)	43/60 (71.7)

Data collected from eight carcinogenicity studies in Wistar Rj:WI (IOPS HAN) rat performed at BayerCropScience (Sophia Antipolis, France) between 2000 and 2004

Other kidney-related findings are increased kidney weights, dilated renal pelvis and hyaline droplets. One question is whether they are related or independent. The nephropathy is clearly age-related, whereas increased kidney weights (<22%) and dilated renal pelvis also are observed in young F1 animals of the 2-generation study. The kidney weight is only affected in males, whereas dilated renal pelvis is observed in both males and females.

The dilated renal pelvis was confirmed microscopically and was accompanied by tubular basophilia and chronic inflammation (no further info in DAR). The DAR states that similar but less prominent effects were observed in the females.

Nephropathy was observed in both sexes, but tembotrione only aggravated the effects in males. If only analysing the cases that were sufficiently severe to be the cause of death, the incidences increased from 4/60 in control males to 11/60, 21/60, 23/60, and 19/60 as the dose increased. Chronic nephropathy was also observed in females, but the incidence was not increased by tembotrione in females.

It cannot be ruled out that these kidney effects are unrelated, and it is therefore not possible to tie them to one mode of action (e.g., male rat specific hyaline droplet formation). The effects may therefore have human relevance, and classification is proposed.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
In vitro studies			
Reverse mutation assay (S.typhimurium TA 1535, TA 1537, TA 98, TA 100 and E. coli CM891)	0, 5, 15, 50, 150, 500, 1500, 5000 µg/plate dissolved in DMSO **Negative** (+/- S-9 mix)	-	May, K;, 2003
In vitro mammalian chromosome aberration test in human lymphocytes	Test 1: 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5 and 10 mM (for cytotoxicity testing) 0, 2,5, 5, 10 mM (for metaphase analysis) Test 2: 1.25, 2.5, 5 and 10 mM (for cytotoxicity testing) 0, 2,5, 5, 7.5 mM (for metaphase analysis) dissolved in DMSO **Positive at cytotoxic concentrations** (10 mM and 7.5 mM, respectively) (+/- S-9 mix)	-	Mason, C.; 2004
In vitro Chinese Hamster Ovary/HPRT locus gene mutation assay	0, 250, 500, 1000, 1200, 1400, 1500, 1600 µg/mL dissolved in DMSO **Negative** (+/- S-9 mix)	-	May, K.; 2005
In vivo studies			
Mouse micronucleus test (CD mice)	0, 500, 1000, 2000 mg/kg bw suspended in Methylcellulose; single oral treatment **Negative**	-	Mehmood, Z.; 2003
UDS assay in rat hepatocytes treated in vivo	0, 1000, 2000 mg/kg bw suspended in aqueous Cremophor; single oral treatment **Negative**	-	Wirnitzer, U.; 2005a

4.9.1 Non-human information

4.9.1.1 In vitro data

There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests. A positive response was observed in the human lymphocyte cultures for chromosomal aberration in vitro at cytotoxic concentrations.

4.9.1.2 In vivo data

Both in vivo test (mouse micronucleus test and in vivo UDS study in the rat) showed clear negative

results. Therefore it can be concluded that Tembotrione has no genotoxic potential.

4.9.2 Human information

Not available.

4.9.3 Other relevant information

Not available.

4.9.4 Summary and discussion of mutagenicity

Tembotrione was tested in a standard battery of genotoxicity and mutagenicity tests in vitro and in vivo. There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests. A positive response was observed in the human lymphocyte cultures for chromosomal aberration in vitro at cytotoxic concentrations. However, in both in vivo test (mouse micronucleus test and in vivo UDS study in the rat) clear negative results were obtained. It can be concluded that tembotrione has no genotoxic potential.

4.9.5 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 (according to both DSD and CLP).

4.9.6 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

4.10 Carcinogenicity

Table 21: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Rat (female) oral via diet, 104 weeks	0, 2, 20, 2500, 5000 ppm (equivalent to 0, 0.1, 1.05, 134 and 280 mg/kg bw/day) NOAEL = ♀ 0.1 mg/kg bw/d Effects/target organs: - body weight gain ↓ - changes in clinical chemistry (Cholesterol, triglycerides, glucose) - ophthalmological changes (corneal opacities, neovascularization, keratitis, epithelial hyperplasia, keratinization, epithelial vacuolization, erosion in cornea) - histological alterations in the liver (biliary hyperplasia/fibrosis, sinusoidal dilatation), sciatic nerve (atrophy, chronic inflammation, vascular mineralization) and pancreas (pancreatic acinar atrophy/fibrosis)	As the two high doses were not tolerated by males this study was only continued with females and a separate study was conducted for males	Kennel, P.; 2005c
Rat (male) oral via diet, 104 weeks	0, 1, 20, 200, 800 ppm (equivalent to 0, 0.04, 0.79, 8.3 and 31.7 mg/kg bw/day) NOAEL = ♂ 0.04 mg/kg bw/d Effects/target organs: - body weight gain ↓ - changes in clinical chemistry (cholesterol) - Ophthalmological changes: (corneal opacities, neovascularization, keratitis) - Squamous cell carcinomas in eyes at 200 and 800 ppm - Kidney and liver weights ↑ - histological alterations in kidneys (chronic nephropathy including tubular cell regeneration, thickened basement membranes [glomerular and tubular], interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralization, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium); thyroid (colloid alteration, pigmentation and cystic hyperplasia) and pancreas (acinar atrophy/fibrosis)	-	Kennel, P.; 2005d
Mouse oral via diet, 80 week	0, 30, 300, 1000, 3000 ppm (equivalent to 0, 4.3, 43, 146, 440 mg/kg bw/day in males and 0, 5.4, 54,179, 552 mg/kg bw/day in females) LOAEL = 4.3 mg/kg bw/d Effects/target organs: - body weight ↓ - changes in hematological parameters (↓ RBC,	-	Langrand-Lerche C.; 2005

	hematocrit, and haemoglobin)- - absolute and relative liver weight ↑ accompanied by histological alterations (hypertrophy, increased number of mitoses, hepatocellular degeneration and eosinophilic inclusion bodies) - Gallstones (often multiple, pinpoint and brown colored) at all dose levels with increased incidence		

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

The long-term toxicity and oncogenic potential of tembotrione was assessed in both, the mouse and rat.

Rat

In the "first" rat study tembotrione was administered via the diet at dose levels of 0, 2, 20, 2500 and 5000 ppm. The two highest dose levels were not tolerated by male animals showing hemorrhagic symptomatology before death, therefore the study was discontinued for the males (a separate combined chronic and carcinogenicity study with tembotrione was conducted in the male rat). In females there was no treatment-related mortality at any dose levels. In female rats the eyes were considered as main target organs (at 20 ppm and above) which were classified in ophthalmological examinations as corneal opacities, neovascularization of the cornea and snow flake-like corneal opacities. Snow flake-like corneal opacities were reversible, whereas other corneal opacities, neovascularization of the cornea and oedema of the cornea were still observed after the recovery period. In addition, non-neoplastic keratitis at 20 ppm or above was observed either unilaterally or bilaterally, and included one or more of the following changes in the cornea: acute inflammation, epithelial hyperplasia, keratinization, epithelial vacuolization and erosion.

The second target organ was the liver: hyperplasia and fibrosis as well as sinusoidal dilatation were found at concentrations of 20 ppm and above, and were associated with biliary hyperplasia/fibrosis. Lesions in the sciatic nerve (atrophy, chronic inflammation and vascular mineralization), and pancreatic acinar atrophy/fibrosis were found significant at concentrations of 2500 ppm and 5000 ppm. There was no indication of any tumorigenic effect of tembotrione in female rats. The NOAEL for the female Wistar rat was 2 ppm (equivalent to 0.10 mg/kg bw/day).

In the "second" rat study, male rats received continuous dietary treatment at 0, 1, 20, 200 and 800 ppm over 2 years. Over the whole study period, the mortality incidence was comparable between the treated and control groups. Also in male rats, ophthalmological changes were found at 20 ppm and at higher dose levels: corneal opacities, neovascularization and oedema of the cornea, and snow flake-like corneal opacities, which increased during the study period. Corneal opacities, oedema of the cornea and snow flake-like corneal opacities were completely reversible, whereas neovascularization of the cornea persisted throughout the 13 weeks of recovery. In addition, keratitis, epithelial hyperplasia, keratinization, epithelial vacuolization, erosion and/or ulceration were observed in dose groups receiving 20 ppm and above. Treatment-related neoplastic changes of the eye (squamous cell carcinoma) were confined to the dose levels of 200 and 800 ppm. The

incidence of neoplastic lesions of the cornea at 200 and 800 ppm was not statistically significant and showed no dose response. These lesions were considered by the study authors to be a result of the keratitis (indirect effect of the corneal inflammation by a non genotoxic mechanism) and not to be toxicologically relevant for human risk assessment.

Table 22: Corneal squamous cell carcinoma (carcinogenicity phase)

Dose levels	0	1	20	200	800
Incidence					
All animals	0/60	0/60	0/60	4/60 NS	2/60 NS

NS: not statistically significant

Further treatment-related effects were seen in kidneys, liver, pancreas and thyroid. Mean kidney weights were significantly higher at 20 ppm and above. This was accompanied by increased incidences of minimal to severe chronic nephropathy with tubular cell regeneration, thickened basement membranes (glomerular and tubular), interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralization, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium. Mean liver weights were statistically significantly increased by 15 to 34% at 20, 200 and 800 ppm, but there was no evidence of histological alterations in the organ. In addition, in the thyroid histological alterations were observed (colloid alterations, pigmentation cystic hyperplasia). In the pancreas, acinar atrophy/fibrosis was observed at 200 and 800 ppm.

A detailed position paper (Semino G, 2006) was submitted by the notifier discussing the findings observed at 20 ppm and providing arguments, why the dose level of 20 ppm can be considered as the NOAEL relevant for human risk assessment:

- Corneal opacity in both sexes, slight increased liver weight, and thyroid colloid alterations are caused by accumulation of tyrosine, a rat-specific mechanism, and are thus endpoints not relevant for human risk assessment.
- Other observations (such as biliary hyperplasia in the females, chronic progressive nephropathy in the males, accumulation of lipofuscin pigment in the thyroid) are spontaneous non-neoplastic changes normally observed in aged rats are therefore also only of limited importance for human risk assessment.The hyaline droplets observed after subchronic administration at high doses consist most likely of α2µ globulin and are thus a species specific effets for male rats.
- Finally, the decrease in body weight gain was not dose-related and not observed throughout the dosing period (body weight was lower only during the last months of the study and the decreased observed at 20 ppm was higher than that occurring at the top dose of 800 ppm).

At the PRAPeR expert meeting 69 (4-8 May 2009), the majority of experts did not support this opinion. Except for the corneal lesions, the effects observed (liver weight and related histopathology and decreased adrenal weight in females, and increased incidence and severity of chronic nephropathy and thyroid alterations in males) were considered relevant for human risk assessment. Furthermore it was noted that blood tyrosine levels have never been measured in the experimental animals at the relevant low dose of 20 ppm. The NOAEL in the male Wistar rat was set at 1 ppm (equivalent to 0.04 mg/kg bw/day). A further study was therefore submitted by the notifier to show that low levels of tembotrione effect blood tyrosin levels, predominantly in male rats. (see 4.12.1.3 *Specific investigations: other studies*)

Mouse

The oncogenic potential of tembotrione was assessed in the mouse following continuous dietary treatment for 18 months. Mice received diets containing 0, 30, 300, 1000 or 3000 ppm of tembotrione. Main target organs were blood, liver and the gallbladder. At dose levels of 30 ppm and above, decreased RBC, decreased haematocrit, and decreased haemoglobin levels were found in females. Liver weight parameters were statistically significantly increased in all treatment groups and both sexes. This was associated with gallbladder stones (often multiple, pinpoint, brown colored) and further microscopic alterations in liver (centrilobular to panlobular hepatocellular hypertrophy, increased number of mitoses, focal/multifocal hepatocellular degeneration, focal/multifocal eosinophilic inclusion bodies) and gallbladder (multifocal/diffuse epithelial hyperplasia, focal/multifocal eosinophilic cytoplasmic alteration, focal/multifocal subepithelial mixed cell infiltrate). There was no indication of treatment-related neoplastic changes at any dose level. The lowest concentration tested 30 ppm (corresponding to 4.3 mg/kg bw/day in males and 5.4 mg/kg bw/day in females) was considered as LOAEL.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

Not available.

4.10.3 Other relevant information

See mechanistic study in chapter *4.12.1.3 Specific investigations: other studies*.

4.10.4 Summary and discussion of carcinogenicity

There was no indication of any tumorigenic effect of tembotrione in either mouse or rat with exception of neoplastic changes of the eye (squamous cell carcinoma) observed in male rats at the dose levels of 200 and 800 ppm (equivalent to 8.3 and 31.7 mg/kg bw/d). The incidence of neoplastic lesions of the cornea at 200 and 800 ppm was not statistically significant and showed no dose response. These lesions were considered by the study authors to be a result of the keratitis (indirect effect of the corneal inflammation by a non genotoxic mechanism) due to rat specific tyrosinaemia and not to be toxicologically relevant for human risk assessment.

The issue of eye tumours was discussed extensively at the PRAPeR expert meeting 69 (4-8 May 2009). Corneal squamous cell carcinomas are very rare tumours and there is no historical control data available. The mechanism is unknown but it is likely to be related to prolonged irritation of the cornea which might be a species specific finding. There is no dose relationship and similar tumours were not found for the comparable compound sulcotrione at doses up to 550 mg/kg bw/d. It was agreed that the RMS Austria should highlight this issue to ECHA.

The RMS is of the opinion that eye tumours observed in male rats are due to eye irritation caused by rat specific tyrosinaemia and are not of relevance (at the dose levels tested) to humans. Therefore no classification and labelling is proposed for carcinogenicity.

4.10.5 Comparison with criteria

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

4.10.6 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

4.11 Toxicity for reproduction

Table 23: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Two-generation study in rats	0, 20, 200 and 1500 ppm (equivalent to 0, 1.3 – 3.3, 13.1 – 31.0 and 98.2 – 228.1 mg/kg bw/d) Parents: LOAEL = 1.3 – 3.3 mg/kg bw/d based on: - ↑ eye lesions - ↓ body weight gain - ↑ liver weight Offspring: LOAEL = 1.3 – 3.3 mg/kg bw/d based on: - ↑ eye lesions - ↓ absolute brain weight - ↑ liver weight - spleen: decreased weight and increased incidence of decreased extramedullary haematopoiesis Reproduction: NOAEL = 98.2 – 228.1 mg/kg bw/d (highest dose tested)	-	*Young, A.D; Fickbohm, B.L.; 2005*
Developmental toxicity study in rats	0, 25, 125, 500 mg/kg bw/d Maternal: LOAEL = 25 mg/kg bw/d based on: - ↓ body weight Fetus: LOAEL = 25 mg/kg bw/d based on: - delayed ossification - increased incidence of variations and anomalies - runts	-	*Wason, S.; 2003a*
Developmental toxicity study in rabbits	0, 1, 10 and 100 mg/kg bw/d Maternal: NOAEL = 1 mg/kg bw/d Effects observed: - mortality (at 100 mg/kg bw/d) - clinical signs Fetus: NOAEL = 1 mg/kg bw/d Effects observed: - delayed ossification - increased incidence of variations and anomalies - dilated cerebral ventricles (at 100 mg/kg bw/d)	-	*Wason, S.; 2003b*

4.11.1 Effects on fertility

4.11.1.1 Non-human information

4.11.2 Two generation reproductive toxicity in the rat (OECD Annex IIA 5.6.1)

Reference:	**Technical Grade AE 0172747: A Two-Generation Reproductive Toxicity Study in the Wistar Rat**
Author(s), year:	Young, A.D; Fickbohm, B.L.; 2005
Report/Doc. number:	201266; M-259850-01-1
Guideline(s):	OECD 416
GLP:	Yes
Deviations:	-
Validity:	Yes

MATERIAL AND METHODS:

Test Material: AE 0172747

Lot/Batch: PFI 0195 (OP2250027)

Purity: 94.0%

Test animals:

Species: Rat

Strain: Wistar Hanover rats (Crl:WI[GLX/BR/HAN]IGS BR)

Age: 6 weeks old

Weight at start: males, mean value range: 276-282 g

females, mean value range: 186.5-188 g

Source: Charles River Laboratories Inc., Raleigh, NC (USA)

Animal assignment and treatment

Four groups of 30 male and 30 female rats each were given 0, 20, 200, and 1500 ppm of AE 0172747 in the diet seven days/week throughout the entire study.

These rats were designated as P-generation. After 10 weeks, animals were mated, females were allowed to litter, and wean their offspring. The offspring were designated as F1 generation.

After weaning and treatment, 30 male and 30 female rats from each F1 group were selected for mating to produce the F2 generation.

The juvenile phase of the F2 generation was evaluated by developmental landmarks, haematological examinations and histopathological investiation of the spleen. The study was complete after the F2 pups had completed post-weaning phase.

Diet preparation and analysis: The concentration of AE 0172747 in the various test diets was determined using liquid chromatography. The homogeneity and stability of the test substance in feed were previously verified at concentrations of 250 and 2500 ppm. The homogeneity and stability for the 20 ppm level in this study were verified concurrently during the study. Prior to the start of the experiment, during and then at the conclusion of the in-life phase of the study, the concentration/stability of the test batch, was determined under storage conditions at the test facility.

Table 24: Corresponding substance intake

Phase of the study	20 ppm in mg/kg bw/day	200 ppm in mg/kg bw/day	1500 ppm in mg/kg bw/day
Males			
Pre-mating (P-gen)	1.4	13.1	98.2
Pre-mating (F1-gen)	1.3	13.5	102.5
Females			
Pre-mating (P-gen)	1.6	15.4	115.4
Pre-mating (F1-gen)	1.6	16.2	123.1
Gestation (P-gen)	1.6	15.9	120.7
Gestation (F1-gen)	1.5	14.8	110.7
Lactation (P-gen)	3.3	31.0	227.2
Lactation (F1-gen)	3.2	30.7	228.1

Clinical observations: Animals were observed for clinical signs daily. Mortality, moribundity, behavioural changes, signs of difficult or prolonged delivery, and overt toxicity were also investigated. A detailed evaluation of clinical signs, and a physical examination was conducted once per week. The anogenital distance of the F2 pups was measured.

Body weight: Parental animals (P and F1) body weights were recorded weekly for both males and females during the premating period. During the mating period and until sacrificed, body weights for the males were recorded at least once per week.

Food consumption was recorded once per week.

Oestrus cycle evaluation: The oestrus cycle was determined by examining daily vaginal smears over a three-week period prior to mating of the P and F1 generation, immediately prior to the cohabitation period.

Sperm analysis: Sperm was collected from left testis and left epididymis for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively at sacrifice in all P- and F1-animals. In addition, an evaluation of the morphology and motility was performed on sperm sampled from the distal portion of the control and high dose groups. Sperm motility and counts were conducted using the Integrated Visual Operating System (IVOS, Hamilton-Thorne

Research, 1998).

Mating: Parental animals (P and F1) were mated by co-housing of one female with one male for up to 14 consecutive days. During the mating phase, vaginal smears were taken each morning and examined for the presence of sperm and/or an internal vaginal plug. Females found to be inseminated (designated day 0 of gestation) were separated.

Parturition and lactation: On gestation day 21, each P or F1 female was examined twice per day for signs of parturition or dystocia. The number of live and stillborn pups (both F1 and F2 generations) was recorded for each litter. Pups were examined, weighed and individually identified by tattoo of the paws. Dead pups were necropsied. Post-partuition mortalities and stillbirths were distinguished.

Offsprings: All pups were counted daily to assess mortality from birth to weaning. Pup body weights were recorded on lactation days 0, 4, 7, 14 and 21. A detailed clinical observation and physical examination was performed on the day the pups were weighed. The size of each litter was standardised to 8 pups, (4 males and 4 females, if possible) on lactation day 4.

Both F1 and F2 pups were observed daily for clinical signs from birth until the start of the premating phase (F1 pups) and until weaning (F2 pups).

The age of vaginal opening and preputial separation was determined for F1 weanlings selected for mating. Based on the delay in preputial separation and vaginal opening observed in the first generation, the anogenital distance was measured in all F2 pups on lactation day 0.

On lactation day 21, one-half of the F2 pups of each sex were kept to evaluate vaginal opening and preputial separation. After sacrifice haematological examinations were performed selected organs weighed and tissues preserved for histopathology.

Gross Necropsy

a) Females: Following the weaning of litters (lactation day 21) each dam (both P and F1 generations) was sacrificed and a gross examination was performed. The uterus was excised and implantation sites counted.

The following tissues were also collected for weight determination and gross examination: eyes, brain, pituitary, liver, kidneys, spleen, thyroid, thymus, adrenals, epididymis, oviduct, uterus with cervix, vagina, as well as ovaries.

A gross necropsy was performed on animals of pre-mature deaths (dams and offspring) as well as inseminated females that did not deliver. These examinations included terminal body weight, oestrus cycle, organ weights and organ preservation.

b) Males: The epididymides (total weight for both, and cauda weight for the side not being utilised for sperm analysis), seminal vesicles (with coagulating glands and their fluids), testes, prostate, uterus (with oviducts and cervix), brain, pituitary, thymus, liver, kidneys, adrenal glands, thyroids, and spleen were removed, weighed, and fixed for gross investigation.

c) Offspring: Culled pups were sacrificed, grossly abnormal pups underwent a gross examination. Remaining offspring of the F1 (not selected for mating) and F2 generation were sacrificed and examined macroscopically for any structural abnormalities or pathological changes, particularly the reproductive system. From one pup/sex/litter from each generation the following reproductive tissues were fixed for a microscopic examination: uterus, ovaries, vagina, cervix, oviduct, testes, epididymides, prostate, coagulating gland, and seminal vesicles. Pups found dead or terminated pre-maturely were examined for possible defects and/or cause of death.

Selected animals of the F1 and F2 generation were sacrificed on day 21 and brain, spleen, thymus, and uterus were weighed. The remaining half of the F2-pups were sacrificed and examined macroscopically for any structural abnormalities or pathological changes, particularly the spleen.

Histopathology

a) Adult Histology: The following tissues from adult animals (P and F1 generations) were examined microscopically: cervix, epididymis (caput, corpus, and cauda), ovaries, prostate, testis, seminal vesicles/coagulating gland, uterus, oviducts, vagina, adrenal glands, liver, kidneys, pituitary, spleen and gross lesions. Reproductive organs were examined in animals with deficits in fertility (those who failed to mate, to conceive, to sire, or to deliver healthy offspring), or affected sperm number, motility, or morphology. Histopathological findings were attributed to treatment in eyes in both generations and the kidneys in the F1 generation, therefore no other tissues were evaluated in the low or mid-dose groups.

b) Offspring: The following tissues from one pup/sex/litter (F1 and F2 generations) were examined microscopically: uterus, ovaries, vagina, cervix, oviduct, testes, epididymides, prostate, coagulating gland, and seminal vesicles. Spleens were investigated of all animals scheduled sacrificed on day 21.

FINDINGS:

General observations

P generation

There were no deaths or animals sacrificed in a moribund condition due to treatment with AE 0172747.

At 1500 ppm body weight of males in the P generation was statistically significantly less than in controls beginning on study day 28 continuing until day 98 with a mean reduction of 6.7%. Body weight gain was also significantly reduced in this dose group (22.7%). Females of the P-generation did not exhibit any significant alteration.

At 200 and 20 ppm, slight variations were notable, but not of toxicological relevance.

Table 25: Body weights and body weight gains in adult P generation

Study period	Dose group ppm			
	Control	20	200	1500
Pre-mating - Males				
Mean body weight (g) – Day 98	432	417.0	411.9	399.9*
Mean weight gain (g) - Days 0-98	156.1	142.6	130.7	120.6
Pre-mating - Females				
Mean body weight (g) – Day 98	283.7	278.3	280.7	277.5
Mean weight gain (g) - Days 0-98	96.7	90.2	94.2	90.0
Gestation – Females				
Mean body weight (g) Day 20	340.0	328.1	328.2	325.5
Mean weight gain (g) - Days 0-20	98.5	90.7	93.9	93.7
Lactation – Females				
Mean weight gain (g) - Days 21	287.1	284.6	282.1	280.8
Mean weight gain (g) - Day 0-21	23.9	23.8	22.5	26.3

* $p \leq 0.05$; ** $p \leq 0.01$

Food consumption in the 1500 ppm treated males was reduced during the premating period compared to controls. In females a reduction was found at 200 ppm and above. There were no effects on food consumption in the 20 ppm groups (males and females).

Table 26: Mean food consumption (g/rat/day) in P generation

Study period	Dose group ppm			
	Control	20	200	1500
Males				
Pre-mating				
Days 0-7	21.7	21.6	21.7	21.5
Days 7-14	21.9	22.2	21.7	20.5*
Females				
Pre-mating				
Days 0-7	15.9	15.6	14.5**	13.7*
Days 7-14	15.9	15.8	15.3	16.1
Gestation				
Days 0-6	17.8	18.9	17.9	17.4
Days 13-20	21.5	21.2	21.9	21.8
Lactation				
Days 0-4	27.6	25.7	26.4	23.1
Days 4-7	40.9	40.8	38.6	24.8**
Days 7-14	52.0	49.9	47.6**	44.2**
Days 14-21	61.0	59.7	56.9	55.8

* $p \leq 0.05$; ** $p \leq 0.01$

Corneal opacities were observed in all treated groups in both males and females. The frequency of occurrence and the onset of this condition were generally dose dependent. The first onset of corneal opacities during the premating phase occurred on week 6 after the start of the treatment and increased markedly in females of all treated groups during lactation. Corneal opacities were not found in control rats.

Table 27: Incidences of corneal opacities in the P generation

Sex	Males	Females
Incedences / Dosage (ppm)		
0	0/30	0/30
20	10/30**	1/30
200	10/30**	11/30**
1500	13/30**	11/30**

** $p \leq 0.01$

There were no compound-related effects on any reproductive and litter parameter (e.g., mating, fertility, or gestation indices, days to insemination, gestation length, or number of implants) in P generation at any dose level tested.

Table 28: Litter data of P adults / F1 offspring

Dosage (ppm) / Parameter	Gestation days	Live	Dead	Implants	Birth index	Live birth index
0 (n=26)	22.0	11.2	0.0	13.0	85.4	99.6
20 (n=25)	22.1	9.9	0.1	11.8	83.5	99.3
200 (n=27)	22.2	10.6	0.0	11.6	92.3	99.6
1500 (n=28)	22.0	10.1	0.2	11.5	89.7	96.2

There were no substance-related effects on oestrus cycle (length or the number of cycles) at any dose level. Sperm motility was comparable to the control group. The mean numbers of sperm samples taken from the epididymis and the testis of the control and 1500 ppm groups were comparable. Sperm morphology was evaluated in the control group and the 1500 ppm group and there were no differences noted.

F1-generation

There were no deaths or animals sacrificed in a moribund condition due to treatment with AE 0172747.

Table 29: Body weights and body weight gains in adult F1 generation

Study period	Dose group ppm			
	Control	20	200	1500
Males				
Pre-mating				
Mean body weight (g) - Day 98	442.9	419.4*	397.1*	387.1*
Mean weight gain (g) - Days 0-98	160.9	148.5	144.2	136.3
Females				
Pre-mating				
Mean body weight (g) - Day 98	266.0	279.4	274.8	269.2
Mean weight gain (g) - Days 0-98	85.9	98.4	102.3	99.6
Gestation				
Mean body weight (g) - Day 20	314.7	328.6	331.2	312.5
Mean weight gain (g) - Days 0-20	86.3	91.2	97.0	86.2
Lactation				
Mean body weight (g) - Day 21	270.5	284.0	279.1	272.3
Mean weight gain (g) - Days 1-21	23.1	20.8	18.8	21.4

* $p \leq 0.05$; ** $p \leq 0.01$

Food consumption in males was comparable to those in the control group. In females food consumptions were declined during the last two periods of lactation in the hightest dose group.

Table 30: Mean food consumption (g/rat/day) in F1 generation

Study period	Dose group ppm			
	Control	20	200	1500
Males				
Pre-mating				
Days 0-7	23.7	23.3	22.9	22.8
Days 63-70	21.0	20.4	20.3	19.5
Females				
Pre-mating				
Days 0-7	16.0	16.5	16.4	16.3
Days 63-70	14.8	16.2**	16.2**	15.3
Gestation				
Days 0-6	14.5	15.6	16.1	15.1
Days 13-20	18.1	20.4**	20.6**	19.6
Lactation				
Days 0-4	25.2	26.5	25.7	23.2
Days 4-7	37.6	37.8	38.0	36.9
Days 7-14	49.3	47.7	48.2	43.4**
Days 14-21	56.6	57.0	55.6	51.4**

* $p \leq 0.05$; ** $p \leq 0.01$

There were no compound-related effects on any reproductive and litter parameter (e.g., mating, fertility, or gestation indices, days to insemination, gestation length, or number of implants) in F1 generation at any dose level tested. There were no substance-related effects on oestrus cycle (length or the number of cycles) at any dose level.

Table 31: Litter data of F1 adults / F2 offspring

Dosage (ppm) / Parameter	Gestation days	Live	Dead	Implants	Birth index	Live birth index
0 (n=26)	22.0	10.3	0.0	11.2	90.5	99.7
20 (n=25)	22.0	10.5	0.2	11.4	93.9	95.9
200 (n=27)	21.9	11.2	0.0	11.8	94.4	99.7
1500 (n=28)	22.0	9.6	0.2	10.9	90.2	97.5

Growth and Developmetal Parameters (F1 and F2 generation)

There were no substance-related clinical observations from birth until weaning observed at any dietary level tested. A decline in body weight and bw gain was noted in both male (at all concentrations tested) and female pups of the F1 generation during the lactation period and persisted throughout adulthood and during the premating period. At 20 ppm there were no relevant

treatment-related effects on body weight in any generation and phase of the study.

Table 32: Body weight changes in F1 pups during lactation (males and females combined)

Body weight or weight change (g)	0 ppm	20 ppm	200 ppm	1500 ppm
Day 0	5.9	6.0	6.0	6.0
Day 0-4	3.8	3.5	3.1*	3.0**
Day 4-7	5.8	5.3	5.0**	4.5**
Day 7-14	16.8	15.4**	15.0**	14.0**
Day 14-21	16.2	15.4	13.1**	13.0**
Total gain (Days 0-21)	42.6	39.6	36.3**	34.6**
% decrease from controls		-7.0%	-14.8%	-18.8%

* $p \leq 0.05$; ** $p \leq 0.01$

F2 pup body weights at birth for the three treated groups were comparable to the control group. Beginning on lactation day 7, the pup body weights for both sexes in the 200- and 1500-ppm dietary groups were less than in the control group. These pup body weights became statistically decreased by lactation day 21 and 14 for the 200- and 1500-ppm groups, respectively. The pup body weight gains were statistically reduced in the 200- and 1500-ppm groups beginning on lactation days 14 and 4, respectively. Body weights and body weight gains for the F2 pups in the 20-ppm group were comparable to the control group for the lactation period.

Table 33: Body weight changes in F2 pups during lactation (males and females combined)

Body weight or weight change (g)	0 ppm	20 ppm	200 ppm	1500 ppm
Day 0	5.8	5.8	5.8	5.9
Day 0-4	3.9	3.8	3.6	3.6
Day 4-7	5.7	5.4	5.4	5.0**
Day 7-14	16.0	15.2	15.3	14.1**
Day 14-21	16.1	16.1	13.8**	12.7**
Total gain (Days 0-21)	41.6	40.7	38.2**	35.5**
% decrease from controls			-8.2%	-14.7%

* $p \leq 0.05$; ** $p \leq 0.01$

Corneal opacities were not observed in the F1 and F2 pups from birth through weaning at any dietary level tested. Onset of post-weaning corneal opacities is presented in the table below. Data show a correlation between the onset of maternal corneal opacities and reduced weight reduction of their pups in both generations. Pups from dams that did not develop corneal opacities during lactation gained weight comparable to the controls.

Table 34: First onset of post-weaning corneal opacities F1 and F2 Generation pups

	20 ppm	200 ppm	1500 ppm
F1 - Post-weaning (days)	31	28	23
F2- Post weaning (days)	34	26	23

In both F1 and F2 generation, delay in preputial separation was observed in the 200 and 1500 ppm

groups and were likely secondary effects of the reduced body weight gain. In the 20 ppm group, delay in preputial separation occurred only in the pups from dams exhibiting corneal opacities during lactation, associated with decrease body weight gain of their offspring. Vaginal opening was statistically delayed in the 1500 ppm group in both generations. The mean anogenital distance of all male and female F2 pups from all litters was nearly identical for all AE 0172747 groups and the control group.

F2 generation during Juvenile Phase

General observations: There were no deaths or animals sacrificed in a moribund condition attributed to treatment with AE 0172747 in the F2-juvenile group.

Corneal opacities were observed in all AE 0172747 treated groups in both males and females.

Hematology parameters were performed just prior to necropsy on only the F2- Juveniles. There were various statistically significant differences when comparing mean values of dosage groups with controls. There was a statistically significant decrease in the total red blood cell count (RBC) and hemoglobin concentration (Hb) in both the male and female 1500 ppm dosage groups and a statistically significant decrease in the RBC's of the 200 ppm female dosage group when compared to controls.

Table 35: Hematologic parameters in F2 generation

Dosage / Parameter	RBC [10^6/mm^3]		Hb [g/dl]	
	Males	**Females**	**Males**	**Females**
0 ppm	8.17	8.29	15.8	16.1
20 ppm	8.25	8.20	16.0	16.2
200 ppm	8.02	7.92*	15.7	16.0
1500 ppm	7.61*	7.87*	15.0*	15.4*

Pathology:

P generation

There were several effects on organ weights. Absolute and relative liver and kidney weights were increased in male rats significantly in all dose groups. In Addition, both sexes show decreased absolute thymus weights in higher concentrations.

Table 36: Liver and kidney weight alteration in male rats of the P generation (day 98)

Dosage / Organ	Liver (g)	Liver/bw ratio	Kidneys (paired)	Kidney/bw ratio
0 ppm	15.225	3.517	1.437	0.334

Dosage / Organ	Liver (g)	Liver/bw ratio	Kidneys (paired)	Kidney/bw ratio
20 ppm	17.459*	4.183*	1.545*	0.372*
200 ppm	17.200*	4.190*	1.566*	0.382*
1500 ppm	17.349*	4.335*	1.522*	0.381*

* $p < 0.05$

Table 37: Thymus weight (g) alteration in male and female rats of the P generation (day 98)

Dosage / Sex	Males	Females
0 ppm	0.404	0.267
20 ppm	0.355	0.232
200 ppm	0.350	0.223*
1500 ppm	0.340*	0.214*

* $p < 0.05$

Corneal opacities, either unilateral or bilateral were confirmed in all three treated groups, but absent in control animals of both sexes in P generation at necropsy. The incidences were noted according to increasing dosage: 0% / 33.3% / 26.7% and 43.3% in males and 0% / 50% / 90% and 80% in females.

Microscopically findings were primarily neutrophilic or mixed cell inflammation involving the cornea or anterior chamber of the eye.

Table 38: Incidences of optical inflammation in male and female rats of the P generation

Dosage / Sex	Males	Females
0 ppm	0%	0%
20 ppm	40%*	59%*
200 ppm	27%*	90%*
1500 ppm	33%*	83%*

* $p < 0.05$

F1 generation (Adults)

In male F1 adult rats weights of brain (absolute and relative, at 200 and 1500 ppm), liver (absolute and relative at 20, 200 and 1500 ppm), absolute thymus (at 20, 200 and 1500 ppm), relative testes (all concentrations), relative seminal vesicle (at 200 and 1500 ppm) and relative left adrenal gland (all concentrations) were significantly altered, but not always in a dose-dependent fashion. Testes were also found to be reduced in size (in the lowest concentration 1/1 and in the highest concentration 1/2, no data are available for controls or the 200 ppm groups).

Table 39: Brain, liver, kidney thymus, kidney and testes weight alterations in male rats of the adult F1 generation (day 35)

Dosage / Organ	Brain (g)	Brain/ bw	Liver (g)	Liver/bw	Thymus (g)	Kidney (g)	Kidney/bw	Testes/bw
0 ppm	2.030	0.461	15.518	3.501	0.445	1.439	0.326	0.421

Dosage / Organ	Brain (g)	Brain/ bw	Liver (g)	Liver/b w	Thymu s (g)	Kidney (g)	Kidney/b w	Testes/b w
20 ppm	1.999	0.479	17.751*	4.241*	0.369*	1.521	0.363*	0.571*
200 ppm	1.919*	0.487*	16.891*	4.255*	0.369*	1.480	0.374*	0.479*
1500 ppm	1.886*	0.492*	17.369*	4.489*	0.365*	1.513	0.391*	0.490*

* $p \leq 0.05$

In females brain (dose-dependent at all concentrations tesed), absolute thymus (at 20 ppm and 1500 ppm), and absolute kidney (at 20 ppm and 200 ppm) weights were altered. Weights of left adrenal gland were increased in the 20 and 200 ppm dose group.

Table 40: Brain, thymus and kidney weight alteration in female rats of the adult F1 generation (day 35)

Dosage / Organ	Brain (g)	Brain/bw	Thymus (g)	Kidneys (paired) (g)	Kidney/bw
0 ppm	1.931	0.728	0.252	1.024	0.386
20 ppm	1.879*	0.676*	0.212*	1.148*	0.414
200 ppm	1.827*	0.669*	0.242	1.104*	0.402
1500 ppm	1.795*	0.671*	0.220*	1.088	0.405

* $p \leq 0.05$

Increased incidences of dilated renal pelvis were observed in all dose levels in both sexes.

Table 41: Incidences of dilated renal pelvis in adult F1 generation

Organ / Dosage	0 ppm	20 ppm	200 ppm	1500 ppm
Males	3.3%	6.9%	16.7%	37.9%
Females	0%	3.3%	20.0%	37.9%

No statistic submitted

Dilation of the renal pelvis was also confirmed microscopically in the 200 and 1500 groups. The incidence in the concurrent control rats was comparable to that of the 20-ppm group. In the highest concentration statistically significantly enhanced renal tubular basophilia was found in male rats as well as a chronic inflammation (already evident at 200 ppm, but not statistically significant). In females these effects were less prominent.

Ovarian follicles (primordial and antral) and corpora lutea were quantitatively evaluated on 10 randomly selected F1 dams in the control and in the 1500 ppm group 21 days after partuition. The number of corpora lutea was statistically significantly reduced in the 1500 ppm group, but without ovarian weight differences when compared to controls.

Corneal opacities, either unilateral or bilateral were confirmed in all three treated groups, but absent in control animals of both sexes in F1 adults at necropsy with the following incidence (according to increasing dosage) 0% / 86.2% / 96.7% and 90% in males and 0% / 70% / 90% and 96.7% in females.

Microscopically findings were primarily neutrophilic or mixed cell inflammation involving the cornea or anterior chamber of the eye. Neovascularization of the cornea was observed in these same rats. All other observations were considered incidental and not related to test substance administration.

Table 42: Incidences of optical inflammation and neovascularization of the adult F1 generation

Dosage / Effect	Inflammation		Neovascularization	
	Males	Females	Males	Females
0 ppm	0%	0%	0%	0%
20 ppm	86%*	72%*	86%*	55%*
200 ppm	100%*	93%*	100%*	93%*
1500 ppm	97%*	97%*	97%*	90%*

* $p \leq 0.05$

F1 generation (Pups)

Absolute and relative brain, thymus, spleen and uterus weights were examined. Absolute and relative weights of brain (absolute at all concentrations, relative at 200 and 1500 ppm) and spleen (at all concentrations tested) were reduced in both sexes, as well as absolute thymus weights (in males and females combined at the highest concentration). Thymus showed certain discoloration in animals receiving the test substance.

Table 43: Alteration of absolute and relative brain and spleen weights in F1 generations (pups)

Organ / Dosage	0 ppm	20 ppm	200 ppm	1500 ppm
Brain (g)				
Males	1.501	1.449*	1.409**	1.360**
Females	1.460	1.396*	1.355**	1.310**
Brain/bw ratio				
Males	3.044	3.151	3.280**	3.315**
Females	3.075	3.205	3.316**	3.323**
Spleen (g)				
Males	0.229	0.190**	0.168**	0.150**
Females	0.233	0.193**	0.169**	0.155**
Spleen/bw ratio				
Males	0.458	0.407*	0.388**	0.362**
Females	0.485	0.434*	0.408*	0.389**

* $p \leq 0.05$; ** $p \leq 0.01$

Decreased spleen weights were associated with a decrease of extramedullary hematopoiesis.

Table 44: Incidences of decrease of extramedullary hematopoiesis in F1 (21-day pups)

Dose group (ppm)	0		20		200		1500	
Sex	M	F	M	F	M	F	M	F

Dose group (ppm)	0		20		200		1500	
No. Animals	25	23	20	21	27	26	27	25
Decreased extramedullary hematopoiesis	0	0	3	3	2	5	9	10
Relative incidences	0%	0%	15%	14%	7%	19%*	33%*	40%*

* $p \leq 0.05$

F2 generation (Pups)

Increased cannibalization could be observed at 200 ppm (3.8%) and 1500 ppm (10.7%) compared to control group (0%).

Weights of selected organs (brain, thymus, spleen and uterus) were determined (no data for liver or kidneys were available). Absolute brain weights were lower than in controls, relative brain weights were not altered. At 1500 ppm absolute brain weight differences were significantly reduced for 14.6% in males and 12.5% in female rats relative brain. At the 200 ppm absolute brain weight reduction was significant (7.8% in males and 9.5% in females) but within historical controls. At both concentrations relative brain weights were not significantly altered. At 20 ppm, no significant changes in brain weights were observed.

For the kidney only data for the male rats in the highest dose are available showing that 1/2 exhibited dilatated renal pelvis.

Spleen weights (absolute and relative) were lower in treated groups when compared to controls. At 1500 ppm significantly reduced spleen weights were found concomitant with extramedullary hematopoiesis. Spleen weights were reduced at the 20 ppm dose level but this was not statistically significant and was accompanied by decreased body weight. At 200 ppm spleen weights were significantly reduced relative to controls in the presence of concurrent weightloss, without histopathological findings, but decreased extramedullary hematopoiesis.

Table 45 Alteration of absolute and relative brain and spleen weights in F2 generations (21-day pups)

Organ / Dosage	0 ppm	20 ppm	200 ppm	1500 ppm
Brain (g)				
Males	1.507	1.476	1.421**	1.370**
Females	1.452	1.431	1.379**	1.332**
Brain/bw ratio				
Males	3.125	3.167	3.179	3.294
Females	3.138	3.184	3.229	3.258
Spleen (g)				
Males	0.230	0.205	0.177**	0.158**
Females	0.231	0.210	0.173**	0.165**
Spleen/bw ratio				
Males	0.474	0.435	0.396**	0.378**
Females	0.497	0.460	0.403*	0.398**

* $p \leq 0.05$; ** $p \leq 0.01$

Table 46: Incidence of decrease of extramedullary hematopoiesis in F2 (21-day pups)

Dose group (ppm)	0		20		200		1500	
Sex	M	F	M	F	M	F	M	F
No. Animals	26	26	26	27	25	26	27	28
Decreased extramedullary hematopoiesis	1	0	2	2	2	2	19	23

Dose group (ppm)	0		20		200		1500	
Relative incidences	4%	0%	8%	7%	8%	8%	70%*	82%*

* $p \leq 0.05$

F2 generation (Juveniles)

Organ weights were determined of 10 animals/sex/group. Absolute and relative brain weights were significantly reduced in the 1500 and 200 ppm groups. Testical malformations were identified in the high dose group in 1/1 (no examination of controls or other dose-levels). Kidneys were found to be dilated (2/2) in males of the 1500 ppm group (no examination of controls, other dose-levels or females). Microscopical examination of the spleens revealed normal development, without decreased extramedullary hematopoiesis.

Corneal opacity was confirmed in all three treated groups the incidences are according to the increased dose levels: 0% / and 100% in all dose groups in males and females also confirmed at microscopical level.

In a position paper with respect to the multigeneration study, the findings observed at 20 ppm such as ocular findings, reduction of brain weight, increase of liver weight, increased kidney weights, incidence of dilated pelvis, reduction in spleen weights, incidence of extramedullary hematopoiesis, as well as reductions in ovarian follicular counts were discussed in connection with historical control data.

Reference:	**Position paper: Tembotrione: two generation reproductive study in the rat**
Author(s), year:	Semino, G.; 2006

Ocular findings:

Corneal opacities were observed in all treated groups in both males and females. The frequency of occurrence and the onset of this condition were dose dependent. The onset of corneal opacities during the pre-mating phase occurred in week 6 after the start of the treatment and increased markedly in females of all treated groups during lactation. Corneal opacities were not found in control rats.

Corneal opacity is a typical effect observed in the rats after dietary exposure to compounds that, like tembotrione, inhibit of 4-hydroxyphenylpyruvate dioxygenase (HPPDase), a key enzyme of tyrosine catabolism. Inhibition of HPPDase in mammals causes increased tyrosine levels in plasma and in the aqueous humour of the eye, which in rats provokes ocular lesions.

The ocular sensitivity of the various species to tyrosine plasma levels and the ability of rats to develop ocular toxicity are attributed to differences in tyrosine catabolism in mammals. It has been shown that the plateau levels of plasma tyrosine after HPPDase inhibitors administration are higher in rats (males > females) than in mice, where they do not reach the threshold for toxic effects, even at the highest doses. In humans, genetically or pharmacologically abolished or highly reduced HPPDase is associated with levels of tyrosinaemia comparable to those observed in mice without the occurrence of signs of ocular toxicity. Therefore, due to the high ability of humans to catabolise tyrosine through an alternative pathway, even when the enzyme HPPDase is inhibited, tyrosine-

related lesions like corneal opacity are of no relevance for human risk assessment.

Changes in organ weights:

Absolute **brain weights** were reduced in the treated groups. However, since relative brain weight changes did not exceed historical controls in any generation at 20 ppm and body weights in the F1 and F2 generation were significantly reduced at 200 and 1500ppm, this finding was not considered to be of toxicological relevance.

Table 47: Relative brain weight (%)

Dose level (ppm)	0	20	200	1500	HCD
Males – F1 pups	3.044	3.151	3.280**	3.315**	2.960 - 3.419
Males – F1 adults	0.461	0.479	0.487*	0.492*	0.445 – 0.494
Males – F2 pups	3.125	3.167	3.179	3.294	2.960 - 3.419
Females – F1 pups	3.075	3.205	3.316**	3.323**	2.912 - 3.411
Females – F1 adults	0.728	0.676*	0.669*	0.671*	0.669-0.783
Females – F2 pups	3.138	3.184	3.229	3.258	2.912 - 3.411

* $p \leq 0.05$: ** $p \leq 0.01$ HCD = Historical Control Data

Absolute liver weight and relative liver weight were increased in male rats in both parental and F1 generation. The change was not dose dependent and not associated with any microscopic findings at the highest dose group (20 and 200 ppm were not examined). Thus, they were not regarded as toxicologically relevant.

Table 48: Liver weight changes in male rats

Dose level (ppm)	0	20	200	1500
Parents - absolute weight (g)	15.225	17.459*	17.200*	17.349*
Parents - relative weight (%)	3.517	4.183*	4.190*	4.335*
F1 - absolute weight (g)	15.518	17.751*	16.891*	17.369*
F1 - relative weight (%)	3.501	4.241*	4.255*	4.489*

* $p \leq 0.05$

Table 49: Histopathology of the liver in male rats

Dose level (ppm)	0	20	200	1500
Parents				
Number of tissues examined	30	0	0	30
Number of abnormalities detected	18	-	-	23
Congestion	1	-	-	0
Inflammation	3	-	-	1
Chronic inflammation	7	-	-	6
Microgranuloma	1	-	-	0
F1				
Number of tissues examined	30	0	0	30
Number of abnormalities detected	30	-	-	26
Chronic inflammation	0	-	-	3

Dose level (ppm)	0	20	200	1500
Microgranuloma	0	-	-	1

In males, a dose related increase in absolute and relative **kidney weights** was observed; relative kidney weights were outside the historical control data in parental and F1 generation at 20 ppm. In the parental generation, no real dose response was observed.
In females, kidney weights w30ere slightly increased in a non-dose related fashion (outside tha historical control data) at 20 ppm in the F1 generation.
In the parental generation, histopathological examination of the kidney did not reveal any changes.
In the F1 generation, the incidence of dilated renal pelvis was increased at 200 and 150 ppm in males and at 1500 ppm in females. At 20 ppm, the incidence of dilated renal pelvis was within the range of historical control data.

Table 50: Kidney weights (absolute and relative)

Dose level (ppm)	0	20	200	1500	HCD
Males					
Parents					
Left Kidney absolute weight (g)	1.419	1.527*	1.551*	1.517*	1.371-1.554
Left Kidney relative bw (%)	0.329	0.367*	0.378*	0.380	0.316-0.347
Right Kidney absolute weight (g)	1.455	1.564*	1.581*	1.528	1.402-1.577
Right Kidney relative bw (%)	0.338	0.376*	0.385*	0.382*	0.316-0.360
F1 generation					
Left Kidney absolute weight (g)	1.414	1.520	1.465	1.514	1.371-1.554
Left Kidney relative bw (%)	0.320	0.363*	0.370*	0.391*	0.316-0.347
Right Kidney absolute weight (g)	1.463	1.521	1.495	1.512	1.402-1.577
Right Kidney relative bw (%)	0.331	0.363*	0.377*	0.391*	0.316-0.360
Females					
Parents					
Left Kidney absolute weight (g)	1.072	1.083	1.080	1.093	0.975 – 1.088
Left Kidney relative bw (%)	0.378	0.389	0.386	0.394	0.352-0.404
Right Kidney absolute weight (g)	1.124	1.122	1.131	1.160	1.029 – 1.141
Right Kidney relative bw (%)	0.396	0.402	0.404	0.418*	0.376 – 0.424
F1 generation					
Left Kidney absolute weight (g)	1.008	1.129*	1.089*	1.072	0.975 – 1.088
Left Kidney relative bw (%)	0.379	0.407	0.396	0.399	0.352-0.404
Right Kidney absolute weight (g)	1.041	1.167*	1.119	1.104	1.029 – 1.141
Right Kidney relative bw (%)	0.392	0.420	0.427	0.410	0.376 – 0.424

* $p \leq 0.05$; ** $p \leq 0.01$ HCD = Historical Control Data

Table 51: Incidence of dilated renal pelvis in the F1 and F2 generations

Dose level (ppm)	0	20	200	1500	HCD
F1 Males	2/30	4/29	15/30	16/30	0/30-5/30
F2 Male Juvenile	0/10	0/10	0/10	1/10	NA
F1 Females	1/30	2/29	4/30	17/30	0/30-3/30
F2 Female Juvenile	0/10	0/10	0/10	0/10	NA

NA = Not available; HCD = Historical Control Data

Males have been shown to be more susceptible to kidney changes than females. In the 90-day rat study, administration of tembotrione at dose levels of 1500 and 7000 ppm provoked an accumulation of hyline droplets in the kidneys without degenerative changes in the tubules which were considered to represent an accumulation of □2u-globulin. As the sequence of renal events leading to its accumulation and subsequent toxicity is male rat specific, the effects on the kidney weight observed in this study are not considered relevant for human risk assessment. Moreover, the occurrence of dilated pelvis was within the range of historical control data in F1 animals and was reversible in the F2 juveniles (no historical control data available).Therefore it can be concluded that the effects observed in the kidneys are not relevant to humans.

The spleen weight in F1 and F2 pups was reduced at 200 and 1500 ppm. At 20 ppm, the effect was statistically significant in F1 pups for males and females, but the data were within the range of historical controls for females (absolute and relative weight). In males, the finding was not considered of toxicological relevance, since in F2-pups, the spleen weight was normal at 20 ppm in both genders.

Table 52: Absolute spleen weight

Dose level (ppm)	0	20	200	1500	HCD
Males – F1 pups	0.229	0.190**	0.168**	0.150**	0.202 - 0.252
Males – F2 pups	0.230	0.205	0.177**	0.158**	0.202 - 0.252
Females – F1 pups	0.233	0.193**	0.169**	0.155**	0.193 - 0.251
Females – F2 pups	0.231	0.210	0.173**	0.165**	0.193 - 0.251

* $p \leq 0.05$; ** $p \leq 0.01$ HCD = Historical Control Data

Table 53: Relative spleen weight

Dose level (ppm)	0	20	200	1500	HCD
Males – F1 pups	0.458	0.407*	0.388**	0.362**	0.422 - 0.503
Males – F2 pups	0.474	0.435	0.396**	0.378**	0.422 - 0.503
Females – F1 pups	0.485	0.434*	0.408*	0.389**	0.416 - 0.503
Females – F2 pups	0.497	0.460	0.403*	0.398**	0.416 - 0.503

* $p \leq 0.05$; ** $p \leq 0.01$ HCD = Historical Control Data

The incidence of **decreased extramedullary hematopoiesis** was increased in the spleen of F1 and F2 pups at 2500 ppm only. Incidence of this finding was comparably low (close to the control value) at 20 and 200 ppm, and in F2 juveniles, no extramedullary hematopoiesis was observed at any dose level showing the reversibility of this effect.

Table 54: Incidence of decreased extramedullary hematopoiesis

Dose level (ppm)	0	20	200	1500
Males				
F1 pups	0/25	3/20	2/27	9/27
F2 pups	1/26	2/26	2/25	19/27
F2 Juvenile	0/10	0/10	0/10	0/10
Females				

Dose level (ppm)	0	20	200	1500
F1 pups	0/23	3/21	5/26	10/25
F2 pups	0/26	2/27	2/26	23/28
F2 Juvenile	0/10	0/10	0/10	0/10

Concerning **reproductive toxicity**, the finding of reduced ovarian follicle counts at 1500 ppm was discussed.

Table 55: Ovarian follicle counts (both ovaries)

	Primordial follicles	Antral follicles	Corpora Lutea
0 ppm	56.7	4.2	40.3
1500 ppm	61.2	2.4	26.8*

* $p < 0.05$

Although the number of corpora lutea was statistically significantly decreased at 1500 ppm, there were no ovarian weight differences or evidence of other reproductive effects or problems, such as oestrus cyclicity, pregnancy rate, number of implantation sites, or organ histopathology. According to "An Evaluation and Interpretation of Reproductive Endpoints for Human Health Risk Assassment" (Daston GP and Kimmel CA, 57-74; 1999), the sole reduction of corpora lutea is not considered to be an adverse effect.

CONCLUSION:

In the 2-generation study tembotrione was administered continuously in the feed to rats at nominal dietary concentrations of 0, 20, 200, and 1500 ppm. The toxicological response of the rat was principally characterised by corneal opacities at all doses in all generations. However, in the F1 and F2 generations the onset of ophthalmologic effects took place only post-weaning, starting from post-weaning day 23 for the highest dose group and later for lower dose groups.

In addition, the liver was a key target tissue, weights were statistically significantly increased in P and F1 generation at all dose levels tested (relative weights were dose-dependent); no data were available for the F2 generation. Absolute brain weights were statistically significantly reduced in F1 (pups and adults, in all groups in a dose dependent manner) as well as in F2 (pups and juvenile, at 200 ppm and 1500 ppm, dose dependently). Increases in relative brain weights were within historical control data in all generations and dose groups. Kidney weights (absolute and relative) were increased at all dose levels in males of the P generation. Relative kidney weights were statistically significantly increased in all dose groups in male rats (dose dependent) of the F1 generation, in females absolute weights were increased (not dose dependent). Dilated renal pelvises were observed at F1 and F2 generations. Reduced spleen weights (all concentrations in both sexes in the F1 pups, and at 200 ppm and above at the F2 pups) were associated with decreased extramedullary hematopoiesis (statistically significant at the 1500 ppm dose). In F1 and F2 pups at the two higher dose groups, a delay in preputial separation and vaginal opening was observed which is likely to have occurred in association with the reduced mean body weight.

Reproductive toxicity was not observed at any dietary level tested, with the exception of the reduced number of corpora lutea in the highest dose group in F1.

A position paper submitted with respect to the 2-generation study provided additional historical control data (HCD) and further information to show that the dose level of 20 ppm is the NOAEL because of the following reasons:

1. ocular toxicity is associated with systemic increased tyrosinaemia, an effect known to be provoked by the class of HPPDase inhibitors, which is of no relevance to humans;

2. relative brain weights were within the HCD;

3. the slightly increased liver weight was not associated with any histopathology finding; moreover, such increments of liver weight are also linked with tyrosinaemia;

4. kidney weight changes were dose-related in males only and subchronic dietary administration of tembotrione was shown to provoke accumulation of α2μ-globulin in rats; the incidence of dilated pelvis was within the HCD;

5. spleen weights were within the HCD, and the decreased extramedullary haematopoiesis was transient at 20 ppm and reversible at higher dose levels (where it was considered treatment-related)

6. the noted decrease in the number of corpora lutea observed at the highest dose level (1500 ppm) has not to be considered adverse since there was no evidence that this finding is correlated with a poor reproductive outcome.

Moreover, all the above variations were more pronounced in the first generation, and (with the exception of eye lesions) no significant necropsy findings were found in the F2-juveniles. This indicates that there was no increased quantitative susceptibility of rat pups following exposure to tembotrione.

Therefore, the NOAEL for parental and offspring systemic toxicity is proposed to be set at the dose level of 20 ppm (equivalent to 1.3 – 3.3 mg/kg bw/day) and a reproductive NOAEL of 1500 ppm (equivalent to 98.2 – 228.1 mg/kg/day) was derived.

During the peer review process, the reduced absolute brain weight and the the findings in liver, kidney, and spleen at 20ppm were considered relevant and treatment related by the majority of experts. Therefore, no parental and offspring NOAEL could be derived from this study.

4.11.2.1 Human information

Not available.

4.11.3 Developmental toxicity

4.11.3.1 Non-human information

Developmental toxicity in rats

Reference:	**AE 0172747 - Developmental toxicity study in the rat by gavage**
Author(s), year:	Wason, S.; 2003a
Report/Doc. number:	SA02226; MO-03-011830; M-111508-01-1
Guideline(s):	OECD 414

GLP:	Yes
Deviations:	-
Validity:	Yes

MATERIAL AND METHODS:

Test Material:	AE 0172747
Lot/Batch:	PFI 0195
Purity:	95.0%
Vehicle:	Aqueous solution of methyl cellulose 400
Test animals:	
Species:	Rat
Strain:	Sprague-Dawley Crl:CD® (SD) IGS BR
Age:	7 – 9 weeks old
Weight at mating:	211 – 286 g
Source:	Iffa-Credo, St Germain-sur-l'Arbresle, France

In a developmental toxicity study tembotrione was administered daily by gavage from gestation day (GD) 6 to 20 to groups of 25 pregnant Sprague-Dawley female rats. The dose levels given were 0, 25, 125 and 500 mg/kg/day suspended in aqueous solution of 0.5% methylcellulose. Stability of the test substance in suspension in the vehicle was determined in a previous study where tembotrione was found to be stable for 30 days under similar conditions to those of the current study.

Clinical observations were recorded daily and body weights were recorded for all females on gestation day 0, 6, 8, 10, 12, 14, 16, 18 and 21. Food consumption was also measured for all the females during the gestation day intervals 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18 and 18-21. At scheduled sacrifice, on gestation day 21, the gravid uterine weight was recorded and the dams were evaluated for number of corpora lutea, number and status of implantations (resorptions, dead and live fetuses). Each female was first subjected to macroscopic examination of the visceral organs and the liver of each pregnant female was weighed. Number and localisation of resorption sites (classified as early and late), number of live and dead fetuses, individual weights of live fetuses: Dead fetuses were defined as fetuses showings distinct digits visible on fore and hind-paws. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulphide to visualise any sites which were not apparent. Live fetuses were removed from the uteri, counted, weighed, sexed and examined externally. Approximately half of the live fetuses from each litter were fixed in Bouin's solution and subsequently dissected for internal examination. The remaining half were eviscerated, fixed in absolute ethanol and stained according to a modification of the Tyl and Marr technique for skeletal examination of bone and cartilage. Structural deviations were classified as malformations, minor anomalies, or common variants according to Palmer (1977).

FINDINGS:

Maternal examinations

General observations: There were no deaths or abortions. At 500 mg/kg/day, treatment-related clinical signs consisted of increased salivation, observed in 72% females on one or more occasions. Vaginal discharge was observed in two females at 500 mg/kg bw and one female at 125 mg/kg bw/day.

In the 500 mg/kg bw/day group, maternal body weight change was significantly lower from gestation day 6 to 10, the difference being more pronounced between gestation day 6 to 8 (- 92%). Furthermore, between gestation day 18 to 21, body weight change was 10% lower than in the control group. In the 125 mg/kg bw/day group, maternal body weight change was 8% lower than in the control group between gestation day 18 to 21.

In the 25 mg/kg bw/day group, body weight change was reduced at the beginning of the dosing period, the effect being statistically significant between gestation day 6 to 8 (- 52%).

Food consumption was reduced at 500 and 125 mg/kg bw/day groups from gestation day 6 to 14 and gestation day 18 to 21, the effect being statistically significantly different with the exception of gestation day 12 to 14 at 125 mg/kg bw/day.

At 25 mg/kg bw/day, food consumption was lower than in the control group between gestation day 6 to 8, though the effect was not statistically significant.

Table 56: Maternal body weight

Dose (mg/kg/day)	0	25	125	500
Mortality	0	0	0	0
Bodyweight (Day 21, g)	416.2	412.2	394.9	392.0
Bodyweight gain (Days 6 – 8, g)	6.2	3.0*	2.3**	0.5**
Corrected bodyweight change (g)	66.4	63.5	59.3	57.5
Food consumption (Day 6 –8, g/kg/day)	47.7	47.8	39.4*	29.8**
Pregnancy rate (%)	92	100	100	100

* $p \leq 0.05$; ** $p \leq 0.01$

Fetal examinations

The mean fetal body weights were significantly lower than the control values at all three treatment levels in a dose-related manner. The number of runt fetuses (bodyweight < 4.0 g) was higher in all treated groups compared with the control group.

Table 57: Fetal weight

Dose (mg/kg/day)	0	25	125	500
Number fetuses/litter	315/23	337/25	330/25	359/25
Bodyweight (combined sexes, g)	5.38	5.20**	4.93**	4.53**
Males	5.50	5.31**	5.11**	4.68**
Females	5.27	5.10**	4.78**	4.42**
% Runt fetuses (< 4 g) per litter examined	4.3	16.0	20.0	44.0
per fetuses examined	0.3	1.5	2.9	10.5

* $p \leq 0.05$; ** $p \leq 0.01$

There were no treatment-related effects observed at the external observation at any dose level.

Visceral observation revealed significantly enlarged thymuses in the 500 mg/kg bw/day group. There were no treatment-related findings of other examined tissues.

Table 58: Incidences of enlarged thymus

Dose (mg/kg/day)	0	25	125	500
Number fetuses/litter	153/23	161/25	159/25	174/24
Inicidences	2/2	4/4	3/3	9/6
% Incidences	1.3/8.7	2.4/16.0	1.8/12.0	5.4/25.0

No statistics performed

Skeletal observations: A series of findings classified as variations was observed in all treated groups. At 500 and 125 mg/kg bw/day groups, poor ossification occurred at several sites (see table below) and extra ossification points on the 14th vertebrae. A few thoracic centra were also poorly ossified: incomplete ossification, unossification with normal cartilages plus bipartite/dumbbell vertebrae with dumbbell cartilages.

At 25 mg/kg bw/day, incomplete ossification was noted in the 7th cervical centrum, 5th and 6th sternebrae, 3rd and 4th proximal phalanges of forepaw, 5th metacarpals, 1st metatarsals and caudal vertebrae. Higher incidences than in the control group were also noted for short 14th ribs and extra ossification point on the 14th vertebrae.

Table 59: % Incidences of fetal skeletal variations

Dose groups (mg/kg/day)	0	25	125	500	0	25	125	500
	Number of fetuses examined				Number of litters examined			
	162	176	171	185	23	25	25	25
Anterior and/or posterior fontanelles enlarged	0	0.7	1.0	1.6	0	4.0	4.0	8.0
Hyoid centrum: incomplete ossification/cartilage normal	0	1.7	2.0	1.1	0	8.0	4.0	8.0
Hyoid centrum: unossified/cartilage normal	0	0	0	0.6	0	0	0	4.0
7th cervical centrum: unossified/cartilage bipartite	0	0	0	0.6	0	0	0	4.0
7th cervical centrum: unossified/cartilage normal	1.7	39.3	38.8	79.6	13.0	72.0	84.0	100
5th and or 6th sternebra(e): incomplete ossification or 5th sternebra: hemisternebra or 5th sternebra: bipartite/cartilage normal	10.6	15.7	16.1	28.6	43.5	56.0	56.0	68.0
5th and/or 6th sternebrae: unossified/cartilage normal	0.6	0.4	2.9	6.8	4.3	4.0	16.0	24.0
14th thoracic rib(s) short	0.5	4.5	14.8	22.9	4.3	20.0	56.0	60.0
Extra ossification point(s) on 14th thoracic vertebra	5.1	10.5	14.5	11.9	13.0	40.0	48.0	60.0
Thoracic centrum: incomplete ossification/caritalage normal	0	0.7	1.6	12.9	0	4.0	8.0	56.0
Thoracic centrum: bipartite and/or dumbbell/cartilage normal	1.8	3.0	2.4	9.3	13.0	16.0	16.0	44.0
Thoracic centrum: unossified/cartilage normal	0	0	0.7	4.3	0	0	4.0	24.0
Forepaw(s): 3rd and/or 4th proximal phalanx unossified/cartialage normal	0.6	5.3	5.0	24.1	4.3	12.0	24.0	40.0
5th metacarpals: incomplete ossification or unossified/cartilage normal	0.6	4.0	4.6	16.2	4.3	12.0	24.0	40.0
1st metatarsals: unossified/cartilage normal	5.0	12.7	13.7	29.6	17.4	32.0	48.0	64.0
Less than 9 vertebare ossified/9 first sacrocaudal vertebrae: cartilage normal	0.5	2.6	18.8	38.7	4.3	12.0	44.0	76.0

Anomalies of the throracic vertebra centra were more prominent in litter examinations at the highest concentration tested.

Table 60: % Incidences of fetal skeletal anomalies

Dose groups (mg/kg/day)	0	25	125	500	0	25	125	500
	Number of fetuses examined				Number of litters examined			
	162	176	171	185	23	25	25	25
Thoracic centrum: bipartite and/or dumbbell/cartilage bipartite or dumbbell	5.4	1.4	3.1	7.8	8.7	8.0	16.0	44.0

CONCLUSION:

In a developmental toxicity study in rats, tembotrione was administered by gavage at doses of 0, 25, 125 and 500 mg/kg bw/d. Maternal bodyweight and bodyweight gains were decreased at all dose levels in a dose-related manner. Fetal delayed ossification occurred at all dose levels, in a dose-related manner too. In addition, the number of runt fetuses was higher in all treated groups compared with the control group. Consequently a NOAEL could not be established in this study for either maternal or developmental toxicity. Therefore 25 mg/kg bw/d can only be considered as the LOAEL in this rat study. However, no teratogenic effects were observed in this study at all dose levels tested.

~~For details see DAR Rev. 2 (June 2009) and Addendum (April 2009).~~

Developmental toxicity in rabbits

Reference:	**AE 0172747 - Developmental toxicity study in the rabbit by gavage**
Author(s), year:	Wason, S.; 2003b
Report/Doc. number:	SA02056; MO-03-011094; M-108558-01-1
Guideline(s):	OECD 414
GLP:	Yes
Deviations:	-
Validity:	Yes

MATERIAL AND METHODS:

Test Material: AE 0172747

Lot/Batch:	PFI 0195
Purity:	95.0%
Vehicle:	Aqueous solution of methyl cellulose 400
Test animals:	
Species:	Rabbit
Strain:	New Zealand White KBL (NZW) IOPS/SPF
Age:	18 weeks old
Weight at mating:	211 – 286 g
Source:	Elevage Scientifique des Dombes (ESD), Chatillon sur Chalaronne, France

Time-mated female New Zealand White rabbits were exposed to AE 0172747by gavage from gestation days (GD) 6 to 28. The doses given were 0, 1, 10 and 100 mg/kg/day in suspension in aqueous solution of 0.5% methylcellulose. Stability of the compound in suspension in the vehicle was determined before the start of the study. All concentrations were checked for all formulations.The time-mated females were allocated to groups (25 per group), the day of mating being day 0 of gestation. The volume of administration was 4 ml/kg based on the most recent body weight recorded. Maternal body weights were recorded for all the females on gestation day 0, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 29. Food consumption was measured for all females on gestation day 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 22, 24, 26, 28 and 29. Clinical observations were recorded daily.

On gestation day 29, surviving females were sacrificed for examination of uterine content. Each female was first subjected to macroscopic examination of the visceral organs and the liver of each pregnant female was weighed.

The reproductive tract was weighed (gravid uterine weight), dissected out and the following parameters recorded: number of corpora lutea, number of implantation sites.

Number and localisation of resorption sites (classified as early and late), number of live and dead fetuses, individual weights of live fetuses: Dead fetuses were defined as fetuses showing distinct digits visible on fore and hind-paws. All the live fetuses were and subjected to an external examination. Uterine horn(s) without visible implantations were immersed in a 10% solution of ammonium sulphide to visualise any sites which were not apparent. Tissues and carcasses were discarded. After visceral examination of the fetuses, the head of fetuses from approximately half of each litter was be immersed in Bouin fluid and the internal structures examined after fixation. The bodies of all fetuses were dissected for soft tissue anormalies and gender determined. Then the fetuses were fixed in absolute ethanol before staining. A modification of the Staples and Schnell staining technique was used and a subsequent skeletal examination was performed.

FINDINGS:

Maternal observations

Clinical signs: At 100 mg/kg/day, five pregnant females died prematurely between gestation day 15 and 22. Few or no faeces were noted on one or more occasion on the days prior to death, for all five

animals. Treatment-related autopsy findings consisted of dark liquid present in the uterine horns or dark contents in the intestines or pale liver in these animals.

At 10 mg/kg/day, one female aborted on gestation day 23 and was subsequently necropsied. Clinical signs consisted of few or no faeces on several occasions.

At 1 mg/kg/day, one female was sacrificed on gestation day 21 for humane reasons. Clinical signs comprised of few or no faeces on several occasions and no urine on gestation day 19.

In addition in the 100 and 10 mg/kg bw/day group, treatment-related signs consisted of no faeces or few faeces. No other treatment-related signs occurred at 1 mg/kg/day.

The pregnancy rates (percentage of pregnant females per group) were comparable in the treated and control groups.

At 100 mg/kg/day, mean maternal body weight change was statistically significantly reduced in comparison with control values from gestation day 6 to 14. For the interval gestation day 6-18, the body weight change was lower (-43%) than control. At 10 and 1 mg/kg/day, body weight change was unaffected by treatment.

At 100 mg/kg/day, mean food consumption was statistically significantly reduced from gestation day 6 to 14, the reduction being more pronounced from gestation day 6 to 8, (-37 to -38%). From gestation day 14 to 18, a slight reduction in food consumption was also noted but was not statistically significant when compared to control value. At 10 mg/kg/day, food consumption from gestation day 6 to 8, was statistically significantly lower (-17%) than the control value. At 1 mg/kg/day, food consumption was unaffected by treatment.

Table 61: Maternal toxicity

Dose (mg/kg/day)	**0**	**1**	**10**	**100**
Mortality	0	1	1	5
Bodyweight (Day 14, kg)	3.64	3.68	3.67	3.55
(Day 28, kg)	3.87	3.92	3.93	3.89
Bodyweight gain (Days 6–29, kg)	0.30	0.31	0.32	0.28
Gravid uterine weight (g)	465.41	490.16	478.28	471.36
Food consumption (Day 6–8, g/kg/day)	47.7	47.8	39.4*	29.8**
Pregnancy rate (%)	96	100	100	100

Fetal examinations

There were no treatment-related effects on litter parameters concerning early and late resorptions, fetal death status and percentage of male/female fetuses. The mean fetal body weight did not show any significant differences when compared to control values.

External observation revealed that effects were comparable between treated and control groups with no evidence of any treatment-relationship.

Visceral observation showed that at 100 mg/kg/day, three fetuses in two litters had dilated cerebral ventricles. Incidences of short innominate artery or the absence of innominate artery also occurred with a higher incidence at this dosage than in the control group.

Skeletal observation clearly showed that at 100 mg/kg/day the following anomalies occurred at a higher incidence than in the controls: extra sternebrae ossification, unossified 1st and 2nd sternebrae,

short 1st ribs, cartilages of the 1st ribs not attached to the sternum, fused cartilages between the 1st and 2nd ribs, cartilages of the 8th ribs attached to the sternum, presence of 14 ribs and presence of 27 pre-sacral vertebrae associated with 13 ribs. The following variations were noted with a higher frequency: incomplete ossification of pubis, unossified atlas centrum, extra ossification site(s) between atlas and axis centrum, incomplete ossification of 1st and 2nd sternebra, unossified 2nd and/or 3rd and/or 4th and/or 5th middle phalanges of hindpaws, short innominate artery also incomplete ossification of the hyoid centrum was found to occur with higher frequency.

At 10 mg/kg/day, the following anomalies occurred with an increased frequency: fused cartilages between the 1st and 2nd ribs, cartilages of the 8th ribs attached to the sternum, 27 pre-sacral vertebrae associated with 13 ribs, unossified 1st and 2nd sternebra and extra sternebral ossification. Variations which occurred with an increased incidence included poor ossification in the atlas, 1st and 2nd sternebra, 1st metacarpal, 5th middle phalanx of forepaws, pubis, astragalus and was reflected in the increased incidence of enlarged fontanelles. Furthermore, extra ossification points between the atlas and axis and also between sternebrae were found to occur with a higher incidence.

At 1 mg/kg/day, there were only few skeletal effects observed. Incomplete ossification of the pubis was considered as variation and the presence of 27 presacral vertebrae associated to 13 thoriacic rib as anomaly.

Table 62: % Incidences of fetal skeletal variatons

group (mg/kg/day)	0	1	10	100	0	1	10	100
	Number of fetus examined				Number of litters examined			
	217	221	233	186	24	24	24	20
Hyoid centrum: incomplete ossification or unossified	17.5	35.3	20.8	39.8	37.5	62.5	37.5	55.0
Atlas centrum: unossified	4.3	2.0	8.9	23.5	12.5	16.7	33.3	70.0
Extra ossification site between atlas and axis centrum	5.2	4.5	15.4	46.3	20.8	29.2	58.3	85.0
1st and 2nd sternebra: incomplete ossification	0	0.3	1.8	7.3	0	4.2	16.7	55.0
Hindpaws: 2nd and/or 3rd and/or 4th and/or 5th middle phalanges: unossified	1.2	0.3	1.5	2.2	8.3	4.2	12.5	20.0
Pubis (unilateral/bilateral): incomplete ossification	0.8	3.2	10.6	11.9	8.3	20.8	50.0	55.0

Table 63: % Incidences of fetal skeletal anomalies

Dosegroups (mg/kg/day)	0	1	10	100	0	1	10	100
	Number of fetuses examined				Number of litters examined			
	217	221	233	186	24	24	24	20
1st and 2nd sternebra: unossified	0	0	1.3	4.2	0	0	12.5	30.0
Extra sternebral ossification	0	0	2.5	4.2	0	0	20.8	15.0
Cartilage of 8th rib (unilateral/bilateral): attached to the sternum	2.1	0.3	2.6	14.2	4.2	4.2	16.7	45.0
1st ribs (unilateral/bilateral): short	1.0	0	1.4	5.6	4.2	0	8.3	35.0
Cartilage of 1st rib (unilateral/bilateral): not attached to the sternum	0.3	0	1.4	5.6	4.2	0	8.3	35.0
Cartilage of 1st and 2nd rib (unilateral/bilateral): fused	2.1	0.6	4.2	10.2	4.2	4.2	20.8	40.0
Presence of 27 presacral vertebrae	1.4	1.2	14.2	51.5	8.3	4.2	54.2	80.0
13 thoriacic rib(s) (unilateral/bilateral) and presence of 27 presacral vertebrae	0.3	1.2	14.2	50.0	4.2	4.2	54.2	80.0

Reference:	Position Paper Tembotrione: Rabbit teratology
Author(s), year:	Mallyon, B., Semino, G.; 2006

A **position paper** was submitted to give further information on the frequency of the visceral findings in historical controls performed with the same strain (*Mallyon B, Semino G; 2006*).

The frequency of the visceral findings "short innominate artery", "absent innominate artery" and "absent caudate lung lobe" observed in the study were well in the range of control data in several studies. Concerning the dilated cerebral ventricles (3 fetuses from 2 litters at 100 mg/kg bw/d), it was only stated that this findings could only be observed at a dose level producing marked maternal toxicity. Moreover, there were no other anomalies or malformations associated with effects on the CNS (e.g.hydrocephaly or Spina bifida). However, treatment-relation could not be excluded.

Table 64: Incidence of variations including historical control incidence

Finding	Dose Groups (mg/kg/day)									
	0	**1**	**10**	**100**	**HCI**	**0**	**1**	**10**	**100**	**HCI**
	Foetuses (%)					**Litters (incidence)**				
Visceral observations										
Innominate artery: short	3.9	2.7	2.1	8.5	0.4-12.7	3/24	4/24	3/24	10/20*	1/22-11/28
Innominate artery : absent	0.0	0.0	0.0	1.4	0.0- 3.8	0/24	0/24	0/24	2/20	0/24-3/20
Caudate lung lobe: absent	1.6	4.3	6.3	3.9	0.8-12.2	3/24	6/24	7/24	5/20	1/21-8/28
Skeletal observations										
Hyoid centrum: incomplete ossification or unossified	17.5	35.3	20.8	39.8	0.8-32.3	9/24	15/24	9/24	11/20	1/21-19/28
Atlas centrum: unossified	4.3	2.0	8.9	23.5	0.0-4.3	3/24	4/24	8/24	14/20***	0/24-3/24
Extra ossification site between atlas and axis centrum	5.2	4.5	15.4	46.3	0.0-5.2	5/24	7/24	14/24*	17/20***	0/23-5/24
1st and 2nd sternebrae: incomplete ossification	0.0	0.3	1.8	7.3	0.0-1.2	0/24	1/24	4/24	11/20***	0/24-2/20
Hindpaws: 2nd and/or 3rd and/or 4th and/or 5th middle phalanges unossified	1.2	0.3	1.5	2.2	0.0-1.7	2/24	1/24	3/24	4/20	0/24-3/22
Pubis (unilateral/bilateral): incomplete ossification	0.8	3.2	10.6	11.9	0.0-3.5	2/24	5/24	12/24**	11/20***	0/23-6/22

HCI: historical control incidence-lower and upper limit
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 65: Incidences of anomalies including historical control incidence

Finding	Dose Groups (mg/kg/day)									
	0	**1**	**10**	**100**	**HCI**	**0**	**1**	**10**	**100**	**HCI**
	Foetuses (%)					**Litters (incidence)**				

Finding	Dose Groups (mg/kg/day)									
	0	1	10	100	HCI	0	1	10	100	HCI
	Foetuses (%)					Litters (incidence)				
Visceral observations										
Dilated cerebral ventricles	0.0.	0.0	0.0	4.6	0.0-1.5	0/24	0/24	0/24	2/20	0/28-1/22
Skeletal observation										
1st and 2nd sternebrae: unossified	0.0	0.0	1.3	4.2	0.0-0.4	0/24	0/24	3/24	6/20*	0/24-1/24
Extra sternebral ossification	0.0	0.0	2.5	4.2	0.0-0.7	0/24	0/24	5/24	3/20	0/24-1/23
Cartilage of 8th rib (unilateral/bilateral): attached to the sternum	2.1	0.3	2.6	14.2	0.0-2.1	1/24	1/24	4/24	9/20**	0/28-1/24
1st ribs (unilateral/bilateral): short	1.0	0.0	1.4	5.6	0.0-1.0	1/24	0/24	1/24	7/20*	0/28-1/21
Cartilage of 1st rib (unilateral/bilateral): not attached to the sternum	0.0-0.3	0.0	1.4	5.6	0.3	1/24	0/24	2/24	7/20*	0/28-1/24
Cartilage of 1st and 2nd rib (unilateral/bilateral): fused	2.1	0.6	4.2	10.2	0.0-2.1	1/24	1/24	5/24	8/20*	0/28-1/24
Presence of 27 pre-sacral vertebrae	1.4	1.2	14.2	51.5	0.0-6.5	2/24	1/24	13/24**	16/20***	0/24-7/20
13 thoracic rib(s) (unilateral/bilateral) and presence of 27 pre-sacral vertebrae	0.3	1.2	14.2	50.0	0.0-6.5	1/24	1/24	13/24**	16/20***	0/28-7/20

HCI: historical control incidence-lower and upper limit

* $p < 0.05$ (Chi squared test), ** $p < 0.01$, *** $p < 0.001$

CONCLUSION:

In a developmental toxicity study in rabbits, tembotrione was given by gavage at dose levels of 0, 1, 10 and 100 mg/kg bw/d. At the dose level of 100 mg/kg bw/d, the pregnant rabbits showed severe maternal toxicity and mortality. Five out of 25 pregnant females died prematurely between gestation day 15 and 22. Due to the severity of these effects (morality), labelling with **R48/22** was considered justified by the experts at the PRAPeR expert meeting 69 (4-8 May 2009). In addition, fetal toxicity was evident at 100 mg/kg bw/d mainly by means of effects on skeletal ossification (delayed ossification, increased variations and anomalies). Also at 10 mg/kg bw/d, slight maternal toxicity was observed together with fetal effects on skeletal ossification and the presence of extra ribs.

Dilated cerebral ventricles were observed in three fetuses of the top dose (3 pups from 2 litters), where clear maternal toxicity was observed. As no fetuses of the control group presented this finding, the incidence of this finding was considered to be related to treatment. There were no other anomalies or malformations associated with effects on the CNS or findings showing a perturbation of the cerebrospinal flux (i.e. hydrocephaly or spinal bifida) in any of the treated groups.

Furthermore, evidence was presented that the skeletal findings in dose group 10 and 100 mg/kg bw/day are attributable to inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPDase) and to the elevation of systemic tyrosine levels, a mechanism with little relevance to humans.

Comparisons were made with the compound mesotrione, showing a similar pattern of delayed fetal

ossification and increase in rib pairs in the absence of teratogenic effects. It was noted that this substance has not been labelled with respect to developmental toxicity by the ECB.

The lowest dose level tested of 1 mg/kg bw/d was considered as NOAEL for both maternal and developmental toxicity in rabbits.

4.11.3.2 Human information

Not available.

4.11.4 Other relevant information

A combination of NTBC and tyrosine administered to rats from gestation day 6 to gestation day 20 produced sustained plasma tyrosinaemia and ocular toxicity (Kennel, P.; 2006). In this group there was an increased incidence of the specific fetal ossification changes as observed also in the rat developmental toxicity study with tembotrione. The results indicate a possible causal relationship between tyrosinaemia and skeletal ossification in the rat.

Oral administration of tembotrione to pregnant rabbits from GD 4 to GD 28 leads to tyrosinaemia (Blanck, O.; 2004).

The different susceptibility to HPPDase induced tyrosinaemia among species and its relevance to humans is well documented by the therapeutic use of NTBC for artificial inhibition of HPPDase at daily dose levels of 1 mg/kg bw/day.

See mechanistic study in chapter *4.12.1.3 Specific investigations: other studies*.

4.11.5 Summary and discussion of reproductive toxicity

No reproductive toxicity was observed in a 2-generation study in rats. In a developmental toxicity study in rats, fetal delayed ossification occurred at all dose levels in a dose-related manner. In addition, the number of runt fetuses was higher in all treated groups compared to the control group. However, no teratogenic effects were observed in this study at all dose levels tested.

In a developmental toxicity study in rabbits, tembotrione produced effects on skeletal ossification (delayed ossification, increased variations and anomalies) in presence of maternal toxicity. Evidence was presented that the skeletal findings in dose group 10 and 100 mg/kg bw/day are attributable to inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPDase) which is involved in the metabolism of the amino acid tyrosine. An inhibition of the enzyme results in elevated systemic tyrosine levels (tyrosinaemia). This could be demonstrated in a separate study by oral administration of 10mg/kg bw/day tembotrione to pregnant rabbits from GD4 to GD28. This daily dose resulted in a marked enhancement of tyrosin levels with a peak at GD 10 when L- tyrosin levels where 6,4 times higher than control levels (Blanck, O.; 2004). The species-specificity towards the susceptibility to tyrosinaemia is linked to the different abilities of the species to metabolize tyrosine via a substitute enzymatic pathway, when the enzyme HPPDase is blocked. This has been demonstrated in vitro using hepatocytes (LiverbeadsTM) from rats, mice, dogs, rabbits and humans (Totis, M.; 2005). The ability of different species to metabolize cellular tyrosine via a substitute enzymatic pathway was measured through detection of the metabolite 4-hydroxyphenyl lactic acid (HPLA). Results showed that human and murine cells are able to use an

alternative pathway for the tyrosine catabolism when HPPDase is inhibited, which on the contrary is far less efficient in rabbits, dogs and rats.

Furthermore, comparisons were made with the compound mesotrione, showing a similar pattern of delayed fetal ossification and increase in rib pairs in the absence of maternal toxicity. It was noted that this substance has not been labelled with respect to developmental toxicity by the ECB.

Dilated cerebral ventricles were observed in three fetuses of the top dose, where severe maternal toxicity (20% mortality) was observed. As no fetuses of the control group presented this finding, the incidence of this finding was considered to be related to treatment. Histological slides were made available by the notifier and no other effects on the CNS were observed. At the PRAPeR expert meeting 69 (4-8 May 2009), the experts considered that the observed dilated cerebral ventricles could be related to delayed brain development.After consideration of the additional information submitted by the notifier, the RMS did not support labelling with the risk phrase 'R63' due to this malformation any longer and the other Member States agreed to this position.

4.11.6 Comparison with criteria

No effects on reproduction were observed in studies conducted with tembotrione. (according to both DSD and CLP).

Developmental effects in the form of dilated cerebral ventricles were regarded to be treatment related and therefore a classification with R63 was proposed in the original DAR.

This classification was discussed during the PRAPeR expert meeting 69 (4-8 May 2009). Histological slides were made avalaible by the notifier and no other effects on CNS were observed. Experts considered that the dilated cerebral ventricles could be related to delayed brain development Furthermore the effects mentioned above occurred only in the presence of clear maternal toxicity so labelling with R63 was not supported any longer.

Since there was clear maternal toxicity observed and enhanced mortality (20% at 100mg/kg bw/d) occurred within the first three weeks of treatment, labelling with **'R48/22'** according to DSD and **STOT RE 2, H373** according to CLP was found to be appropriate. For detailed comparison with criteria see chapter 4.7., *Repeated dose toxicity.*

4.11.7 Conclusions on classification and labelling

Taken together, no classification and labelling for effects of tembotrione regarding reproductive toxicity neither under directive 67/548/EEC nor under regulation (EC) No. 1272/2008 is proposed. The proposal of classification and labelling with R48/22 due to mortality observed in rabbits receiving 100 mg/kg bw/d is discussed in chapter *4.7 Repeated dose toxicity.*

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal The DS did not propose classification for reproductive toxicity. There were no indications of effects on fertility in a 2-generation study in rats. In the developmental studies in rats and rabbits, there were indications of delayed skeletal ossification. However, this delay was thought to be related to the tyrosinaemia, and in rabbits it also occurred in the presence of maternal mortality. Three cases of dilated cerebral ventricles were found at the top dose in the rabbit study, in the absence of any other effects on the CNS, and the effect could therefore be caused by a general delay in the brain development.

Comments received during public consultation

No classification was proposed for reproductive toxicity. Three comments were received. Two member states proposed classification for developmental toxicity and one industry organisation supported no classification.

RAC assessment and comparison with the classification criteria

Fertility

The only effect that could possibly be linked to fertility was a statistically significantly reduced number of corpora lutea in high dose F1 animals of the rat 2-generation study (26.8 vs. 40.3 in controls). There were no effects on ovarian weight, or on number of primordial and antral follicles. No historical control data were given in the CLH report. The CLH report argues that the sole reduction of corpora lutea is not considered an adverse effect. The RAC is of the opinion that a reduced number of corpora lutea can be an adverse finding, but that this isolated finding, in successfully reproducing animals, is not sufficient for classification for effects on fertility.

Developmental toxicity

The pup weights in the rat 2-generation study were not affected at birth, but growth was dose-dependently decreased during the lactation phase by up to 19% (at a dose of 100-200 mg/kg/day), from day 4 and 7 in F1 and F2, respectively. The decreased growth rate was accompanied by developmental delays (time of preputial separation and vaginal opening). Except for ocular toxicity, no other effects were noted in the parental animals. The effects in pups could thus qualify as developmental toxicity or possibly lactational toxicity. Severe ocular effects could be seen in pups of all treated groups, indicating that the pups suffered from tyrosinaemia. Whether the reduced growth rate could be related to the tyrosinaemia is not clear, but cannot be ruled out if a higher sensitivity of young versus older animals is assumed and considering the ocular toxicity observed in the pups.

In the rat developmental toxicity study, pup body weights were significantly and dose-dependently reduced at sacrifice on gestation day 21 by 3, 8 and 16% in low, mid, and high dose groups, respectively (25, 125, and 500 mg/kg/day). Many dose-dependent variations related to poor ossification were noted, some even in the low dose group (without effects on the maternal body weight). However, statistical significance is not reported and historical control incidences are not included in the CLH report. The CLH report refers to a study by Kennel (2006) to disregard the skeletal variations. The Kennel study is said to show increased incidences of delayed ossification in rats treated with tyrosine and an HPPD-inhibitor, but no data is given in the CLH report. The full study report was provided during the opinion development process and was assessed by the RAC (see in depth analysis below). The RAC notes that induced tyrosinaemia and tembotrione treatment cause similar effects on body weight and skeletal ossification, and concludes that the skeletal variations caused by tembotrione are caused by the tyrosinaemia.

Delayed ossifications were also found in the rabbit developmental toxicity study, Although maternal body weights were not affected, 20% dam mortality was noted at the highest dose, indicating that developmental effects noted at the top dose (100 mg/kg/day) could be caused by the excessive maternal toxicity and should therefore not be considered for classification. This includes the three findings of dilated cerebral ventricles (in 2 litters), occurring in the absence of other findings in the CNS. However, statistically significantly increased litter incidences of variations (extra ossification sites between atlas and axis centrum, incomplete ossification of pubis) and anomalies (presence of 27 presacral vertebrae in combination with 13 thoracic rib) were also noted at the mid dose (10 mg/kg/day). The incidences of these findings were roughly twice the highest historical control rates. In a separate study, the dose level of 10 mg/kg/day has been shown to lead to elevated concentrations of tyrosine (6-fold) in pregnant rabbits. The CLH report suggests that the delayed ossification is caused by tyrosinaemia, but the effects of induced tyrosinaemia have not been studied in rabbits.

The consistent findings of skeletal variations and anomalies, and of reduced growth of rats during the gestational and lactational phase, with secondary effects on sexual development, do not provide an undisputable argumentation for classification of developmental toxicity. However, although it has not been shown in the CLH report that tyrosinaemia decreases the growth rate, the Kennel study (2006) shows that provoked tyrosinaemia decreases the pup body weight and delays the ossification of rats at the time of birth, making it likely that the decreased growth rate also after birth could be related to the tyrosinaemia. This assumption is supported by the occurrence of eye damage in the pups, a key effect of tyrosinaemia, clearly indicating that the pups suffered from tyrosinaemia also during the lactation phase. The corneal opacities were first observed at day 23, with a similar LOAEL (the lowest dose tested) as in the dams of the 2-generation study.

The RAC therefore concludes that tembotrione affects skeletal development in rats (variations) and rabbits (anomalies and variations), and decreases pre- and postnatal growth rates in rats, at doses not affecting e.g. maternal body weights. The MoA is likely to be tyrosinaemia, leading to effects characterised by a decreased growth rate of the pups.
The criteria state:"*The classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other effects or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effect. However, when there is mechanistic information which raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."*

The effects are adverse, and could be considered for category 1B, but they are not very severe (decreased growth rate). The adverse effects on reproduction occur at doses causing tyrosinaemia in the dams, and the maternal tyrosinaemia is the likely specific mode of action for the reproductive effects. Thus, the reproductive effects are not considered to be secondary non-specific consequences of other toxic effects. Similarly, the MoA is relevant for humans, but it can also be expected that humans are less sensitive than rats. See also the 'In depth analyses by RAC' of repeated dose toxicity in the BD. Because of these uncertainties, category 1B does not seem appropriate. Still, classification is warranted, and the RAC therefore proposed classification with Repr. 2 - H361d (CLP) (Rep. Cat. 3; R63 according to DSD) in consideration of these uncertainties.

Supplemental information - In depth analyses by RAC

The study by Kennel (2006) on the effects of a provoked tyrosinaemia on rat foetal development has been further analysed by RAC. Pregnant rats were exposed to a combination of 2% L-tyrosine in the diet and a specific inhibitor of 4-hydroxy-phenylpyruvate dioxygenase (NTBC), leading to a 63-fold accumulation of tyrosine in the blood as compared to control rats. The treatment lead to smaller pups at sacrifice (mean fetal body weight was statistically significantly decreased by 7%) and a plethora of fetal skeletal variants related to a delayed ossification. The skeletal variants observed were very similar to the ones seen in rats after exposure to 25-125 mg/kg/day tembotrione, and the decrease in pup weight was also similar (7% vs 3-8% for 25-125 mg/kg/day tembotrione).

Blood concentrations of tyrosine were unfortunately not measured in the tembotrione developmental toxicity study. However, effects of tembotrione on blood concentrations of tyrosine has been studied in non-pregnant rats (Debruyne, 2009), showing 11-fold and 21 – fold increases in blood tyrosine 21 days after exposure to 0.5 and 1.7 mg/kg/day tembotrione via the diet. Higher doses of tembotrione may thus lead to such high concentrations of tyrosine that the effects on pup weight and ossification can be presumed to be caused by tyrosinaemia.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 66: Summary table of relevant neurotoxicity studies

Method	Results	Remarks	Reference
Acute neurotoxicity in rats	0, 200, 500, 2000 mg/kg bw NOAEL = 200 mg/kg bw Adverse effects: - clinical signs - reduced motor activity - decreased arousal	-	Sheets, L.P.; Gilmore, R.G.; Elcock, L.E. 2005
Subchronic 90 day neurotoxicity in rats	0, 20, 250 and 2500 ppm (equivalent to 0, 1.33, 16.4 and 160 mg/kg bw/d for males and 0, 1.75, 21.0 and 224 mg/kg bw/d for females) NOAEL = 16.4 (♂) NOAEL = 224 (♀) Adverse effects: - Reduced body weight in males - No effect in females	-	Gilmore, R. G.; Elcock, L. E., 2005
Developmental neurotoxicity in rats	0, 10, 200 and 1500 ppm (equivalent to 0, 0.8, 16.3, 118 mg/kg bw/d) NOAEL= 0.8 (Dams) Adverse effects in dams: - Corneal opacities, - reduced body weight NOAEL = 0.8 (offspring) Adverse effects in offspring: - Corneal opacities - decreased bodyweight - decreased acoustic startle in males - delayed preputial separation - lower brain weight	-	Sheets, L. P.; Gilmore, R. G.; Hoss, H. E., ; 2005

In an acute neurotoxicity study with rats administration of tembotrione produced evidence of toxicity in males and females at 500 mg/kg and at 2000 mg/kg bw but not at 200 mg/kg bw in either gender. Evidence of toxicity was confined to clinical signs and decreased activity together with decreased arousal rate on the day of treatment, with incomplete recovery during the next few days and complete recovery by day 14. There were no lesions in any tissue investigated or evidence of specific neurotoxicity at any dose. Based on these results, the "neurotoxic" NOAEL for tembotrione after acute oral administration was 200 mg/kg bw for both sexes.

Also in a rat 90-day neurotoxicity study, dietary concentrations of up to 2500 ppm of tembotrione produced no evidence of neurotoxicity. Decreased body weight and food consumption were

observed in males at 2500 ppm. There were no other signs of toxicity at the lower dose levels in males and at any dose levels in females. The study NOAEL was set at 2500 ppm for females (224 mg/kg bw/day) and at 250 ppm (16.4 mg/kg bw/day) for males.

In a developmental neurotoxicity study, tembotrione was administered via diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats at concentrations of 0, 10, 200 and 1500 ppm during gestation. No reproduction parameters were affected by treatment at any dietary dose level. However, in dams, clinical signs were apparent at 1500 ppm, and body weight, body weight gain and food consumption were reduced at 1500 and 200 ppm. Corneal opacities were also evident during lactation at the mid and high dose groups. There were no signs of maternal effects at 10 ppm.

In the offspring, at 1500 and 200 ppm, dose-related decreased body weights were observed in both sexes from PND 11 to 21 and after weaning (when exposure was discontinued), with a recovery of body weight effect in females at study termination. As a consequence of general body weight reduction, brain weight was also reduced. There was also a slight delay in preputial separation that was correlated with body weight decrease at 1500 and 200 ppm. Corneal opacities were observed in postweaning males on PND 35-45, but resolved at the end of observation. Concerning neurotoxicity the only finding was a significant decrease in acoustic startle response at the mid and high dose levels. There were no compound-related effects at 10 ppm.

In conclusion the dose level of 10 ppm (equivalent to an average maternal intake of 0.8 mg/kg bw/day) was the NOAEL for both maternal and postnatal developmental effects.

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

A series of mechanistic studies has been conducted investigating the effects on and of tyrosinaemia.

Table 67: Summary table of relevant mechanistic studies

Reference	Results	Remarks	Author(s); year
AE 0172747 - Effects on blood coagulation parameters with and without administration of vitamin K1	The coagulopathy observed in rats after tembotrione (AE 0172747) oral administration was mediated by an effect on vitamin K1 clotting factors.	-	Blanck, O.; 2005
AE 0172747 – In vitro inhibition of HPPDase using Liverbeads™ from different species	Human and mouse hepatocytes produced more HPLA (a metabolite of cellular tyrosine metabolism) than those of the rabbit, dog and rat in presence of either AE 0172747, L-Tyrosine or both. Human and mouse are able to use an alternative pathway for the tyrosine catabolism when HPPDase is inhibited, whereas in rabbit, dog and rat, this alternative pathway is much less efficient.	-	Totis, M.; 2005
Tyrosine – Exploratory 14-day (ocular toxicity) study in the rat and mouse	Addition of 5% tyrosine to the diet of male CD rats resulted in the rapid onset (48 hours) of corneal opacities with a "snow-flake"	-	Esdaile, D.J.; 1995

	appearance (a superficial keratitis). Corneal lesions were correlated with elevated plasma tyrosine levels. One of five Brown - Norway male rats at 5% tyrosine was found to have slight bilateral opacities at 14 days and a high plasma tyrosine level. No corneal opacities were seen in female rats, in mice of either sex or in any group given 2% tyrosine diets.		

Effects of diets enriched with tyrosine on selected organs in rats	Dietary administration of 10 µg/kg/day NTBC + 2% L-tyrosine or of 10µg/kg/day NTBC alone induced clear increases of plasma tyrosine levels in rats. Treatment-related clinical signs however, were only observed in animals treated with NTBC + L-tyrosine: The ophthalmological findings include oedemas of the cornea (9/10 males), congestive irisitis (3/10 males), unilateral or bilateral keratitis (9/10 males, 1/10 females) and "snow flake" corneal opacity (10/10 males and 3/10 females). Furthermore, relative liver weights were significantly increased and slight effects on the pancreas and on the thyroid gland were observed.	-	Blanck,, O.; 2006
Effects of tyrosinaemia on selected organs in rats	Administration of 2% L-tyrosine and NTBC resulted in an increase in blood tyrosine (24-fold increase in males and 18-fold in females). Treatment-related clinical signs were observed in only in this group and consisted of eye effects (half closed eyes, oedema of the cornea and "snow flake" corneal opacity, anterior synechia (iris), bilateral keratitis), effects on the pancreas (interstitial inflammation, higher incidence of acinar degeneration/apoptosis of the pancreas), and effects on the thyroid gland (minimal to slight colloid alteration).	-	Blanck,, O.; 2006
Effect of tyrosinaemia on pregnancy and embryo-fetal development in the rat	Co-administration of L-Tyrosine plus NTBC provoked maternal tyrosinaemia which causes a general delay of ossification in fetuses, whereas each substance alone did not affect fetal development. In fact there is supportive evidence from both literature and experimental data that a threshold of plasma tyrosine concentration exists (~1000 nmol/L), below which tyrosine-related findings will not be observed	-	Kennel, P.; 2006
AE 0172747 Effect on blood tyrosine level in pregnant rabbit after oral administration by gavage	Oral administration of 10 mg/kg bw/day tembotrione to pregnant rabbits from GD4 to GD28 leads to tyrosinaemia. No treatment related clinical signs, abortions, moribundity, or mortality was observed. Maternal corrected body weight change was slightly decreased in treated animals. No abnormalities were noted at the post mortem examinations.	-	Blanck, O.; 2004
Position paper: Tembotrione systemic toxicity	It was stated that due to the similarity in tyrosine kinetics between mice and humans, and based on the absence of these types of lesions in the mice studies, it can be concluded that tyrosine-related lesions observed in the rat studies are not relevant for human risk assessment.	-	Semino, G.; 2006
Tembotrione: Effect on Blood Tyrosine Level in the Rat by Dietary Administration	Administration of low levels of tembotrione to rats causes significant alterations in blood tyrosine levels. Males are more sensitive to tembotrione-induced tyrosinaemia than female	-	Debruyne E.; 2009

	rats. In male rats the critical threshold of 1000 nmol for tyrosine-related effects is exceeded already at a dose level of 6 ppm.		

The potential of tembotrione to affect blood coagulation parameters was assessed in male rats receiving 1000 mg/kg bw/d by gavage for 3 days (Blanck, O.; 2005). Prothrombin time, activated partial thromboplastin time and the coagulation time of the Vitamin K1 clotting factors were prolonged after treatment with tembotrione and at necropsy these animals had hemorrhagic foci. Concomitant administration of vitamin K1 prevented these effects of tembotrione as all parameters measured were within the background values. This study confirmed that the coagulopathy observed in male rats after tembotrione oral administration was mediated by an effect on vitamin K1 clotting factors.

The main effect of systemic exposure to tembotrione in mammals is inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPDase), which is involved in the metabolism of the amino acid tyrosine. Prolonged inhibition of this enzyme results in increased plasma tyrosine levels (tyrosinaemia). The species-specificity towards the susceptibility to tyrosinaemia is linked to the different abilities of the species to metabolize tyrosine via a substitute enzymatic pathway, when the enzyme HPPDase is blocked. This has been demonstrated in vitro using hepatocytes (LiverbeadsTM) from rats, mice, dogs, rabbits and humans (Totis, M.; 2005). The ability of different species to metabolize cellular tyrosine via a substitute enzymatic pathway was measured through detection of the metabolite 4- hydroxyphenyl lactic acid (HPLA). Results showed that human and murine cells are able to use an alternative pathway for the tyrosine catabolism when HPPDase is inhibited, which on the contrary is far less efficient in rabbits, dogs and rats.

The main consequence of sustained tyrosinaemia is the onset of eye lesions in the cornea with a "snow flake" appearance (a superficial keratitis). Evidence of the correlation between tyrosinaemia and ocular toxicity has been provided in a study in which high dietary intake of free L-tyrosine (5%) leaded to increased plasma tyrosine and the rapid onset of corneal opacities in male rats (Esdaile, D.J.; 1995). In contrast, no corneal opacities were observed in female rats, in mice and in animals given 2% L-tyrosine diets. Furthermore, in mice high intake of tyrosine did not lead to increased plasma tyrosine.

A second study (Blanck, O.; 2006) demonstrated that dietary administration of 2% L-tyrosine and 10 µg/kg/day NTBC (a drug inhibitor of HPPDase) to rats also induced ophthalmological findings such as oedemas of the cornea, keratitis and "snow flake" corneal opacities. Furthermore, increased relative liver weights and slight effects on the pancreas and on the thyroid gland were observed.

Another study on the effects of tyronsinaemia on selected organs (Black, O.; 2006) confirmed that elevated plasma tyrosine levels following administration of 2% L-tyrosine and 10 µg/kg bw/day NTBC over a prolonged period of time lead to keratitis of the eye, focal/multifocal acinar degeneration and apoptosis of the exocrine pancreas, and to colloid alterations of the thyroid follicles.

These studies support the hypothesis that the alterations only will occur if a certain threshold dose is exceeded. In all studies, male rats were more sensitive and showed higher severity of eye lesions than females. Accordingly, all dietary toxicity studies with tembotrione showed that the onset of eye lesions occurred in females either at higher doses than in males or after longer exposure time.

Sustained tyrosinaemia in the rat might also be responsible for most delayed skeletal ossification seen in the rat developmental toxicity study. Test animals were dosed with either dietary tyrosine, NTBC or a combination of NTBC and tyrosine from gestation day gestation day 6 to gestation day 20 (Kennel, P.; 2006). Sustained plasma tyrosinaemia and ocular toxicity were seen only in the

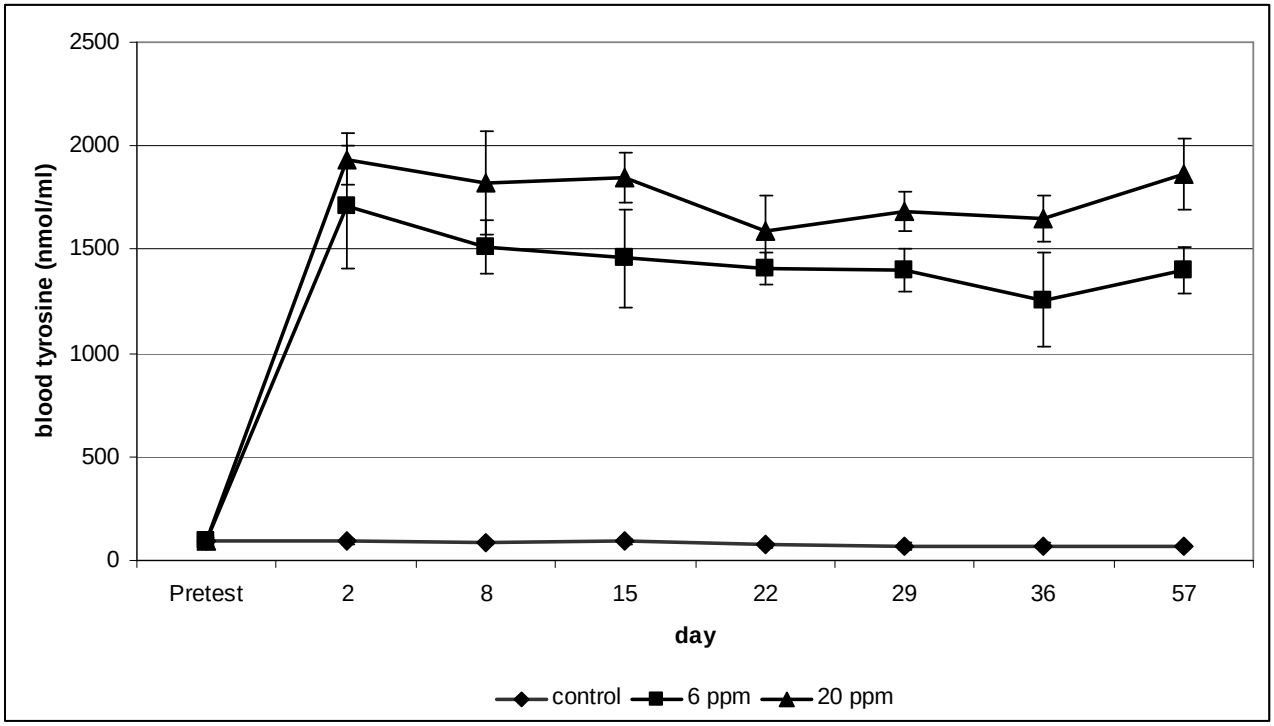
group receiving both NTBC and tyrosine. In this group there was an increased incidence of the specific fetal ossification changes observed also in the rat developmental toxicity study with tembotrione. The results indicate a possible causal relationship between tyrosinaemia and skeletal ossification in the rat.

In a rabbit study (Blanck, O.; 2004), after administration of 10 mg/kg bw/day from gestation day 4 to 28, no treatment related clinical signs, abortions, moribundity, or mortality was observed. Maternal corrected body weight change was slightly decreased in treated animals (-0.16 kg vs. -0.03 kg in controls). All dams used in the course of this study were pregnant with 9-11 foetuses per dam in the treated and 5 to 12 per dam in the control group. No abnormalities were noted at the post mortem examinations. L-tyrosine blood levels were markedly increased in the treated group (up to 6.4 times higher than in the control), with the highest levels measured at GD 10 and 15. In conclusion, oral administration of tembotrione to pregnant rabbits from GD 4 to GD 28 leads to tyrosinaemia.

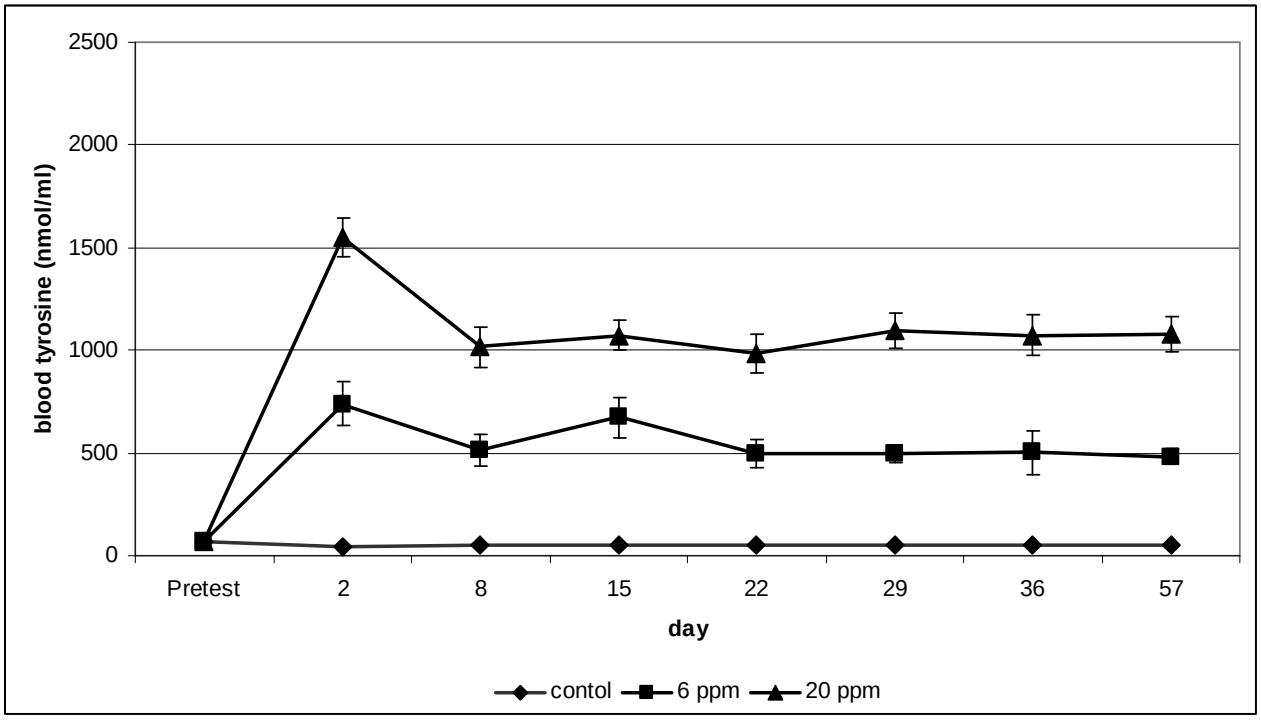
In the position paper "Tembotrione systemic toxicity" (Semino, G.; 2006), the notifier states that the typical tyrosine-related lesions on the eye, pancreas and thyroid observed in the rat studies are not relevant for human risk assessment. Mammalian species vary widely in their ability to maintain lower steady state levels of tyrosine, when HPPDase is inhibited. Rats and dogs are not able to easily excrete excess tyrosine and thus in these species administration of HPPDase inhibitors causes accumulation of tyrosine above the threshold of 1000 nmol/L. On the contrary in human and mice tyrosine aminotransferase (TAT) becomes the principal enzyme through which tyrosine can be metabolised when HPPD is inhibited, thus preventing tyrosine concentrations reaching toxic levels.

During the expert discussion (PRAPeR Round 14, Meeting 69, 4th-8th of May, 2009) it was noted that blood tyrosine levels had never been measured at low dose levels (6 and 20 ppm), and that it was therefore doubtful if such low levels of tembotrione would significantly alter blood tyrosine levels. Therefore, a mechanistic study was conducted by the notifer to show that administration of low levels of tembotrione to rats causes significant alterations in blood tyrosine levels (Debruyne, E.; 2009): Up to the highest dose level tested of 20 ppm, tembotrione did not induce any mortality or clinical signs, and had no effect on body weight, body weight gain, or food consumption. Tembotrione induced a marked dose-related increase in blood tyrosine concentration in rats in both sexes from Study Day 2 onwards; thereafter the tyrosine concentrations remained relatively stable over the entire study period. The results confirm that males are more sensitive to tembotrione-induced tyrosinaemia than female rats and that in male rats the critical threshold of 1000 nmol for tyrosine-related effects is exceeded already at a dose level of 6 ppm.

Blood tyrosine levels in male rats



Blood tyrosine levels in female rats



4.12.1.4 Human information

Not available.

4.12.2 Summary and discussion

Neurotox

Short term (dog, 90-days feeding) and long term (female rat, 104-weeks feeding) toxicity studies revealed signs of neurotoxicity (digestion chambers in nerves and atrophy, chronic inflammation and vascular mineralization of sciatic nerves, respectively). Therefore, neurotoxicity investigations were performed.

No evidence of neurotoxicity was observed in rats in an acute neurotoxicity study and in a 90-day neurotoxicity study. In a developmental neurotoxicity study in rats, the only finding concerning neurotoxicity was a significant decrease in acoustic startle response in the offspring at the mid and high dose levels (equivalent to 16.3 and 118 mg/kg bw/d, respectively). There were no compound-related effects at the lowest dose level of 10 ppm (equivalent to an average maternal intake of 0.8 mg/kg bw/d) which was considered as the NOAEL for both maternal and postnatal developmental effects. No classification is proposed.

Mechanistic studies

The main effect of systemic exposure to tembotrione in mammals is inhibition of the enzyme 4-hydroxy-phenylpyruvate dioxygenase (HPPDase), which is involved in the metabolism of the amino acid tyrosine. Prolonged inhibition of this enzyme results in increased plasma tyrosine levels (tyrosinaemia). The species-specificity towards the susceptibility to tyrosinaemia is linked to the different abilities of the species to metabolize tyrosine via a substitute enzymatic pathway, when the enzyme HPPDase is blocked.

Typical tyrosine-related lesions on the eye, pancreas and thyroid observed in the rat studies are not relevant for human risk assessment. Mammalian species vary in their ability to maintain lower steady state levels of tyrosine, when HPPDase is inhibited. Rats and dogs are not able to easily excrete excess tyrosine and thus in these species administration of HPPDase inhibitors causes accumulation of tyrosine above the threshold of 1000 nmol/L. On the contrary in human and mice tyrosine aminotransferase (TAT) becomes the principal enzyme through which tyrosine can be metabolised when HPPD is inhibited, thus preventing tyrosine concentrations reaching toxic levels.

4.12.3 Comparison with criteria

Considering the criteria for classification and labelling according to both CLP and DSD no classification for tembotrione considering neurotoxic effects is considered necessary.

4.12.4 Conclusions on classification and labelling

Directive 67/548/EEC: no classification proposed

Regulation (EC) No. 1272/2008: no classification proposed

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 68: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis rate OECD 111 USEPA 161-1 OPPTS 835.2110	The rate of hydrolysis has been determined in a sterile aqueous buffer solutions at pH 4, 7 and 9 and at 50°C and 25°C. The test substance was stable to hydrolysis under the conditions of the test.	Purity (> 99 %,)	M-107923-01-1 Fliege R., 2003
Direct phototrans-formation EEC 95/36 SETAC Europe Section 10 USEPA 162-1	The photo-degradation has been studied in sterile aqueous buffer solution at pH 7 at 25°C under artificial sunlight for a total of 10 days. The test substance was moderately photolysed with an experimental half-life of 56.3 days under the conditions of the test. A minor metabolite (glutaric acid, M4) was found at 7% at the end of the irradiation period.	Purity (> 99 %,)	M-063564-01-1 Hellpointner, E., 2004
Lifetime in the top layer of aqueous systems (calculated and real) EEC 94/37 and 95/36 German UBA ECETOC (1992)	Calculated half-life expressed as summer days: 269 days (Athens/Greece) Real photolytic half-lives in the environment according to software GCSOLAR (days) Degree latitude (°N): 30 / 40 / 50 / 60 Spring 10 / 11 / 13 / 17 Summer 9 / 9 / 10 / 11 Fall 15 / 19 / 31 / 60 Winter 20 / 34 / 72 / 234	pure substance 989 g/kg	M-063564-01-1 Hellpointner, E., 2004 M-105933-01-1 Hellpointner E., 2003
Ready biodegradability CD 92/69/ EEC C.4-E, OECD 301 D	Tembotrione (AE 0172747) is considered to be not readily biodegradable	purity 98.9 %	Weyers, A., 2005
Aerobic aquatic metabolism in sediment system 'Rhein' US-EPA: N: 162-4, PMRA (6.2.C.2), OECD 308	Based on the 1st tier approach, the $DegT_{50}$ of tembotrione (AE 0172747) in the total system was calculated to be 176 and 65.9 days for the 'Nidda' and 'Rhein' system, respectively, with a geometric mean of 108 days (following SFO kinetics, $R^2 > 0.98$).	(geometric mean in 2 Sytems)	Nicolaus, B., 2004
SETAC (1995), OECD 307 (2000) SETAC (1995), OECD 307 (2000) US-EPA §162-1, N (1982)	The route of aerobic degradation (metabolism) of tembotrione (AE 0172747) in soils was established in three laboratory studies: Under aerobic viable conditions and temperatures of 20 – 25 °C tembotrione (AE 0172747) degraded with a half-life time of 4.3 – 56.4 days (n = 6) following SFO kinetics (R2 > 0.86).		Fliege, R., 2003a Fliege, R., 2003b Dominic, A. R., Arthur, E. L., Mislankar, S. G., 2005

Method	Results	Remarks	Reference
OECD Guideline 307 OECD Guideline 307	The route of anerobic degradation (metabolism) of tembotrione in soils was established in two laboratory studies: Under anaerobic conditions degradation of tembotrione (AE 0172747) significantly slowed down, calculated DT50 was 278 days (n = 2, mean of both labels, SFO kinetics, R2 > 0.60).		Mathew, A. E., Desmarteau, D. A., 2004 Mathew, A. E., Desmarteau, D. A., 2005

5.1.1 Stability

Hydrolytic degradation (OECD Annex IIA 7.5)

Reference:	**Abiotic hydrolysis of ^{14}C-AE 0172747 in buffered aqueous solutions at pH 4, pH 7, and pH 9**
Author(s), year:	Fliege, R., 2003
Report/Doc. number:	MEF-083/03
Guideline(s):	US EPA, N: §161-1 (1982); US EPA: OPPTS 835.2120 (1998); OECD 111 (1981)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	[Cyclohexyl-U-^{14}C]-tembotrione (AE 0172747), 6.97 MBq mg^{-1}, ≥ 96.9 % radiochemical purity (HPLC)
Reference substances:	Tembotrione (AE 0172747, unlabelled), M6 (AE 0456148), M1 (AE 0968400), M7 (AE 1124336)
Test systems:	pH 4: 0.01 M citrate buffer (adjusted with 1 M NaOH) pH 7: 0.01 M tris(hydroxymethyl)aminomethane buffer (adjusted with 1 M HCl) pH 9: 0.01 M borate buffer (adjusted with 1 M NaOH) All buffers sterilized (autoclaved) and checked for sterility throughout the test duration. Oxygen content reduced by nitrogen bubbling
Test temperature:	50 °C (pre-test) and 25 °C (main test) in the dark
Test duration:	5 days (pre-test) and 30 days (main test)
Sample concentration:	1.8 mg L^{-1}
Co-solvent:	Acetone, < 0.1 % of total volume
Analysis:	LSC, HPLC-UV/RAM, TLC LOD < 1 % of AR

FINDINGS:

Material balance was in the range of 94.1 – 103.5 % of AR in both experiments. No distinct time-dependent degradation of tembotrione (AE 0172747) was observed, tembotrione (AE 0172747) accounted for 91.8 – 99.1 % of AR throughout the pre-test and 89.9 – 101.0 % of AR in the main test. No single radioactive species other than the parent test item occurred at levels > 2 % of AR.

CONCLUSION:

Tembotrione (AE 0172747) is hydrolytically stable under abiotic environmental conditions, its hydrolytic half-life can be expected to exceed on year.

Photolytic degradation (OECD Annex IIA 7.6)

Reference:	**Phototransformation of AE 0172747 in sterile water buffered at pH 7**
Author(s), year:	Hellpointner, E., 2004
Report/Doc. number:	MEF-412/03
Guideline(s):	94/37/EC, 95/36/EC, SETAC (1995)
GLP:	Yes
Deviations:	None
Validity:	Yes

MATERIAL AND METHODS:

Test substances:	[Cyclohexyl-U-^{14}C]-tembotrione (AE 0172747), 6.38 MBq mg^{-1}, > 99 % radiochemical purity [Phenyl-U-^{14}C]-tembotrione (AE 0172747), 5.21 MBq mg^{-1}, > 99 % radiochemical purity
Reference substances:	Tembotrione (AE 0172747, unlabelled)
Test systems:	Sterile pH 7.0 buffer (0.01 M phosphate), adjusted with NaOH, sterility was checked throughout the experiment
Test temperature:	25 ± 1 °C
Test duration:	10 days continuous irradiation (equivalent to 47.8 solar midsummer days in Athens, Greece, 38.0 °N) or dark incubation
Sample concentration:	1.0 mg L^{-1}
Co-solvent:	Acetonitrile (0.2 %, v/v)
Test system:	Xenon arc lamp (Suntest), cut-off < 290 nm, 640 W m^{-2} (300 – 800 nm), 20 cm above test vessel Light intensity measured by spectroradiometer
Volatile traps:	1 x soda lime and 1 x polyurethane foam
Analysis:	LSC, HPLC-UV/RAM, HP-TLC, TLC (for polars), LC-MS/MS (ESI+/-), NMR LOQ = 1.0 % of AR
Kinetic evaluation:	Simple first order (SFO) kinetics, ModelManager 1.1, curve fit based on mean values of both labels

FINDINGS:

Mass balance was in a range of 100.3 – 105.5 % of AR for irradiated samples and 101.5 – 104.6 % of AR for dark control samples. In irradiated samples $^{14}CO_2$ increased to approx. 2 % of AR by 10 DAT (both labels), in dark controls a maximum amount of 0.1 % of AR was detected as $^{14}CO_2$, no significant amounts of other volatiles were found. Tembotrione (AE 0172747) showed moderate photo-degradation under the experimental conditions, no degradation was observed in dark samples. One major product, pentanedioic acid (glutaric acid, AE 1275213), was found at maximum levels of 6.8 % of AR at study end using Cy-He labelled tembotrione (AE 0172747). All other peak zones (all unidentified) were < 5 % of AR. It is likely that solar irradiation mainly affects the Cy-He moiety of tembotrione (AE 0172747).

Table 69: Photo-transformation of tembotrione (AE 0172747) in sterile water buffered at pH 7 [% of AR].

Label	Conditions	DAT	CO_2	Volatiles	Tembotrione (AE 0172747)	Peak B	Peak C	Peak D	Others[b]
Cy-He	Irradiated	**0**	nd[a]	nd	100.0	nd	nd	nd	nd
		1	0.2	nd	102.2	nd	< 1.0	nd	< 1.0
		2	0.3	nd	97.6	nd	1.1	nd	2.2
		3	0.4	nd	97.7	nd	1.6	nd	2.3
		6	1.0	nd	93.5	1.3	**1.8**	1.2	5.4
		8	1.5	nd	93.3	**1.6**	1.5	nd	7.1
		10	1.9	nd	87.5	**1.6**	1.5	**1.6**	**9.6[c]**
	Dark	**0**	nd	nd	100.0	nd	nd	nd	nd
		3	< 0.1	nd	102.2	nd	nd	nd	nd
		10	0.1	nd	104.6	nd	nd	nd	nd
Ph	Irradiated	**0**	nd	nd	99.0	**< 1.0**	nd	nd	nd
		1	1.0	nd	100.5	**< 1.0**	nd	1.6	nd
		2	0.2	nd	98.1	nd	0.0	2.1	nd
		3	0.3	nd	98.7	nd	< 1.0	2.7	nd
		6	0.7	nd	94.6	nd	2.1	3.3	3.5
		8	1.0	nd	92.7	nd	2.6	3.1	4.8
		10	1.0	nd	88.3	nd	**3.0**	**3.4**	**6.2**
	Dark	**0**	nd	nd	99.0	nd	nd	nd	nd
		10	0.1	nd	101.3	nd	nd	nd	nd

[a] nd denotes not detected.
[b] Predominately polar compounds, each < 5 % of AR by RP-18 TLC, except pentanedioic acid (glutaric acid, AE 1275213).
[c] Amounts of pentanedioic acid (glutaric acid, AE 1275213) = 6.8 % of AR (only investigated in 10 DAT sample).

Table 70: Calculated photolytic DT50 and DT90 of tembotrione (AE 0172747) in 0.01 M phosphate buffer at pH 7 using SFO kinetics (mean value of both labels).

	DT_{50} [days]	DT_{90} [days]	R^2	Kinetics
Irradiated (mean of both labels)	56.3[a]	187[a]	0.92	SFO
Dark	Stable	Stable	-	SFO

[a] Interpolated value.

CONCLUSION:
Tembotrione (AE 0172747) showed moderate photochemical degradation under experimental conditions, resulting in a DT50 of 56.3 days (SFO kinetics, R2 = 0.92). This half-life corresponds to 269 solar summer days in Athens (Greece). No degradation product exceeded 5 % of AR, with the exception of pentanedioic acid (glutaric acid, AE 1275213, 6.8 % of AR at study end). Solar radiation under environmental conditions does not significantly contribute to the overall degradation of tembotrione (AE 0172747) in aqueous solutions.

COMMENTS (RMS):
DT50 values were calculated by the notifier including both labels, an additional calculation by the RMS shows, that the two labels show a similar rate of degradation.

Table 71: Calculated photolytic DT_{50} and DT_{90} of tembotrione (AE 0172747) (AE 0172747, both labels separate) in 0.01 M phosphate buffer at pH 7 using SFO kinetics (calculated by the RMS).

Conditions	Cy-He			Ph			Kinetics
	DT_{50} [days]	DT_{90} [days]	R^2	DT_{50} [days]	DT_{90} [days]	R^2	
Irradiated	53.2	178	0.90	60.0	199	0.92	SFO

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

5.1.2.2 Screening tests

Ready biodegradability of the active substance (OECD Annex IIA 7.7)

Reference:	**AE 0172747 - Biodegradation**
Author(s), year:	Weyers, A., 2005
Report/Doc. number:	1352 N/05 C
Guideline(s):	CD 92/69/EEC C.4-E, OECD 301 D
GLP:	Yes
Deviations:	No
Validity:	Yes

MATERIAL AND METHODS:

Test substance:	Tembotrione (AE 0172747, unlabelled), purity 98.9 %
Reference substance:	Na-benzoate
Inoculum:	Secondary effluent of a domestic sewage treatment plant (5 mL L^{-1})
Treatments:	• Blank control • Reference substance: Na-benzoate (2.9 mg L^{-1}) • Test substance: Tembotrione (AE 0172747, 5.2 mg L^{-1}) • Toxicity control: Tembotrione (AE 0172747, 5.2 mg L-1) and Na-benzoate (2.9 mg L^{-1})
Analysis:	Chemical oxygen demand (COD)
Incubation conditions:	22 ± 2 °C, 28 days

FINDINGS:

Table 72: Biodegradation of tembotrione (AE 0172747) and reference compound [% of theoretically possible degradation].

DAT	Reference substance	Test substance	Toxicity control
7	73	7	30
14	71	7	33
21	80	5	35
28	83	4	32

Within 28 days a degradation of 4 % was determined for tembotrione (AE 0172747). The reference substance (Na-benzoate) has reached level for ready biodegradability by 14 days.

CONCLUSION:

Tembotrione (AE 0172747) is considered to be not readily biodegradable.

COMMENTS (RMS):

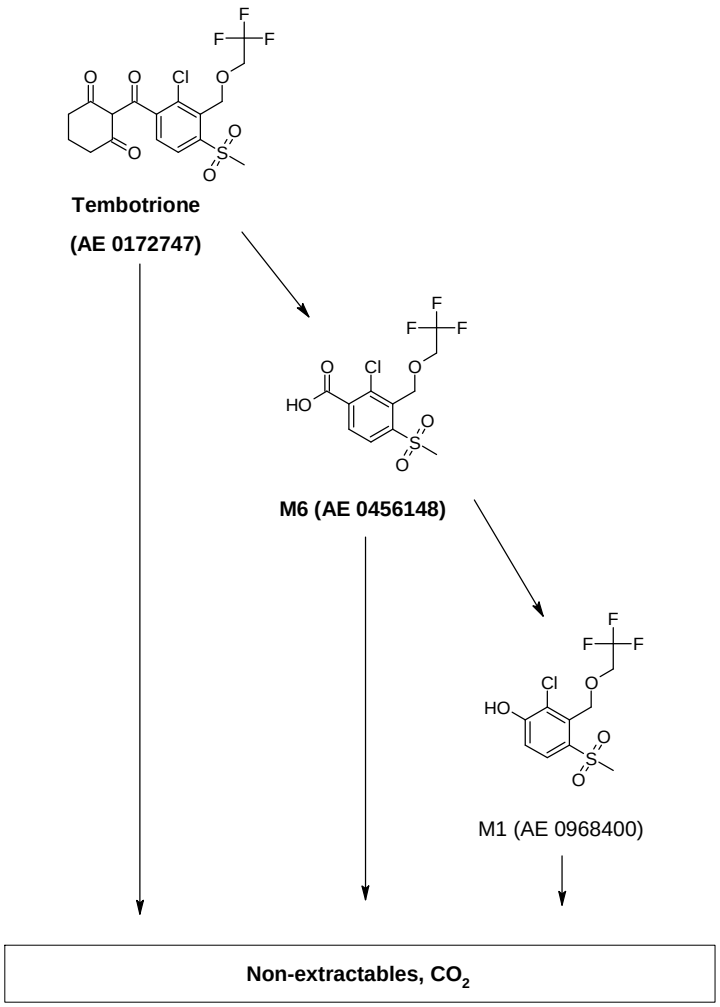
None.

5.1.2.3 Simulation tests

Reference:	**[Cyclohexyl-U-^{14}C]- and [phenyl-U-^{14}C]-AE 0172747: Aerobic aquatic metabolism in sediment system 'Rhein'**
Author(s), year:	Nicolaus, B., 2004
Report/Doc. number:	MEF 391/03
Guideline(s):	US-EPA: N: 162-4, PMRA (6.2.C.2), OECD 308
GLP:	Yes
Deviations:	None
Validity:	Yes

Dark water/sediment studies were conducted under aerobic conditions with two contrasting (pH, texture) natural systems, 'Nidda' and 'Rhein', using Cy-He and Ph labelled tembotrione (AE 0172747). The 'Nidda' system represents a silt loam sediment with an organic carbon content of 4.2 %, a high amount of microbial biomass (1505 µg microbial C g-1) and a pH of 5.9 (CaCl2). The 'Rhein' system is characterized by a sand sediment with a pH of 7.1 (CaCl2), with a similar organic carbon content of 4.5 % but a lower microbial biomass (452 µg microbial C g-1).
Ph labelled tembotrione (AE 0172747) did hardly release 14CO2, whereas formation of 14CO2 using Cy-He labelled parent was pronounced (63 and 67 % of AR at study termination). As frequently observed in other studies, the Cy-He moiety is much more accessible to microbial mineralization. In the water/sediment system the Ph moiety of tembotrione (AE 0172747) is of high persistence. Formation of NER was relatively small with a maximum amounts of 9 - 22 % of AR.
In the total system, decline of tembotrione (AE 0172747) followed SFO kinetics with DegT50 of 176 days and 65.9 days in 'Nidda' and 'Rhein' system, respectively (geometric mean is 108 days). Dissipation in the water phase was calculated to be within 17.5 and 45.1 days. To derive reasonable degradation rates of tembotrione (AE 0172747) in the water and in the sediment phase, the two water/sediment systems were implemented into an inverse modelling using TOXSWA 1.2. Assuming no degradation of tembotrione (AE 1072747) in the water phase (DegT50 set to 1000 days, conservative assumption owing to results from hydrolysis), degradation rates of 153.2 and 17.0 days in the sediment were derived for 'Nidda' and 'Rhein' system, respectively (geometric mean 51.0 days). This 'couple' of degradation values for tembotrione (AE 0172747) in the water and sediment phase is considered appropriate for further surface water risk assessment.

The only metabolite occurring in the water/sediment studies > 10 % of AR was M6 (AE 0456148), which steadily accumulated in both systems towards the end of the experiment (maximum occurrence 61.8 % of AR in the total system of 'Nidda' and 96.7 % of AR in the 'Rhein' system. According to multi-compartment modelling M6 (AE 0456148) is considered to be stable in the total system.
As a minor metabolite M1 (AE 0968400) occurred at a maximum of 4.4 % of AR in the total system of the 'Rhein' system at study termination.



Proposed degradation pathway of tembotrione (AE 0172747) in water/sediment systems (metabolites > 10 % of AR are indicated in bold).

Table 73: Summary on DT50 and DT90 [days] for the dissipation and degradation of tembotrione (AE 0172747) and M6 (AE 0456148) in laboratory water/sediment studies.

Substance / system	Water				Sediment		Total system	
	Degradation		Dissipation		Degradation		Degradation	
	$DegT_{50}$	$DegT_{90}$	DT_{50}	DT_{90}	$DegT_{50}$	$DegT_{90}$	$DegT_{50}$	$DegT_{90}$
Tembotrione (AE 0172747)								
'Nidda'	1000	3320	17.5	58.0	153	508	176	584
'Rhein'	1000	3320	45.1	150	17.0	56.4	65.9	219
Geometric mean	**1000**	**3320**	**28.1**	**93.3**	**51.0**	**169**	**108**	**358**
M6 (AE 0456148)								
'Nidda'	nc	nc	nc	nc	nc	nc	Stable	Stable
'Rhein'	nc	nc	nc	nc	nc	nc	Stable	Stable
Geometric mean	**nc**	**nc**	**nc**	**nc**	**nc**	**nc**	**Stable**	**Stable**

Table 74: Summary on maximum occurrence [% of AR] of tembotrione and metabolites in water/sediment studies (based on individual replicates, data stated in brackets give day of maximum occurrence, values shaded in grey were used for surface water risk assessment).

Compartment	Tembotrione (AE 0172747)	M6 (AE 0456148)	M1 (AE 0968400)
Water	-	77.4 (141)	3.0 (365)
Sediment	67.7 (61)	22.1 (365)	1.4 (365)
Total	-	96.7 (141)	4.4 (365)

5.1.3 Route and rate of degradation in soil

Under **aerobic, viable conditions** the metabolism of tembotrione (AE 0172747) was shown to proceed via loss of the cyclohexane dione moiety to form the benzoic acid M6 (AE 0456148, maximum occurrence in the lab 72.4 % of AR by 14 DAT). The carboxylic acid function of M6 (AE 0456148) is subject of further conversion into the phenol type component M1 (AE 0968400, max. 14.9 % of AR by 30 DAT). The formation of the methyl phenol metabolite M7 (AE 1124336, max. 8.7 % of AR by 182 DAT) could be explained by methylation of the phenol M1 (AE 0968400). The metabolism of tembotrione (AE 0172747) is mainly driven by microbial processes.

In contrast to experiments with four EU soils, the carboxy benzylic alcohol M2 (AE 1392936) was additionally observed in two US soil at maximum amounts of 4.2 % of AR over an incubation period of 179 days. This metabolite results from a hydrolysis at the ether bond (1,1,1-trifluoroethyl moiety) of the benzoic acid M6 (AE 0456148). The increase of M2 (AE 1392936) in one of the two US soils from 179 DAT onwards to a maximum occurrence of 17.1 % of AR by 270 DAT is mainly attributed to the reduced microbial biomass and is not considered relevant for further risk assessment. From results of all other degradation rate studies (one US soil, four EU soils) and from results of six representative field studies (not detected > LOD) it can be concluded that M2 (AE 1392936) is unlikely to exceed 5 % of AR in viable soils.

Apart from the observation of metabolite M2 (AE 1392936) in the two US soils, the metabolic profiles were similar at all temperatures and in all soils, but varied in quantity and by time for each component.

The incubation of Cy-He labelled tembotrione (AE 0172747) resulted in rapid mineralization with no intermediate degradates.

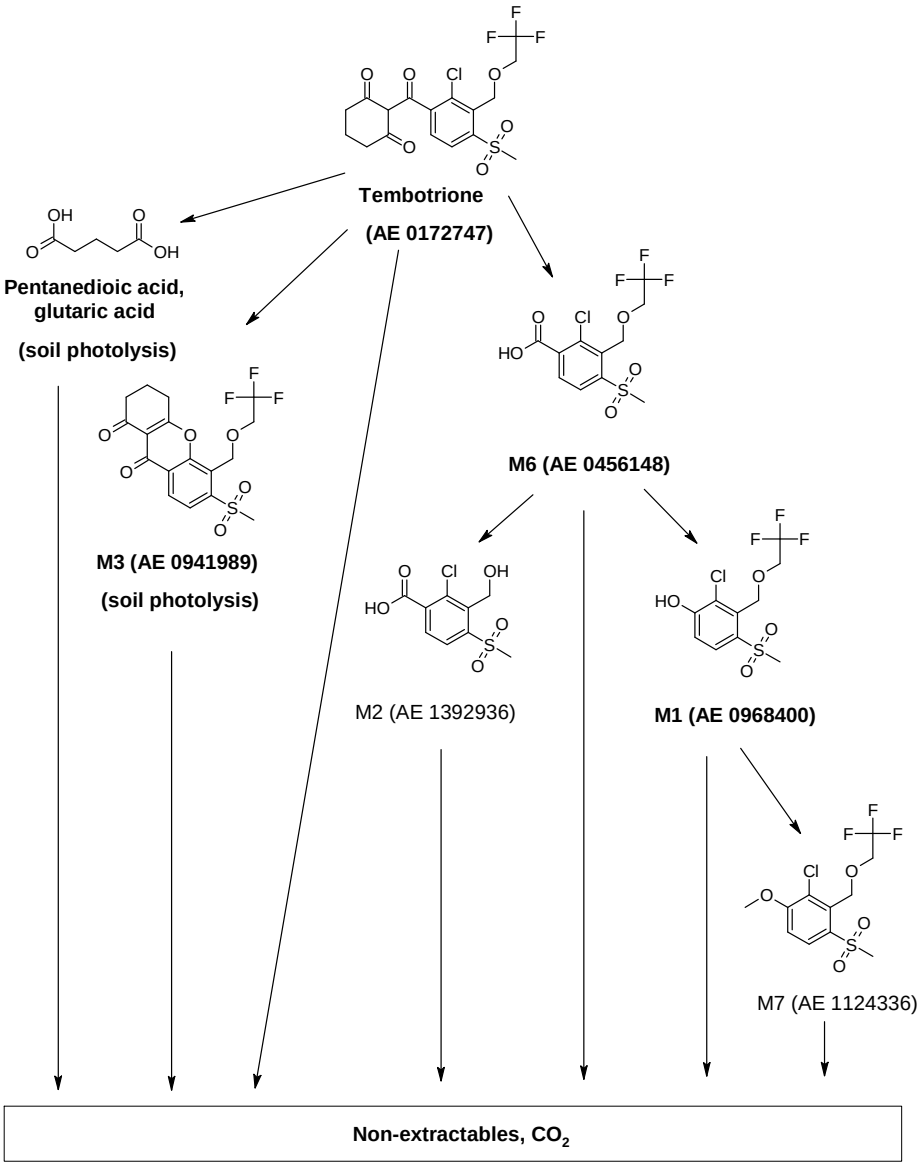
The occurrence of non-extractable residues (maximum 20 – 41 % of AR using Ph label and 19 – 26 % of AR using Cy-He label) is in line with a significant formation of $^{14}CO_2$ (13 – 55 % of AR by 120 DAT using Ph label and 37 – 77 % of AR by 120 DAT using Cy-He label). Formation of $^{14}CO_2$ was generally more pronounced using Cy-He labelled tembotrione (AE 0172747) indicating a lower persistence of the cyclohexane dione moiety in comparison to the phenyl moiety.

Under **aerobic, sterile conditions** no degradation of tembotrione (AE 0172747) occurred (one study) indicating that the metabolism of tembotrione (AE 0172747) in viable soils (kept under dark conditions) is mainly driven by soil microbial activity.

Under **anaerobic conditions** degradation of tembotrione (AE 0172747) significantly slowed down (both labels), metabolites present at the onset of the anaerobic phase (M6, AE 0456148) did hardly increase or decrease indicating that the overall degradation of tembotrione (AE 0172747) and its soil metabolites is almost negligible under anaerobic conditions. This assumption is confirmed by

extremely low formation of $^{14}CO_2$ (both labels) after onset of the anaerobic phase. However, amounts of NER increased during the anaerobic phase.

Photo-degradation on the soil surface investigated by use of both labels in an acidic soil (Pikeville) was shown to be significant under the conditions of the test. Ph labelled tembotrione (AE 0172747) degraded to the soil metabolite M6 (AE 0456148) and the photolytic metabolite M3 (AE 0941989, maximum 15.3 % of AR) under irradiated conditions. Using Cy-He labelled tembotrione (AE 0172747) the photolytic metabolites pentanedioic acid (glutaric acid, maximum 13.8 % of AR) and M3 (AE 0941989, maximum 17.9 % of AR of AR) were found.



Proposed metabolic pathway of tembotrione (AE 0172747) in aerobic, viable soils including soil photolysis (metabolites > 10 % of AR are indicated in bold letters).

Table 75: Summary on maximum observed amounts [% of AR] of metabolites of tembotrione (AE 0172747) in laboratory soil studies, numbers in brackets give day of maximum occurrence).

Study	Aerobic (20 – 25 °C)	Aerobic (10 °C)	Anaerobic	Soil photolysis
M6 (AE 0456148)	72.4 (14)	70.6 (35)	na[a]	22.0 (9)
M1 (AE 0968400)	14.9 (30)	23.3 (120)	-	4.5 (9)[b]
M2 (AE 1392936)	4.2 (179)[c]	-	-	-
M7 (AE 1124336)	8.7 (182)	-	-	-
M3 (AE 0941989)	-	-	-	15.9 (3)
Glutaric acid (AE 1275213)	-	-	-	13.8 (3)

[a] na denotes not applicable, amounts of metabolite formed during aerobic phase ('aging') did not significantly change during anaerobic phase.
[b] M1 (AE 0968400) co-elutes with AE 0172747-phenol acid, both tentatively characterized.
[c] Maximum occurrence considered relevant in soil.

Rate of degradation in soil

The laboratory soil degradation rate of tembotrione (AE 0172747) was investigated in total 6 soils with a representative range of properties (pH, organic C, texture, origin), at varying temperature (10, 20 and 25 °C) and varying incubation conditions (aerobic, anaerobic and sterile) using Ph and Cy-He labelled tembotrione (AE 0172747).

- pH ($CaCl_2$): 5.6 – 7.8
- Organic C: 1.3 – 4.5 %
- Clay content: 3 – 44 %
- Temperature: 10, 20 and 25 °C
- Incubation conditions: aerobic, anaerobic (water logged), sterile

Under **aerobic viable conditions** and temperatures of 20 – 25 °C tembotrione (AE 0172747) degraded with a half-life time of 4.3 – 49.2 days (n = 5) following SFO kinetics (R^2 > 0.92, chi^2 error ≤ 12.4 %). In one acidic soil ('Pikeville') best fit was achieved by DFOP kinetics with a conservative SFO $DegT_{50}$ of 103 days (based on the degradation rate k_2 of the slower degrading DFOP compartment). Respective $DegT_{90}$ values were 14.2 – 342 days. No significant differences in the degradation rates between the two label positions of tembotrione (AE 0172747) were observed. Degradation of tembotrione (AE 0172747) was strongly depending on soil pH, highest degradation rates were observed in more alkaline soils. No dependency on amounts of organic or microbial C could be observed. After normalization to 20 °C and pF2, DT_{50} of tembotrione (AE 0172747) was in a range of 3.8 – 80.5 days (n = 6) . In respect to the distinct pH dependence of degradation, the geometric mean $DegT_{50}$ is not considered appropriate for deriving an end point for the laboratory degradation rate of tembotrione (AE 0172747). Based on linear regression, a pH specific $DegT_{50}$ for tembotrione (AE 0172747) can be derived by the linear function: $DegT_{50}(pH, H_2O) = -31.1 \cdot pH(H_2O) + 249$ days, with min / max values of 3.8 / 66 days, respectively. Similar pH dependence was also observed in the field dissipation trials (used for higher tier risk and exposure assessment) and adequately taken into account to derive FOCUS scenario specific modelling end points for tembotrione (AE 0172747).

At 10 °C degradation half live of tembotrione (AE 0172747) in one soil was 15.3 days (n = 1) following SFO kinetics (R^2 = 0.998, chi^2 error = 2.9 %). Respective $DegT_{90}$ was 50.7 days.

The six aerobic soil degradation studies, conducted at 20 or 25 °C, were subjected to extensive rate evaluation (multi-compartment modelling based on SFO kinetics for all metabolites and SFO or

DFOP kinetics (depending on best fit) for the parent) including the soil metabolites M6 (AE 0456148), M1 (AE 0968400), M7 (AE 1124336) and M2 (AE 1392936).

The degradation rate of metabolite M6 (AE 0456148), directly formed from the parent, was calculated to be within a range of 5.1 – 73.0 days (n = 5, SFO kinetics, $R^2 > 0.71$, chi^2 error ≤ 32.2 %). Respective $DegT_{90}$ values were 17.1 – 242 days. The kinetic evaluation of M6 (AE 0456148) in the 'Pikeville' soil (parent following DFOP kinetics) with a $DegT_{50}$ of 177 days is not considered valid (p of t-test > 0.10) and is therefore taken into account for the laboratory endpoint $DegT_{50}$ of this metabolite. Metabolite M6 (AE 0456148) was formed from the parent with a formation fraction of 0.363 – 1.000 with an arithmetic mean of 0.851. Normalization to 20 °C and pF2 gave a $DegT_{50}$ in the range of 3.4 – 63.9 days with a geometric mean of 11.6 days. Degradation of M6 (AE 0456148) was higher in soils rich in microbial C. No clear dependency on soil pH was observed.

Soil metabolite M1 (AE 0968400), which is formed from M6 (AE 0456148) with a formation fraction of 0.178 – 0.362 (arithmetic mean 0.283), showed a $DegT_{50}$ in a range of 11.9 – 39.8 days (n = 3, SFO kinetics, $R^2 > 0.82$, chi^2 error ≤ 39.1 %), respective $DegT_{90}$ was in a range of 39.6 – 132 days. For three soils no reliable degradation data could be obtained (low occurrence, bad visual and statistic fit). After normalization to 20 °C and pF2 a geometric mean $DegT_{50}$ of 21.8 days was achieved.

Degradation of M1 (AE 0968400) leads to the formation of M7 (AE 1124336), which was formed with a formation fraction of 0.272 – 1.000 (arithmetic mean 0.757). M7 (AE 1124336) degraded with a $DegT_{50}$ in a range of 11.5 – 60.8 days (n = 3, SFO kinetics, $R^2 > 0.91$, chi^2 error ≤ 16.6 %) . Respective $DegT_{90}$ values were in a range of 38.1 – 202 days. For three soils no reliable degradation data could be obtained owing to low occurrence. Since two of the three evaluated $DegT_{50}$ values are affected by a low p of t-test (based on visual assessment the fit is acceptable) the moisture and temperature normalized worst case $DegT_{50}$ (i.e. 53.4 days) is considered appropriate as conservative modelling endpoint for M7 (AE 1124336).

The metabolite M2 (AE 1392936), deriving from M6 (AE 0456148) via the loss of the trifluoroethyl moiety, was only detected in the two US soils. Kinetic evaluation using the multi-compartment approach gave no statistically relevant degradation rates. Therefore, a separate degradation study using M2 (AE 1392936) as parent was conducted on three soils. Obtained $DegT_{50}$ was in a range of 7.9 – 15.6 days (n = 3, SFO kinetics, $R^2 > 0.99$, chi^2 error ≤ 7.0 %, geometric mean 11.3 days). Respective $DegT_{90}$ values were 26.2 – 51.9 days. After normalization to pF2 and 20 °C a geometric mean DT_{50} of 10.7 days was calculated. Degradation studies were conducted on similar soils within a narrow pH range of 7.1 – 7.4. No degradation data in more acidic soils are available for M2 (AE 1392936). However, M2 (AE 1392936) is not considered as a major metabolite in soil and from molecule structure it can be deduced that M2 (AE 1392936) will be only present in the ionized form at an environmental relevant pH range of 5 – 9. No distinct differences in degradation rates are expected in soils with different pH values. Therefore, the available information on the degradation rate of M2 (AE 1392936) is considered sufficient for a final risk assessment. The formation fraction of M2 (AE 1392936), used for groundwater risk assessment, is based on one reliable value from multi-compartment modelling (i.e. 0.147).

For the major soil photolysis metabolite M3 (AE 0941989) a separate degradation study using the metabolite as parent was conducted on three soils. M3 (AE 0941989) degraded rapidly with a $DegT_{50}$ in a range of 1.1 – 1.3 days (n = 3, SFO kinetics, $R^2 > 0.99$, chi^2 error ≤ 19.0 %, geometric mean 1.1 days). Respective $DegT_{90}$ values were 3.5 – 4.3 days. These studies were conducted on similar soils with a narrow pH range of 7.1 – 7.4. To obtain further degradation rates in more acidic soils, the soil photolysis study (soil pH 4.6) with tembotrione (AE 0172747) as parent was subjected to detailed multi-compartment modelling. The $DegT_{50}$ of metabolite M3 (AE 0941989) was

calculated to be 5.2 and 9.8 days (two parent labels) following SFO kinetics ($R^2 > 0.89$). Under irradiation M3 (AE 0941989) was formed from the parent with an arithmetic mean formation fraction of 0.521. Finally, normalization of all available degradation rate data of M3 (AE 0941989) to pF2 and 20 °C gave a range of 1.1 – 6.2 days with a geometric mean of 1.6 days. Owing to the reduced microbial activity under irradiation conditions the degradation rates of M3 (AE 1392936) obtained from soil photolysis study (with tembotrione (AE 0172747) as parent) are considered as conservative values.

Under **anaerobic conditions** degradation of tembotrione (AE 0172747) significantly slowed down, calculated $DegT_{50}$ was 278 days (n = 2, geometric mean of both labels, SFO kinetics, $R^2 > 0.60$, chi^2 error ≤ 5.8 %). Amounts of the major soil metabolite M6 (AE 0456148) formed before the onset of the anaerobic phase (5 days of aerobic 'soil ageing') did not significantly change during the anaerobic incubation phase. Therefore, tembotrione (AE 0172747) and metabolites (at least M6, AE 0456148) are considered fairly stable in soil under anaerobic conditions.

Under **aerobic sterile conditions** no degradation of tembotrione (AE 0172747) occurred (n = 1) indicating that degradation of tembotrione (AE 0172747) is mainly driven by microbial activity.

Dissipation of tembotrione (AE 017274) during **photolysis on the soil surface**, conducted with the acidic 'Pikeville' soil, clearly followed biphasic kinetics with a rapid initial decline to approx. 60 % of AR by 3 DAT, followed by a distinct slower dissipation afterwards. Assuming that the initial phase is attributed to the impact of irradiation only (representing the fast declining compartment of DFOP kinetics), the experimental net half-life of tembotrione (AE 0172747) was 0.7 days in this soil. This experimental net degradation half-life corresponds to 3.8 environmental solar days in Athens (Greece, EU) at 38 °N, indicating that photolysis on the soil surface may contribute to the overall degradation of tembotrione (AE 0172747) in soil.

Table 76: Summery on laboratory and temperature and moisture normalized DegT50 and DegT90 of tembotrione (AE 0172747) in laboratory soil degradation studies conducted at 20 – 25 °C under aerobic conditions.

Soil	Texture	pH ($CaCl_2$)	pH (H_2O)	Organic C [%]	Clay (USDA) [%]	Non-normalized		Normalized	Kinetics
						$DegT_{50}$ [days]	$DegT_{90}$ [days]	$DegT_{50}$ [days]	
'SL S'[a]	Silt loam	7.1	7.7	1.72	19.9	4.3	14.2	4.2	SFO
'Flint Hall'	Clay	7.8	8.2	2.54	44.4	5.7	18.9	3.8	SFO
'SL 2.3'	Sandy loam	6.4	7.4	1.33	10.7	14.8	49.0	12.9	SFO
'LS 2.2'	Loamy sand	5.6	5.9	2.76	4.4	49.2	163.3	49.2	SFO
'Pikeville'[a]	Loamy sand	5.6	6.3	1.57	3	103	342	80.5	SFO (DFOP, k_2)[b]
'Northwood'	Silt loam	7.3	7.6	4.52	18	6.6	21.9	6.6	SFO
Geometric mean						**na**	**na**	**na** (13.2)	

na denotes not appropriate for endpoint owing to pH dependence (linear regression my be used to derive pH dependent $DegT_{50}$ values, the number in brackets indicates the geometric mean without taking into account pH dependence)

[a] Ph and Cy-He label handled as replicates.

[b] Conservative SFO $DegT_{50}$ based on the slower degrading DFOP compartment (k_2)

Table 77: Summery on laboratory DT50/DegT50 and DT90/DegT90 of tembotrione (AE 0172747) in laboratory soil degradation studies conducted at 10 °C, under sterile and anaerobic conditions and under soil irradiation.

Study type	Soil	pH (H_2O)	Label	DT_{50}/ $DegT_{50}$ [days]	DT_{90}/ $DegT_{90}$ [days]	R^2	Kinetics
Aerobic degradation at 10 °C	'SL S'	7.7	Ph	14.5	48.2	0 .999	SFO
Aerobic sterile degradation	'SL S'	7.7	Ph	Stable		-	-
Anaerobic degradation	'Hattersheim'	8.3	Ph	257	853	0.602	SFO
	'Hattersheim'	8.3	Cy-He	301	1000	0.783	SFO
Geometric mean				**278**	**924**	**-**	**-**
Soil photolysis – irradiated	'Pikeville'	5.3	Ph	0.8	2.7	0.81	SFO (DFOP, k_1)[a]
	'Pikeville'	5.3	Cy-He	0.7	2.3	0.60	SFO (DFOP, k_1)[a]
Soil photolysis – dark	'Pikeville'	5.3	Ph	65.2	217	0.82	SFO
	'Pikeville'	5.3	Cy-He	114	379	0.33	SFO
Soil photolysis – net	'Pikeville'	5.3	Ph	0.8	2.7	-	SFO
	'Pikeville'	5.3	Cy-He	0.7	2.3	-	SFO
Soil photolysis – net, environmental conditions	'Pikeville'	5.3	Ph	4.1	13.4	-	SFO
	'Pikeville'	5.3	Cy-He	3.5	11.8	-	SFO
Geometric mean (net, environmental conditions)				**3.8**	**12.6**	-	-

[a] Based on the rapid dissipating compartment of DFOP kinetics with the dissipation rate k_1

Table 78: Summery on non-normalized laboratory DegT50 and DegT90 of soil metabolites of tembotrione (AE 0172747) in laboratory soil degradation studies conducted at 20 – 25 °C under aerobic conditions using tembotrione (AE 0172747) as parent (multi-compartment modelling).

Soil	M6 (AE 0456148)		M1 (AE 0968400)		M7 (AE 1124336)		M2 (AE 1392936)		Parent kinetics
	$DegT_{50}$ [days]	$DegT_{90}$ [days]	$DegT_{50}$ [days]	$DegT_{90}$ [days]	$DegT_{50}$ [days]	$DegT_{90}$ [days]	$DegT_{50}$ [days]	$DegT_{90}$ [days]	
'SL S'	14.5	48.1	11.9	39.6	11.5	38.1	-[a]	-	SFO
'Flint Hall'	5.1	17.1	38.1	127	26.2	86.9	-	-	SFO
'SL 2.3'	73.0	242	39.8	132	60.8	202	-	-	SFO
'LS 2.2'	11.5	38.3	ne[b]	ne	ne	ne	-	-	SFO
'Pikeville'	ne	ne	ne	ne	ne	ne	ne	ne	DFOP
'Northwood'	6.0	19.8	ne	ne	ne	ne	ne	ne	SFO
Geometric mean	**13.0**	**43.2**	**26.2**	**87.2**	**26.4**	**87.5**	**ne**	**ne**	**-**

[a] Metabolite not observed.
[b] ne denotes not evaluable owing to low occurrence or low statistical significance (t-test).

Table 79: Summery on temperature and moisture normalized DegT50 of soil metabolites of tembotrione (AE 0172747) in laboratory soil degradation studies conducted at 20 – 25 °C under aerobic conditions using tembotrione (AE 0172747) as parent (multi-compartment modelling).

Soil	M6 (AE 0456148)	M1 (AE 0968400)	M7 (AE 1124336)	M2 (AE 1392936)	Parent kinetics
	$DegT_{50}$ norm. [days]	$DegT_{50}$ norm. [days]	$DegT_{50}$ norm. [days]	$DegT_{50}$ norm. [days]	
'SL S'	14.1	11.6	11.2	-[a]	SFO
'Flint Hall'	3.4	25.5	17.5	-	SFO
'SL 2.3'	63.9	34.8	53.4	-	SFO
'LS 2.2'	11.5	ne[b]	ne	-	SFO

'Pikeville'	ne	ne	ne	ne	DFOP
'Northwood'	6.0	ne	ne	ne	SFO
Geometric mean	**11.6**	**21.8**	**nc**	**ne**	**-**
Worst case	**nc**	**nc**	**53.4**	**-**	**-**

[a] Metabolite not observed.
[b] ne denotes not evaluable owing to low occurrence or low statistical significance (t-test).

Table 80: Summery on formation fractions [0 … 1] of soil metabolites of tembotrione (AE 0172747) in laboratory soil degradation studies conducted at 20 – 25 °C under aerobic conditions using tembotrione (AE 0172747) as parent (multi-compartment modelling).

Soil	M6 (AE 0456148)	M1 (AE 0968400)	M7 (AE 1124336)	M2 (AE 1392936)	Parent kinetics
	P → M6	M6 → M1	M1 → M7	M6 → M2	
'SL S'	1.000	0.362	0.272	-[a]	SFO
'Flint Hall'	1.000	0.178	1.000	-	SFO
'SL 2.3'	1.000	0.310	1.000	-	SFO
'LS 2.2'	1.000	ne[b]	ne	-	SFO
'Pikeville'	0.363	ne	ne	ne	DFOP
'Northwood'	0.741	ne	ne	0.147	SFO
Arithmetic mean	**0.851**	**0.283**	**0.757**	**0.147**	**-**

[a] Metabolite not observed.
[b] ne denotes not evaluable owing to low occurrence or low statistical significance.

Table 81: Summery on laboratory DegT50 and DegT90 and normalized DegT50 of soil metabolite M2 (AE 1392936) in laboratory soil degradation studies conducted at 20 °C under aerobic conditions using the metabolite as parent, based on SFO kinetics.

Soil	Texture	pH (H_2O)	Organic C [%]	$DegT_{50}$ [days]	$DegT_{90}$ [days]	R^2	$DegT_{50}$ norm. [days]
'Flint Hall'	Clay loam	8.3	2.2	7.9	26.2	0.994	7.4
'Shelley Field'	Clay loam	7.9	1.9	11.7	38.7	0.996	10.6
'Laacher Hof AXXa'	Sandy loam	7.5	2.3	15.6	51.9	0.991	15.6
Geometric mean				**11.3**	**37.5**	**-**	**10.7**

Table 82: Summery on laboratory DT50 and DT90 and temperature and moisture normalized DT50 of soil photolysis metabolite M3 (AE 0941989) in laboratory soil degradation studies conducted at 20 °C under aerobic conditions using the metabolite as parent and soil photolysis studies using tembotrione (AE 0172747) as parent, based on SFO kinetics.

Soil	Type of study	Texture	pH (H_2O)	Org. C [%]	DT_{50}/ $DegT_{50}$ [days]	DT_{90}/ $DegT_{90}$ [days]	R^2	Formation fraction (from parent) [0 … 1]	DT_{50} norm. [days]
'Flint Hall'	Aerobic degradation	Clay loam	7.9	2.2	1.05	3.48	0.985	-	1.05
'Shelley Field'		Clay loam	7.5	1.9	1.29	4.27	0.998	-	1.29
'Laacher Hof AXXa'		Sandy loam	7.3	2.3	1.08	3.58	0.992	-	1.08
'Pikeville'	Soil photolysis	Loamy sand	5.3	1.2	5.2	17.3	0.948	0.535	3.3
'Pikeville'		Loamy sand	5.3	1.2	9.8	32.5	0.894	0.507	6.2
Overall geometric mean[a]					**1.8**	**6.0**	**-**	**-**	**1.6**

Soil	Type of study	Texture	pH (H_2O)	Org. C [%]	DT_{50}/ $DegT_{50}$ [days]	DT_{90}/ $DegT_{90}$ [days]	R^2	Forma-tion fraction (from parent) [0 … 1]	DT_{50} norm. [days]
Arithmetic mean					**-**	**-**	**-**	**0.521**	**-**

[a] The two labels of the Pikeville soil (soil photolysis) were averaged by geometric mean before averaging all soils

5.1.4 Summary and discussion of degradation

Degradation in water:

Abiotic degradation:

Hydrolysis

Tembotrione was **hydrolytically stable at a pH range of 4 to 9** in the hydrolysis study.

Photolysis

Under the influence of irradiation in sterile aqueous buffer solution at pH 7, 25°C , tembotrione was **moderately photolysed with an experimental half-life of 56.3 days** under testconditions.

Calculated half-life expressed as summer days: 269 days (Athens/Greece)
Real photolytic half-lives in the environment according to software GCSOLAR (days) in dependence of season and degree latitude are in the range of 9 to 243 days

Degradation in water:

Biotic degradation

The results of a **readily biodegradability** study indicate, that tembotrione **is not readily biodegradable**. (Within 28 days a degradation of 4 % was determined for tembotrione)

In **water/sediment DegT50** in the total system was calculated to be **176 and 65.9 days** for the 'Nidda' and 'Rhein' system, respectively, with a **geometric mean of 108 days** (following SFO kinetics, R2 > 0.98). According to inverse modelling (2nd tier approach) this overall degradation in the total system corresponds to a (fixed) degradation half-life of 1000 days in the water phase and a mean degradation half-life of 51.0 day in the sediment.

Degradation in soil (not relevant for classification and labeling)

Under **aerobic** viable conditions and temperatures of 20 – 25 °C tembotrione (AE 0172747) degraded with a half-life time of 4.3 – 56.4 days (n = 6) following SFO kinetics (R2 > 0.86).

Under **anaerobic** conditions degradation of tembotrione (AE 0172747) significantly slowed down, calculated DT50 was 278 days (n = 2, mean of both labels, SFO kinetics, R2 > 0.60). Amounts of the major soil metabolite M6 (AE 0456148) formed before the onset of the anaerobic phase (5 days of aerobic 'soil ageing') did not significantly change during the anaerobic incubation phase. Therefore, tembotrione (AE 0172747) and metabolites (at least M6, AE 0456148) are considered fairly stable in soil under anaerobic conditions

Conclusion:

Tembotrione is hydrolytically stable at environmentally relevant pH values from pH 4 to pH 9.
Photodegradation of Tembotrione was moderate with an experimental half-life of 56.3 days under the test conditions.
Tembotrione is not readily biodegradable, and does not meet the criterion for rapid degradation with a DT50 whole system of 108 days in a water/sediment study.
Based on available data a non rapid degradation is proposed for tembotrione.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Reasonable adsorption/desorption coefficients (K_{FOC}, 1/n values) were determined for Ph labelled tembotrione (AE 0172747) in soil batch equilibrium experiments using 4 EU soils, 2 US soils and one sediment with a representative spectrum of properties (pH, carbon content, texture). Results from the sediment were not taken into consideration further owing to its high carbon content (12.5 %). Obtained K_{FOC} values were in a range of 20 – 131 L kg^{-1} . Respective 1/n values were in a range of 0.871 – 0.993 (arithmetic mean 0.907). Adsorption of tembotrione (AE 0172747) to soil was depending on soil pH with higher adsorption in more acidic soil (soil pH < 6). This behaviour of tembotrione (AE 0172747) was adequately taken into account for groundwater and surface water risk assessment.

Adsorption/desorption batch equilibrium experiments were also conducted for all metabolites requiring further consideration for the risk assessment. According to these experiments the acidic metabolite M6 (AE 0456148) shows low adsorption in three more acidic soils with a pH < 6.5, K_{FOC} values were in a range of 0.7 – 3.7 L kg^{-1} with an arithmetic mean of 2.7 L kg^{-1}. No reasonable K_{FOC} could be obtained for 2 more alkaline soils (pH ~ 7.2). Based on K_D (K_{OC}) values of the highest concentration tested (1 mg L^{-1}) no clear dependency of adsorption of M6 (AE 0456148) on soil pH could be deduced. In order to derive a robust adsorption endpoint for M6 (AE 0456148) the invalid K_{FOC} values of the 'Sarotti' and 'Northwood' soil were replaced by the more reliable K_D value based on the highest test item concentration (1.0 mg L^{-1}) in combination with a 1/n value of 1.0 (PRAPeR 32 agreed default value for linear adsorption). Finally, an arithmetic mean K_{FOC} value of 1.9 L kg^{-1} and an arithmetic mean 1/n value of 0.978 were derived.

Adsorption/desorption batch equilibrium experiments conducted with metabolite M1 (AE 0968400) using a representative set of five soils gave K_{FOC} values in a range of 18 – 123 L kg^{-1}. Similar to the parent, the adsorption of M1 (AE 0968400) was significantly depending on soil pH with higher K_{FOC} values in more acidic soils. 1/n values were in a range of 0.708 – 0.823 with an arithmetic mean of 0.767. Soil pH dependency of adsorption of M1 (AE 0968400) was adequately taken into account for groundwater risk assessment.

No adsorption (batch equilibrium experiments) was obtained for the acidic metabolite M2 (AE 1392936) in three more alkaline soils (pH 7.0 – 7.4). Adsorption was also extremely low in one more acidic soil (pH 6.0) with a valid K_{FOC} value of 0.11 L kg^{-1}, 1/n = 0.953. The arithmetic mean K_{FOC} value of all four soils, used for further risk assessment, was 0.03 L kg^{-1}, the 1/n value of the three alkaline soils was set to the PRAPeR 32 default value of 1.0 for linear adsorption, resulting in a final arithmetic 1/n value of 0.988.

According to batch equilibrium experiments with five representative soils, M7 (AE 1124336) shows K_{FOC} values in a narrow range of 201 – 332 L $kg^{-}1$ with an arithmetic mean of 277 L kg^{-1}. 1/n values were in a range of 0.804 – 0.903, arithmetic mean 0.860. No dependency on soil pH could be found for this metabolite.

Soil photolysis metabolite M3 (AE 0941989) was also tested in batch equilibrium experiments using four representative soils. K_{FOC} values for this metabolite were in a range of 400 – 1743 L kg^{-1} (arithmetic mean 878 L kg^{-1}) indicating low mobility of this metabolite in soils. 1/n values were close to 1 with a range of 0.993 – 1.032 (arithmetic mean 1.012). Adsorption of M3 (AE 0941989) did not depend on soil pH.

Table 83: Summary on Freundlich adsorption constants for tembotrione (AE 0172747) and metabolites.

Substance	N	K_{FOC} [L kg^{-1}]		1/n		Soil pH depen-dency
		Range	Arithmetic mean	Range	Arithmetic mean	
Tembotrione (AE 0172747)	6	20 - 131	**na** (66)	0.871 – 0.993	**0.907**	Yes
M6 (AE 0456148)	5	0.0 – 3.7[a]	**1.9**	0.944 – 1.000	**0.978**	ne[b]
M1 (AE 0968400)	5	18 - 123	**na** (66)	0.708 – 0.823	**0.767**	Yes
M2 (AE 1392936)	4	0.00 – 0.11	**0.03**	0.913 – 1.000	**0.988**	ne[b]
M7 (AE 1124336)	5	201 - 332	**277**	0.804 – 0.903	**0.860**	No
M3 (AE 0941989)	4	400 - 1743	**878**	0.993 – 1.032	**1.012**	No

na denotes not applicable owing to pH dependence which was taken into account for the exposure and risk assessment assuming linear regression (the number in brackets indicates the arithmetic mean without taking into account pH dependence)
[a] In case of M6 (AE 0456148) K_{OC} values are stated for 'Sarotti' and 'Northwood' soil instead of K_{FOC} values.
[b] ne denotes not evaluable owing to the overall low adsorption resulting in partly invalid K_{FOC} values, for both metabolites no reasonable K_{FOC} values are available for more alkaline soils (soil pH approx. 7).

5.2.2 Volatilisation

The low vapour pressure (1.1 x 10^{-10} hPa at 20 °C) and low Henry constant (1.71 x 10^{-10} Pa m^3 mol^{-1}) of tembotrione (AE 0172747) indicate low tendency for volatilisation. Additionally, tembotrione (AE 0172747) degrades fast by photochemical oxidative degradation (half-life according to the Atkinson method = 2.93 hours assuming a12-hour day and a OH^- concentration of 1.5 x 10^{-6} cm^{-3}) once released into the atmosphere. Thus, no significant residues are expected in the atmosphere after use of tembotrione (AE 0172747) as a herbicide.

5.2.3 Distribution modelling

Nop data available

5.2.4 Summary and discussion evironmental distribution

Data element: Evironmental Distribution (not relevant for classification and labelling)
Adsorption/Desorption $K_{F,OC}$ values were calculated to be in the range of 20 – 131 L kg-1 with an arithmetic mean of 66 L kg-1. Respective 1/n values were in a range of 0.871 – 0.993 (arithmetic mean 0.907). Adsorption of tembotrione (AE 0172747) to soil was depending on soil pH with higher adsorption in more acidic soil (soil pH < 6).
Volatilisation The low vapour pressure (1.1 x 10-10 hPa at 20 °C) and low Henry constant (1.71 x 10-10 Pa m3 mol-1) of tembotrione (AE 0172747) indicate low tendency for volatilisation. Additionally, tembotrione (AE 0172747) degrades fast by photochemical oxidative degradation (half-life according to the Atkinson method = 2.93 hours assuming a12-hour day and a OH- concentration of 1.5 x 10-6 cm-3) once released into the atmosphere. Thus, no significant residues are expected in the atmosphere after use of tembotrione (AE 0172747) as a herbicide.

5.3 Aquatic Bioaccumulation

Table 84: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient *n*-octanol/water EEC/A8 OECD 107 & 117 Flask-shaking with HPLC method	pH = 2 logPow = 2.16 at 23 °C pH = 7 logPow = - 1.09 at 24 °C pH = 9 logPow = - 1.37 at 23 °C	Purity: (989 g/kg)	M-108278-01-1 Mühlberger B., 2003b

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log P_{ow} of tembotrione was found to be 2.2, at pH 2, -1.1 at pH 7 and -1.4 at pH 9. Hence no bioconcentration estimation is required.

5.3.1.2 Measured bioaccumulation data

The log P_{ow} of tembotrione was found to be 2.2, at pH 2, -1.1 at pH 7 and -1.4 at pH 9. Hence no bioconcentration study is required.

5.3.2 Summary and discussion of aquatic bioaccumulation

Partition coefficient n-octanol/water

				Test guideline / design	pH	GLP (y/n)	Reliability *
Log octanol/water partition coefficient (Log Kow):				EEC/A8 OECD 107 & 117 Flask-shaking with HPLC method	4 7 9	y	
pH	2 (23 °C)	**7.0** **(24 °C)**	9.0 (23 °C)				
Log P_{ow}	2.16	**1.09**	1.37				

Conclusion: Log K_{ow} = 1.09; (pH=7; 24°C), indicate low potential for bioaccumulation,.

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5.4 Aquatic toxicity

Table 85: Summary of relevant information on aquatic toxicity

Method	Results						Remarks	Reference
	testorganism	testcondition	exp. time	endpoint	NOEC (mg ai/L)	EC50/LC50 (mg ai/L)		
US-EPA OPPTS 850.1075, OECD 203	*Oncorhynchus mykiss*	s	96 h	Mortality	100	> 100	n L	Dorgerloh, 2003a
US-EPA OPPTS 850.1075, OECD 203	*Lepomis acrochirus*	s	96 h	Mortality	100	> 100	n L	Dorgerloh, 2003b
US-EPA OPPTS 850.1075	*Cyprinodon variegatus*	ss	96 h	Mortality	100	> 100	n	Lima, 2003a
OECD 210, US-EPA OPPTS 850.1400	*Pimephales promelas*	f	34 d	Fry survival	0.604	-	mm	Dogerloh, 2003c
OECD 202, US-EPA 72-2	*Daphnia magna*	*s*	*48 h*	*Immobility*	*18*	*49.8*	*n*	*Sowig, 2002a*
OECD 211, US-EPA 850.1300	*Daphnia magna*	*ss*	*21 d*	*Reproduction* *Growth*	*5*	-	*n*	*Dogerloh, 2003d, accept.*
OECD 219 (proposal for a new guideline, April 2003)	*Chironomus riparius*	s	28 d	Emergence Develop. rate	2 32	12.5 > 32	n	Dogerloh, 2005e.
OECD 201, US-EPA J § 123-2, EU C.3	*Pseudokirch.subcapitata*	s	96 h	Biomass Growth rate	0.2 0.2	0.38 0.75	mm	Gosch & Ebeling, 2002
OECD 201, US-EPA J § 123-2, EU C.3	*Anabaena flos-aquae*	s	96 h 72 h	Biomass (96 h) Growth rate (72 h)	16 39	64 71	mm	Hoberg, 2003a
OECD 201, US-EPA J § 123-2, EU C.3	*Navicula pelliculosa*	s	72 h [b]	Biomass Growth rate	5.6 5.6	10.8 47.9	n	Sowig & Gosch, 2003,
OECD 201, OPPTS 850.5400, EU C.3	*Skeletonema costatum*	s	72 h	Biomass Growth rate	0.96 2.6	5.1 4.5	mm	Hoberg, 2003b, pac. [a]
OECD draft 1998, US-EPA J § 123-2	*Lemna gibba*	ss	7 d	Biomass Growth rate	0.0032 0.0032	0.00599 0.00848	n	Sowig, 2003
Under principal consideration of OECD 221 (proposal 2004)	*Lemna gibba* [a]	s	7 d	Yield [b] Growth rate	0.0024 0.0024	Not calculated	mm	Dogerloh, 2004a
US EPA 72-3, OPPTS 850.1035 (draft)	*Americamysis bahia*	*f*	*96 h*	*Mortality* *Subl. effects*	*0.046*	0.1	*mm*	*Lima, 2003b*
US EPA 72-3, OPPTS 850.1025 (draft)	*Crassostrea virginica*	*f*	*96 h*	*Shell growth*	*n.d.*	14	*mm*	*Dionne, 2003,(partly accept)*

[a] Modified exposure study with sediment present in the test system, water spiked

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference:	**Acute Toxicity of AE 0172747 technical substance to fish (*Oncorhynchus mykiss*) (product code AE 0172747 001C97 0001)**
Author(s), year:	Dorgerloh, M. (2003a)
Report/Doc. number:	DOM 23034; M-106511-01-1
Guideline(s):	US-EPA OPPTS 850.1075, OECD 203
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance: Tembotrione (AE 0172747), batch: LE 356, purity: 97.4 % (w/w)

MATERIAL AND METHODS:

Test species:	Rainbow trout (*Oncorhynchus mykiss*) Wet weight (mean ± s.d.): 1.0 ± 0.3 g Length (mean ± s.d.): 4.5 ± 0.3 cm Loading: 0.75 g fish/L
Test concentrations:	Nominal: 0 (control) and 100 mg/L (pure a.s.) Mean measured: -- and 102 mg/L (pure a.s.)
No. of replicates:	One replicate with 30 fish per treatment and control
Test type / duration:	Static, 96 hours
Dilution water:	Reconstituted water, hardness: 40 – 60 mg/L as $CaCO_3$
Test conditions:	Temperature: 11.9 – 12.2 °C pH: 7.0 – 7.2 Dissolved oxygen: 93 – 101 % saturation (no absolute values provided) Photoperiod: 16 hours light and 8 hours darkness
Observations:	Mortalities and signs of poisoning were recorded after 4, 24, 48, 72 and 96 hours.
Analytical measurements:	For chemical analysis of the test substance samples were taken at test initiation, after 48 hours and at test termination (96 h). Analytical method: HPLC/UV
Statistical evaluation:	No evaluation performed (limit test, no mortalities observed)

FINDINGS:

Analytical results:	Measured concentrations were in the range of 101 – 102 % of nominal
Sublethal effects:	No effects observed
Mortalities:	No mortalities observed

CONCLUSION:
LC_{50} (96 h): > 100 mg/L
NOEC (96 h): 100 mg/L
Based on a nominal limit concentration.

COMMENT (RMS)
A rather high number of fish was used per test aquarium. However, the loading was within the limits provided in OECD 203 (≤ 1 g fish/L) and OPPTS 850.1075 (≤ 0.8 g fish/L) for static test systems and hence the study is considered acceptable.

Reference:	**Acute Toxicity of AE 0172747 technical substance to fish (*Lepomis macrochirus*) (product code AE 0172747 001C97 0001)**
Author(s), year:	Dorgerloh, M. (2003b)
Report/Doc. number:	DOM 22065; M-079090-01-1
Guideline(s):	US-EPA OPPTS 850.1075, OECD 203
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance: Tembotrione (AE 0172747), batch: LE 356, purity: 97.4 % (w/w)

MATERIAL AND METHODS:

Test species:	Bluegill sunfish (*Lepomis macrochirus*) Wet weight (mean ± s.d.): 0.6 ± 0.2 g Length (mean ± s.d.): 3.4 ± 0.4 cm Loading: 0.45 g fish/L
Test concentrations:	Nominal: 0 (control) and 100 mg/L (pure a.s.) Mean measured: -- and 100 mg/L (pure a.s.)
No. of replicates:	One replicate with 30 fish per treatment and control
Test type / duration:	Static, 96 hours
Dilution water:	Reconstituted water, hardness: 40 – 60 mg/L as $CaCO_3$
Test conditions:	Temperature: 22.0 – 23.4 °C pH: 7.2 – 7.3 Dissolved oxygen: 95 – 99 % saturation (no absolute values provided) Photoperiod: 16 hours light and 8 hours darkness
Observations:	Mortalities and signs of poisoning were recorded after 4, 24, 48, 72 and 96 hours.
Analytical measurements:	For chemical analysis of the test substance samples were taken at test initiation, after 48 hours and at test termination (96 h). Analytical method: HPLC/UV
Statistical evaluation:	No evaluation performed (limit test, no mortalities observed)

FINDINGS:

Analytical results:	Measured concentrations were in the range of 99.5 - 100 % of nominal
Sublethal effects:	No effects observed
Mortalities:	No mortalities observed

CONCLUSION:

LC_{50} (96 h): > 100 mg/L

NOEC (96 h): 100 mg/L

Based on a nominal limit concentration.

COMMENT (RMS)

A rather high number of fish was used per test aquarium. However, the loading was within the limits provided in OECD 203 (≤ 1 g fish/L) and OPPTS 850.1075 (≤ 0.8 g fish/L) for static test systems and hence the study is considered acceptable.

Reference:	**AE 0172747 – acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under static-renewal conditions**
Author(s), year:	Lima, W. (2003a)
Report/Doc. number:	13798.6109; M-086607-01-1
Guideline(s):	US-EPA OPPTS 850.1075
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

RANGE FINDING TEST:

In two range finding tests 10 fish per treatment level and control were exposed for 96 hours. No effects on fish were observed at test concentrations of 10, 50 and 100 mg/L (static test) and 1.6, 3.1, 6.3, 13 and 25 mg/L (flow-through conditions).

Test substance:	Tembotrione (AE 0172747), batch: 2250016 and 2250027, purity: 95 and 94 % (w/w)

MATERIAL AND METHODS:

Test species:	Sheepshead minnow (*Cyprinodon variegatus*) Mean wet weight: 0.17 g (range: 0.08 – 0.42 g) Mean length: 19 mm (14 – 25 mm) Loading: not stated
Test concentrations:	Nominal: 0 (control and solvent control), 13, 22, 36, 60 and 100 mg/L (pure a.s.) Mean measured: --, 13, 23, 38, 65 and 100 mg/L (pure a.s.) Solvent: Dimethylformamide, 0.5 mL/L in the solvent control and all test solutions
No. of replicates:	Two replicates with 10 fish each per treatment level and control
Test type / duration:	Static-renewal, renewal after 48 hours, test duration: 96 hours
Dilution water:	Natural filtered seawater
Test conditions:	Temperature: 21 – 23 °C pH: 7.1 - 7.9 Salinity: 33 – 34 ‰ Dissolved oxygen: 5.0 – 7.5 mg/L (> 60 % air saturation) Photoperiod: 16 hours light and 8 hours dark with a transition period
Observations:	Mortalities and signs of poisoning were recorded after 6, 24, 48, 72 and 96 hours.
Analytical measurements:	For chemical analysis of the test substance samples were taken at the beginning of the test from replicates A (new solutions), at 48 hours from replicates B (new solutions) and at test termination again from replicates A (old solutions). Analytical method: HPLC/UV
Statistical evaluation:	No evaluation performed (no mortalities observed)

FINDINGS:

Analytical results:	Measured concentrations were in the range of 100 – 112 % of nominal concentrations.
Sublethal effects:	No effects observed
Mortalities:	No mortalities observed

CONCLUSION:

LC_{50} (96 h): > 100 mg/L
NOEC (96 h): 100 mg/L
Based on nominal concentrations.

5.4.1.2 Long-term toxicity to fish

Fish early life stage toxicity test (OECD IIA 8.2.3, IIIA 10.2.5.2)

Reference:	**Early-life stage toxicity of AE 0172747 technical substance to fish (*Pimephales promelas*) (product code AE 0172747 00 1C94 0003)**
Author(s), year:	Dorgerloh, M. (2003c)
Report/Doc. number:	DOM 22076; M-091065-01-1
Guideline(s):	OECD 210, US-EPA OPPTS 850.1400
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: PFI 0215-01, purity: 93.6 % (w/w)
MATERIAL AND METHODS:	
Test species:	*Pimephales promelas*, freshly fertilised eggs (less than 24 hours old) of five pairs
Treatments:	Nominal: 0 (control and solvent control), 0.625, 1.25, 2.50, 5.00, 10.0 mg/L pure a.s.) Mean measured: --, 0.604, 1.15, 2.33, 5.00 and 9.81 mg/L (pure a.s.) Solvent: Dimethylformamide, 0.1 mL/L in the solvent control and all test solutions
No. of replicates:	4 replicates with 25 eggs each per treatment and control
Test type / duration:	Flow-through system, 24 volume additions per day, test duration: 34 days (29 days post hatch)
Dilution water:	Reconstituted water, hardness: 2.6 – 2.9 °dH (46 - 52 mg/L as $CaCO_3$)
Test procedure:	*Incubation*: Eggs were incubated in egg cups constructed from stainless steel pipes (diameter: 5.5 cm) with a screened bottom perforated with holes (hole diameter: 0.8 mm). These cups were suspended in replicate test chambers and oscillated vertically two times per minute. The approximate test chamber volume was 3.8 L. After completion of hatch (5 days after start of exposure) number of larvae were thinned to 15 individuals per egg cup. *Feeding*: Shortly after the larvae had hatched, feeding was started with live newly hatched brine shrimp nauplii (ad libitum, 1 – 2 times a day). Excess food and faecal material were siphoned from the aquaria. *Loading at test end*: 0.05 g fish/L test solution flowing through a test chamber per day or 1.1 g fish/L volume of a single test chamber.
Test conditions:	Temperature: 23.7 – 25.5 °C pH: 7.0 – 7.3 Dissolved oxygen: 101 – 116 % saturation Photoperiod: 16 hours light and 8 hours darkness, 30 minutes transition period
Observations:	Embryo mortality was observed daily. Percent egg hatchability was evaluated on day 5 of exposure (when all eggs had hatched). Fry was observed for abnormalities and mortalities on weekdays. At test end standard length, wet weight and dry weight of surviving fry were determined.
Analytical	For chemical analysis of the test substance samples were taken from

measurements:	alternating test chambers of all test levels on days 0, 7, 14, 21, 28 and 34. Analytical method: HPLC/UV
Statistical evaluation:	T-test to compare control and solvent control data NOEC: Time to hatch, hatching success, fry survival and overall survival data were arcsine transformed before analysis using the Williams-test. Length and weight were analysed with Williams test without transformation of data.
FINDINGS:	
Analytical results:	Mean measured concentrations ranged from 92 -100 % of nominal concentrations.
Sublethal effects:	At 5.00 and 9.81 mg/L the following morphological effects were frequently observed: kyphoscoliosis (lateral curvature of the spine with vertebral rotation) associated with a flexed spinal column.
Time to hatch:	At all treatment levels hatching started on day 3 and continued until day 5. There was no significant difference in any treatment group compared to pooled control data.

Table 86: Effects of tembotrione on hatching success, fry survival, overall survival and growth of Pimephales promelas.

Tembotrione mean measured [mg pure a.s./L]	Hatching success [a] (mean ± s.d.) [%]	Fry survival [b] (mean ± s.d.) [%]	Overall survival [b] (mean ± s.d.) [%]	Length [b] (mean ± s.d.) [mm]	Dry weight [b] (mean ± s.d.) [mg]
Water control	90 ± 6.8	92 ± 3.3	82 ± 7.3	24.3 ± 1.6	66.2 ± 16.3
Solvent control	87 ± 3.8	90 ± 11.5	80 ± 7.3	24.5 ± 1.6	67.0 ± 15.1
0.604	93 ± 6.8	82 ± 14.8	77 ± 18.4	24.4 ± 1.8	70.8 ± 19.4
1.15	88 ± 5.7	75 ± 13.7 *	67 ± 16.5	23.7 ± 2.6	69.9 ± 23.9
2.33	90 ± 5.2	48 ± 13.7 *	44 ± 14.5 *	22.3 ± 2.4 *	52.9 ± 17.0 *
5.00	92 ± 5.7	43 ± 12.8 *	39 ± 9.7 *	20.8 ± 2.5 *	42.4 ± 17.7 *
9.81	92 ± 3.3	25 ± 11.4 *	23 ± 10.7 *	19.3 ± 3.3 *	30.8 ± 17.9 *

[a] After 5 days of exposure
[b] After 34 days of exposure (29 days exposure post hatch), survival based on eggs incubated
* Denotes statistically significant difference from pooled controls (α = 0.05, Williams Test)

CONCLUSION:
NOEC (34 d): 0.604 mg/L
LOEC (34 d): 1.15 mg/L (reduced fry survival)
Based on mean measured concentrations (pure a.s.)

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference:	**Acute toxicity to *Daphnia magna* (water flea) under static testing conditions AE 0172747, substance, technical**
Author(s), year:	Sowig, P. (2002a)
Report/Doc. number:	CE02/023; M-078343-01-1
Guideline(s):	OECD 202, US-EPA 72-2
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: LE 356, purity: 97.4 % (w/w)

MATERIAL AND METHODS:

Test species:	*Daphnia magna*, < 24 hours old
Treatments:	Nominal: 0 (control), 10, 18, 32, 56 and 100 mg/L (pure a.s.) Mean measured: --, 11.50 15.68, 31.78, 55.13, and 98.95 mg/L (pure a.s.)
No. of replicates:	2 replicates with 10 daphnids each per treatment and control
Test type / duration:	Static, 48 hours
Dilution water:	Reconstituted water, hardness: no reliable value provided in the study report (see comment of RMS below)
Test conditions:	Temperature: 19.4 – 19.9 °C pH: 7.7 – 7.9 Dissolved oxygen: 8.1 – 9.2 mg/L (> 60 % saturation) Photoperiod: not stated in the study report
Observations:	Daphnids were examined after 24 and 48 hours for immobilisation and behavioural abnormalities.
Analytical measurements:	At the beginning of the test and at test termination (48 h) samples from all test levels were analysed for the test substance. Analytical method: HPLC/UV
Statistical evaluation:	EC_{50} for immobility: Probit analysis NOEC: Directly derived from the raw data

FINDINGS:

Analytical results:	Measured concentrations were in the range of 88 – 119 % of nominal.
Sublethal effects:	No effects observed.
Immobilisation:	Up to a test concentration of 18 mg/L no immobile daphnids were observed. At 32, 56 and 100 mg/L 30, 60, and 85 % immobilisation was found after 48 hours.

CONCLUSION:
EC_{50} (48 h): 49.8 mg/L (95 % CL: 40.8 – 61.9 mg/L)

NOEC (48 h): 18 mg/L
Based on nominal concentrations.

COMMENT (RMS):
In the study report a total hardness of 1.66 mg $CaCO_3$/L is stated for the dilution water. This value is unrealistically low. Since the conductivity of the test solutions was around 515 µS/cm and the acid binding capacity of the dilution water was 0.67 mmol HCl/L it can be assumed that the hardness of the test water was in an acceptable range.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:	**Influence of AE 0172747 (tech.) on development and reproductive output of the water flea *Daphnia magna* in a static renewal laboratory test system**
Author(s), year:	Dorgerloh, M. (2003d)
Report/Doc. number:	DOM 23002; M-111125-01-1
Guideline(s):	OECD 211, US-EPA 850.1300
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: PFI 0215-01, purity: 93.6 % w/w
MATERIAL AND METHODS:	
Test species:	*Daphnia magna*, less than 24 hours old
Test concentrations:	Nominal: 0 (control), 0.078, 0.16, 0.31, 0.63, 1.25, 2.5, 5 and 10 mg/L (pure a.s.) Measured (time weighted): --, 0.08, --, 0.32, --, 1.26, --, 5.09 and -- mg/L (pure a.s.), Time weighted concentrations were calculated according to the following procedure: Area = (conc.fresh - conc.aged) / (Ln(conc.fresh) - Ln(conc.aged)) The time weighted mean is then the total area from all measured renewal periods divided by the duration of the test.
No. of replicates:	10 replicates with 1 daphnid each per treatment
Test type / duration:	Static-renewal system, renewal of test solutions three times per week Test duration: 21 days
Dilution water:	Reconstituted water (M-7 medium), hardness: about 178 - 214 mg/L as $CaCO_3$
Test conditions:	Temperature: 20.1 – 21.6 °C pH: 7.8 – 8.9 Dissolved oxygen: 8.4 – 9.5 mg/L (> 60 % saturation) Photoperiod: 16 hours light and 8 hours darkness Feeding: daily (except on days 3 & 4) with *Scenedesmus subspicatus* (TOC content of 0.2 mg TOC per test vessel with 100 mL corresponding to 10^8 cell/L). On day 2 the threefold amount was fed for days 3 & 4.
Observations:	Parental endpoints: Immobilisation, presence of males (daily except on days 3 and 4), length and dry weight at the end of the test Reproduction endpoints: Alive and immobile neonates produced by each parent animal and aborted eggs (daily from appearance of first offspring emergence).
Analytical measurements:	For chemical analysis of the test substance freshly prepared test media from all treatment levels were sampled on days 0, 9 and 19. Old test media were

	sampled from test levels of 0.078, 0.31, 1.25 and 5 mg/L on days 2, 12, and 21. Analytical method: HPLC/UV
Statistical evaluation:	NOEC: Data were arc sine transformed and then subjected to Dunnett's test, t-test after Bonferroni-Holm or Bonferroni-U-test after Holm as appropriate.

FINDINGS:

Analytical results:	Measured concentrations of freshly prepared test solutions ranged from 96 to 106 % of nominal. The measured concentrations of aged test solutions (0.078, 1.16, 0.31 and 5 mg/L) ranged from 98 to 107 % of nominal.

Table 87: Effects of tembotrione on survival of parents and reproduction of Daphnia magna after 21 days of exposure.

Tembotrione (nominal) [mg/L]	Adult survival [%]	1st day of offspring emergence	Alive young / surviving parent (mean ± s.d.)	Alive young /surviving parent / reprod. day (mean ± s.d.)	Adult length (mean ± s.d.) [mm]	Adult dry weight (mean ± s.d.) [mg]
Water control	100	9.5	87 ± 30	7.0 ± 2.4	4.3 ± 0.1	0.90 ± 0.09
0.078	100	9.4	82 ± 11	6.5 ± 1.0	4.3 ± 0.2	0.79 ± 0.16
0.16	100	9.7	82 ± 17	6.6 ± 1.2	4.2 ± 0.1	0.82 ± 0.12
0.31	100	9.6	81 ± 22	6.4 ± 1.3	4.2 ± 0.2	0.83 ± 0.14
0.63	100	9.9	70 ± 29	5.5 ± 2.1	4.2 ± 0.2	0.75 ± 0.16
1.25	60 [b]	9.5	64 ± 15	5.1 ± 1.1	4.0 ± 0.1*	0.88 ± 0.14
2.5	90 [a]	9.1	72 ± 10	5.6 ± 0.8	4.2 ± 0.1	0.72 ± 0.13
5.0	100	9.7	76 ± 18	6.2 ± 1.8	4.1 ± 0.2	0.82 ± 0.16
10	90 [a]	9.7	71 ± 10	5.8 ± 1.0	4.1 ± 0.1*	0.81 ± 0.16

[a] The one daphnid that died in these groups was accidentally injured during handling by the personnel, thus the immobilisation of these daphnids is not treatment related.
[b] all four daphnids in this group died between day 7 and day 10, no explanation is given in the study report.
* Denotes statistically significant difference from the control (alpha = 0.05)

Offspring from prematurely died parent animals and neonates which were found dead were excluded from any calculations and statistical evaluation as recommended in the OECD guideline 211.
Rates of aborted eggs ranged from 0.1 to 5.4 % of the total amount of alive offspring per study group. Time point and amount of aborted eggs showed no treatment relation. Additionally on day 10 two females of the lowest test concentration produced dead or immobilised offspring (= 0.2 % of the total reproductive output).

CONCLUSION:
NOEC (21 d): 5 mg/L
LOEC (21 d): 10 mg/L (reduced parental body length at test end)
Based on nominal concentrations.

COMMENT (RMS):
The RMS accepted a NOEC of 5 mg/L as stated in the study report because the statistical significant reduction in body length at 1.25 mg/L was followed by two test concentrations at which no statistically significant reduction in length was found. Additionally at 1.25 mg/L the reduction in length was rather low (7 % compared to the control) and reproductive parameters were not affected up to the highest test concentration of 10 mg/L. The effect on body length at 10 mg/L is considered relevant because it is the highest test concentration and no information on possible effects at even higher concentrations is available.

5.4.3 Algae and aquatic plants

Reference:	**Algal growth inhibition- *Pseudokirchneriella subcapitata*** **AE 0172747; substance, technical**
Author(s), year:	Gosch, H.; Ebeling, M. (2002)
Report/Doc. number:	CE02/024; M-078348-01-1
Guideline(s):	OECD 201, US-EPA J § 123-2, EU C.3
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: LE 356, purity: 97.4 % (w/w)
MATERIAL AND METHODS:	
Test species:	*Pseudokirchneriella subcapitata*
Test concentrations:	Nominal: 0 (medium control), 0.10, 0.18, 0.31, 0.55, 0.97, 1.75, 3.12 mg/L (pure a.s.) Mean measured: --, 0.07, 0.13, 0.20, 0.41, 0.53, 1.18, 2.11 mg/L (pure a.s.)
No. of replicates:	3 replicates per test concentration and 6 replicates for the control
Initial loading:	10^4 cells/mL
Test type / duration:	Static, 96 hours
Nutrient medium:	AAP-medium adjusted to a pH of 7.5
Test conditions:	Continuous illumination at 66 ± 4.1 µE m^{-2} s^{-1} (wide spectrum fluorescent lamps) Shaking rate: 100 cycles/minute Temperature: 24.0 – 25.1 °C pH at test start: 7.3 – 7.5, pH at test end: 7.5 - 9.8
Observations:	Cell concentrations were evaluated by Coulter Multisizer II after 24, 48, 72 and 96 hours. Morphology of algal cells was examined using counting chambers and a microscope.
Analytical measurements:	For chemical analysis of the test substance samples of test solutions from all treatment levels were taken at test start and test end. Analytical method: HPLC/UV
Statistical evaluation:	EC_{50}: Binomial probability method NOEC: Duncan's Multiple Range Test.
FINDINGS:	
Analytical results:	In freshly prepared test media test item concentrations were 53 – 73 % of nominal. At test termination measured concentrations were in the range of 56 – 82 % of nominal concentrations. Mean measured concentrations were found to be 55 – 76 % of nominal.
Morphological effects:	Not reported

Table 88: Effects of tembotrione on biomass (area under growth curve) and growth rate of Pseudokirchneriella subcapitata after 72 hours and 96 hours of exposure.

Tembotrione (mean measured) [mg/L]	Inhibition after 72 hours [%]		Inhibition after 96 hours [%]	
	Biomass (AUC)	Growth rate	Biomass (AUC)	Growth rate
0.07	-30 *	-10 *	-21 *	-3
0.13	-8	4	-8	-1
0.20	1	1	3	1
0.41	39 *	22 *	58 *	23 *
0.53	56 *	35 *	77 *	40 *
1.18	70 *	50 *	88 *	63 *
2.11	72 *	59 *	91 *	75 *

AUC…Area Under growth Curve
* Denotes statistically significant difference from the control (alpha = 0.05)

Table 89: Toxicity of tembotrione for the freshwater alga Pseudokirchneriella subcapitata. Toxicity values are based on mean measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [mg/L]	95 % CL for EC_{50} [mg/L]
Biomass (AUC)	72 h	0.2	0.48	0.41 – 0.53
	96 h	0.2	0.38	0.20 – 0.41
Growth rate	72 h	0.2	1.18	0.53 – 2.11
	96 h	0.2	0.75	0.53 – 1.18

CONCLUSION:
The following toxicity values are indicative for the toxicity of tembotrione to *Pseudokirchneriella subcapitata.*
E_bC_{50} (96 h): 0.38 mg/L
E_rC_{50} (96 h): 0.75 mg/L
NOEC (96 h): 0.2 mg/L (based on inhibition of biomass and growth rate)
Based on mean measured concentrations.

COMMENT (RMS):
The NOEC for algal growth and average specific growth rate was set considering only inhibition of algal growth in the test concentrations relative to the control. However, at the test concentration of 0.07 mg/L a stimulation of algal growth was observed.

Reference:	**AE 0172747 – Acute toxicity test with freshwater blue-green alga (*Anabaena flos-aquae*)**
Author(s), year:	Hoberg, J.R. (2003a)
Report/Doc. number:	13798.6104; M-091552-01-1
Guideline(s):	OECD 201, US-EPA OPPTS 850.5400 (Draft), EU C.3
GLP:	Yes
Deviations:	No clear exponential growth was demonstrated for control and solvent control cultures. However, the validity criterion of OECD 201 (1984) was met (16-fold increase in algal cells over a period of 72 hours).
Validity:	Acceptable

Test substance: Tembotrione (AE 0172747), batch: PFI 0215, purity: 94 % (w/w)

RANGE FINDING TEST:

Algae were exposed to a medium control, a solvent control, 0.001, 0.010, 0.10, 1.0 and 10 mg a.s./L (2 replicates per treatment level). Respective cell densities were 47, 75, 61, 77, 56, 54 and 56 x 10^4 cells/mL after 96 hours.

DEFINITIVE TEST:

MATERIAL AND METHODS:

Test species:	*Anabaena flos-aquae*
Treatments:	Nominal: 0 (medium control and solvent control) 1.0, 2.6, 6.4, 16, 40, and 100 mg/L (pure a.s.) Mean measured: --, 1.0, 2.7, 6.5, 16, 39 and 100 mg/L (pure a.s.) Solvent: Dimethylformamide, 0.1 mL/L in the solvent control and all test solutions
No. of replicates:	3 replicates per test concentration, medium and solvent control
Initial loading:	10^4 cells/mL
Test type / duration:	Static system, 96 hours
Nutrient medium:	AAP medium adjusted to a pH of 7.5 ± 1
Test conditions:	Continuous illumination, light intensity: 1900 – 2400 lux, PAR at test initiation: 26 – 31 µE m^{-2} s^{-1} (fluorescent bulbs) Shaking rate: 100 rpm Temperature: 24.0 °C pH at test start: 4.6 – 7.4, pH at test end: 4.8 – 8.5 Lowest pH values were observed in the 100 mg/L vessels.
Observations:	After 24, 48, 72 and 96 hours cell counts and morphological examinations of algal cells were conducted using a haemocytometer and a compound microscope. Solutions were vigorously pipetted multiple times to break up the filaments of *Anabaena flos-aquae* into its cells prior to removing a sample for cell counts.
Analytical measurements:	For chemical analysis of the test substance samples from test solutions of each treatment and control level were taken at test start and test end (96 h). Analytical method: HPLC/UV
Statistical evaluation:	Comparison of control and solvent control: t-test, if a significant difference at alpha < 0.05 was found inhibition for the respective parameter was compared to the solvent control cultures only. NOEC: Williams' Test or Kruskal-Wallis' Test as appropriate EC_{50}: Linear regression of response (percent inhibition) vs. exposure concentration.

RECOVERY TEST:

After 96 hours a sample of the pooled replicates from the 100 mg/L treatment level was taken and diluted with AAP medium to yield a subculture with a nominal concentration of 1.0 mg/L. This subculture was incubated under the same conditions as applied for the definitive test for six days.

FINDINGS:

Analytical results: Mean measured concentrations were in the range of 98 - 102 % of nominal concentrations.

Morphological effects: After 96 hours cells from the 100 mg/L treatment level were smaller than cells from the control. Cells from the remaining test concentrations were unaffected.

Table 90: Effects of tembotrione on biomass (area under growth curve, AUC) and growth rate of Anabaena flos-aquae after 72 hours and 96 hours of exposure.

Tembotrione (mean measured) [mg/L]	Inhibition after 72 hours [%]		Inhibition after 96 hours	
	Biomass (AUC) [a]	Growth rate [a]	Biomass (AUC) [a]	Growth rate [b]
1	31	-4	-1	-13
2.7	10	-3	-6	-9
6.5	17	-6	9	3
16	27	6	24	4
39	23	7	26 *	12
100	79 *	77	81 *	42 *

* Denotes statistically significant difference from the control (alpha = 0.05)
[a] Based on a comparison of the treatment data and solvent control data
[b] Based on a comparison of treatment data and pooled control data

Growth rate 72 h: The Kruskal Wallis test did not detect a significant reduction in growth rate in any treatment level due to the high variability in growth rates of control cultures. Thus, in the study report the NOEC for growth rate was empirically estimated to be 39 mg/L the highest concentration tested resulting in < 10 % inhibition.

Table 91: Toxicity of tembotrione for the freshwater alga Anabaena flos-aquae. Toxicity values are based on mean measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [mg/L]	95 % CL for E_{50} [mg/L]
Biomass (AUC)	72 h	39 [a]	67 [a]	41 - 77
	96 h	16 [a]	64 [a]	60 - 71
Growth rate	72 h	39 [ab]	71 [a]	60 - 92
	96 h	39 [c]	> 100 [c d]	60 - 92

[b] With a Kruskal-Wallis test the NOEC would be set to 100 mg/L, however since at 100 mg/L 77 % reduction in average specific growth rate compared to the solvent control was observed the NOEC was empirically set at 39 mg/L (7 % reduction in growth rate)
[c] Based on a comparison of treatment data and pooled control data
[d] 42 % inhibition at 100 mg/L

RECOVERY TEST:

Within 6 days in the subculture the cell density had increased to 7.25 x 10^4 cells/mL. This result indicates that tembotrione has rather an algistatic than an algicidal effect on the growth of *Anabaena flos-aquae*.

CONCLUSION:
The following toxicity values are regarded as indicative for the toxicity of tembotrione to *Anabaena flos-aquae.*
E_bC_{50} (96 h): 64 mg/L
E_rC_{50} (72 h): 71 mg/L
NOEC (96 h): 16 mg/L (based on inhibition of biomass)
NOEC (72 and 96 h): 39 mg/L (based on inhibition of growth
Based on mean measured concentrations.

COMMENTS (RMS):
No clear exponential growth was demonstrated for control and solvent control cultures. Additionally growth rates of control and solvent control cultures for individual days were highly variable. The notifier stated that for *Anabaena flos-aquae* exponential growth should not be expected since it is a filamentous alga generally expanding only from the terminal ends of the filament which limits its growth potential relative to single cell algae. The RMS accepted this statement and the study was considered acceptable since it met the OECD 201 (1984) validity criterion of a 16-fold increase in cell numbers in the control cultures over a period of 72 hours. In the presented study *Anabaena flos-aquae* cell densities observed in the control and solvent control were 10^4 cells/mL at test start and 18 x 10^4 and 27 x 10^4 cells/mL at test end.

Reference:	**Algal growth inhibition - *Navicula pelliculosa* AE 0172747; substance technical**
Author(s), year:	Sowig, P.; Gosch, H. (2003)
Report/Doc. number:	CE02/025 M-108273-01-1
Guideline(s):	OECD 201, US-EPA J § 123-2, EU C.3
GLP:	Yes
Deviations:	An initial lag phase was observed (for the first 24 hours of the test growth rates of the control cultures were negative in all but one replicate). Therefore E_rC_{50} and E_bC_{50} values were calculated for the exposure period between 24 – 96 hours of exposure (a 72 hour exposure period).
Validity:	Acceptable

Test substance: Tembotrione (AE 0172747), batch: LE 356, purity: 97.4 % (w/w)

MATERIAL AND METHODS:

Test species:	*Navicula pelliculosa*, freshwater diatom
Test concentrations:	Nominal: 0 (medium control), 1.8, 3.2, 5.6, 10, 18, 32 and 56 mg/L (pure a.s.) Mean measured: --, 1.61, 3.04, 5.34, 9.32, 16.92, 29.87 and 52.46 mg/L (pure a.s.)
No. of replicates:	4 replicates per test concentration and 8 replicates for the control
Initial loading:	10^4 cells/mL
Test type / duration:	Static, 120 hours
Nutrient medium:	AAP medium adapted for the specific demands of diatoms, the pH of the medium after aeration was 7.5
Test conditions:	Continuous illumination at 66 ± 4.1 µE m^{-2} s^{-1} (wide spectrum fluorescent lamps) Shaking rate: 100 cycles/minute Temperature: 24.0 – 25.2 °C

	pH at test start: 7.3 – 7.6, pH at test end: 7.7 – 8.6
Observations:	After 24, 48, 72, 96 and 120 hours cell concentrations were determined by Coulter Multisizer II. Morphology of algal cells was examined using counting chambers and a microscope.
Analytical measurements:	For chemical analysis of the test substance samples of each treatment level and the control level were taken at test start and test end (120 h). Analytical method: HPLC/UV
Statistical evaluation:	EC_{50}: Probit analysis NOEC growth rate: Welch t-test or Williams multiple sequential t-test as appropriate NOEC AUC: not stated

FINDINGS:

Analytical results:	Measured concentrations ranged from 94 – 99 % of nominal at test start and from 87 – 99 % at test end.
Morphological effects:	Not reported

Table 92: Effects of tembotrione on biomass (area under growth curve, AUC) and growth rate of Navicula pelliculosa for the time period of 24 to 96 hours from test start (a 72 hour exposure period).

Tembotrione (nominal) [mg/L]	Inhibition of growth rate [%]	Inhibition of biomass (AUC) [%] [a]
1.8	-22	-11.3
3.2	-19	-3.3 [a]
5.6	26	12.3
10	54 *	20.1 *
18	77 *	27.8 *
32	87 *	46.3 *
56	85 *	48.9 *

* significantly different at alpha = 0.05

[a] The RMS derived a value of 3.1 % inhibition from the raw data. However, this minor difference would not have affected the outcome of the statistical NOEC determination and the EC_{50} estimate and hence this minor difference is considered negligible.

Table 93: Toxicity of tembotrione for the freshwater alga Navicula pelliculosa. Toxicity values are based on nominal concentrations.

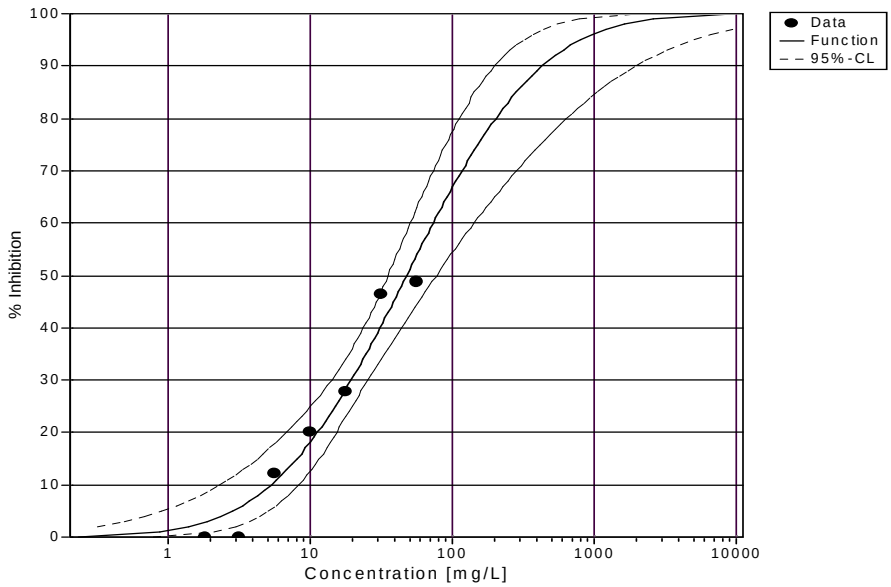
Endpoint	Time scale	NOEC [mg/L]	LOEC [mg/L]	EC_{50} [mg/L]	95 % CL for E_{50} [mg/L]
Biomass (AUC)	24 - 96 h	5.6	10.0	10.8	7.7 – 15.1
Growth rate	24 - 96 h	5.6	10.0	47.9	35.4 – 77.6

CONCLUSION:
E_bC_{50} (24 - 96 h): 10.8 mg/L
E_rC_{50} (24 - 96 h): 47.9 mg/L
NOEC: 5.6 mg/L (based on inhibition of average specific growth rate and biomass)
Based on nominal concentrations.

COMMENTS (RMS):
Low doses of 1.8 and 3.2 mg/L seem to have stimulated algal growth. The EC_{50} values for growth rate and biomass were estimated only for % inhibition ignoring any positive effects on growth.

An initial lag phase was observed for algal growth (for the first 24 hours of the test growth rates of the control cultures were negative in all but one replicate). A similar situation was observed in test substance treatments. Additionally growth was not exponential any more at the last day of exposure (from 96 – 120 h). Therefore the RMS did not accept the E_rC_{50} and E_bC_{50} estimates given in the study protocol. The notifier revised the statistical part of the study and provided E_rC_{50} and E_bC_{50} estimates based on the time interval between 24 and 96 hours from test start and thus for an exposure period of 72 hours. For this period exponential growth in control cultures is given and the OECD validity criterion of a 16 fold increase in cell numbers is satisfied. Though this is not a common procedure the RMS is of the opinion that this is acceptable due to the fact, that a clear dose response relationship is given for the parameters area under the growth curve (AUC) and growth rate after 96 hours test duration (see figure below). In the study description above only the revised evaluation of the raw data for the time interval between 24 and 96 hours from test start is given.

Daily growth rates were highly variable in control cultures. The notifier stated that the Navicula test system is more difficult to handle than the green algae test system and therefore more variability may occur. For this reason the test was run with 8 control replicates. Additionally the dose response curve of the test shows for growth rates (24 – 96 hours) a clear concentration/effect relationship with narrow confidence limits and hence sound E_bC_{50} and E_rC_{50} estimates can be obtained from the results. The RMS agrees with this argumentation and considers the study acceptable.



Concentration-effect curve showing the influence of the test item on growth rate of the introduced *Navicula pelliculosa* as observed after 96 h (growth rates were calculated for the time period between 24 and 96 hours of exposure and hence for a 72 hour exposure period).

Reference:	**AE 0172747 – Acute toxicity to the marine diatom, *Skeletonema costatum*, under static conditions**
Author(s), year:	Hoberg; J. R. (2003b)
Report/Doc. number:	13798.6105; M-091555-01-1
Guideline(s):	OECD 201, OPPTS 850.5400, EU C.3
GLP:	Yes
Deviations:	Lower light intensity as recommended in the guidelines
Validity:	Acceptable

RANGE FINDING TEST

Two replicates per test level were exposed to concentrations of 0.001, 0.001, 0.1, 1.0, 10 mg/L, a control and a solvent control. After 96 hours respective cell densities were 110, 101, 109, 92 and 58 x 10^4 cells/mL.

Test substance: Tembotrione (AE 0172747), batch: PFI 0215, purity: 94.0 % (w/w)

MATERIAL AND METHODS:

Test species:	*Skeletonema costatum*, marine diatom
Test concentrations:	Nominal: 0 (medium and solvent control), 1.0, 2.6, 6.4, 16, 40, 100 mg/L (pure a.s.) Mean measured: --, 0.96, 2.6, 6.2, 16, 39, 97 mg/L (pure a.s.) Solvent: Dimethylformamide, 0.1 mL/L in the solvent control and all test solutions
No of replicates:	3 replicates per test concentration and 6 replicates for the controls
Initial loading:	7.7 x 10^4 cells/mL
Test type / duration:	Static, 96 hours
Dilution water:	Artificially enriched seawater (AES) medium prepared with sterile, filtered, natural seawater. The medium was adjusted to pH 8.0 ± 0.1
Test conditions:	Continuous illumination at 51 - 65 µE m^{-2} s^{-1}, 4000 – 4600 lux (fluorescent lamps) Shaking rate: 60 rpm Temperature: 19 - 22 °C pH at test start: 7.4 – 8.1, pH at test end: 7.6 – 8.8 Salinity: 30 ± 2 g/L
Observations:	Cell concentrations were determined by a haemocytometer and a compound microscope after 24, 48, 72 and 96 hours. At the same time points morphology of algal cells was examined using counting chambers and a microscope.
Analytical measurements:	For chemical analysis of the test substance samples of each treatment level and the control level were taken at test start and test end (96 h). Analytical method: HPLC/UV
Statistical evaluation:	Comparison of daily cell numbers between control and solvent control: T-test NOEC: William's test EC_{50}: Linear regression of response (percent inhibition) vs. exposure concentration

RECOVERY TEST:

After 96 hours a sample of the pooled replicates from the 100 mg/L treatment level was taken and diluted with freshly prepared AES medium to prepare a subculture with a nominal concentration of

1 mg/L. The estimated cell density of this solution was 19.5 x 10^4 cells/mL. The subculture was incubated under the same conditions as applied for the definitive test for nine days**.**

FINDINGS:

Analytical results:	Measured concentrations ranged from 98 – 104 % of nominal at test start and from 94 – 100 % at test end.
Morphological effects:	At test termination cells exposed to treatment levels ≥ 6.2 mg/L were observed to be small and light in colour. Cell fragments were also observed at these treatment levels.

Table 94: Effects of AE 0172747 on biomass (area under growth curve, AUC) and growth rate of Skeletonema costatum after 72 and 96 hours of exposure.

Tembotrione (mean measured) [mg/L]	Inhibition of biomass (AUC) [%]		Inhibition of growth rate [%]	
	72 h [a]	96 h [a]	72 h [a]	96 h [b]
0.96	19	9	7	4
2.6	24 *	7	6	1
6.2	61 *	73 *	92 *	49 *
16	71 *	82 *	103 *	63 *
39	82 *	87 *	144 *	55 *
97	84 *	91 *	148 *	69 *

[a] Inhibition relative to pooled control
[b] Inhibition relative to solvent control since a difference between control and solvent control data was found for growth rate data after 96 hours of exposure.
* Denotes statistically significant difference from the control (alpha = 0.05)

Table 95: Toxicity of AE 0172747 for the marine alga Skeletonema costatum. Toxicity values are based on mean measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [mg/L]	95 % CL for E_{50} [mg/L]
Biomass (AUC)	72 h	0.96	5.1	4.0 – 9.7
	96 h	2.6	4.9	4.6 – 5.5
Growth rate	72 h	2.6	4.5	4.1 – 4.7
	96 h	2.6	7.1	5.7 - 11

RECOVERY:
The results from the recovery test indicated that the test substance has an algicidal effect.

CONCLUSION:
The following toxicity values are regarded indicative for the toxicity of tembotrione to *Skeletonema costatum.*
E_bC_{50} (72 h): 5.1 mg/L
E_rC_{50} (72 h): 4.5 mg/L
NOEC (72 h): 0.96 mg/L (based on inhibition of biomass)
$NOEC_r$ (72 and 96 h): 2.6 mg/L (based on inhibition of average specific growth rate)

Based on mean measured concentrations.

COMMENTS (RMS):
During the study a light intensity of 4000 – 4600 lux was applied. This is a lower light intensity than recommended in the OECD guideline (approximately 8000 lux). In the study protocol it is stated that based on the experience of the conducting laboratory, algal growth is improved using the applied light source and intensity compared to the intensity recommended in the guideline. The RMS is of the opinion that this deviation from the guideline is acceptable since adequate algal growth in control vessels was given.

In the original study report 72 hour data were excluded from the 0 – 96 hour total biomass data since the 72 hour cell counts were atypical (72 hour cell density counts in concentrations ≥ 6.2 mg/L decreased from the 48 hour observation and then increase again at 96 hours). The RMS did not accept the omission of the 72 hour cell density data for the calculations of areas under growth curves because from the raw data it could be assumed that this intermittent decrease in cell numbers was treatment related. Therefore the notifier provided revised EC_{50} estimates for biomass based on areas under growth curves for 72 and 96 hours of exposure including the 72 hour cell density data. In the result tables above only revised values are provided which are regarded acceptable by the RMS.

Reference:	**Duckweed (*Lemna gibba* G3) growth inhibition test with recovery phase AE 0172747; substance, technical**
Author(s), year:	Sowig P. (2003)
Report/Doc. number:	CE02/026; M-108152-01-1
Guideline(s):	OECD draft 1998, US-EPA J § 123-2
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch no.: LE 356, purity: 97.4 % (w/w)
MATERIAL AND METHODS:	
Test species:	*Lemna gibba*
Test concentrations:	Nominal: 0 (medium control) 1.0, 1.8, 3.2, 5.6, 10, 18 and 32 µg/L (pure a.s.) Mean measured: --, 0.94, 1.6, 2.9, 5.1, 9.3, 17.1 and 30.9 µg/L (pure a.s.)
No. of replicates / loading:	Treatment phase: 6 replicates with 12 fronds (3 – 4 plants) per test flask Recovery phase: 3 replicates with 12 fronds from each treatment level.
Test type / duration:	Semi-static, 7 days exposure followed by a 7 days recovery period, renewal of the test solutions on days 3 and 5 (treatment phase), renewal of untreated nutrient solutions on days 10 and 12 (recovery phase)
Nutrient medium:	20X-AAP medium adjusted to a pH of 7.5 ± 1
Test conditions:	Continuous illumination at 100 112 µE m^{-2} s^{-1} (wide spectrum fluorescent lamps) Temperature: 23.0 – 24.5 °C pH fresh solutions: 7.6 – 7.9, pH old solutions: 8.3 - 9.1 Dissolved oxygen: 7.4 – 9.4 mg/L Hardness: 280 mg/L as $CaCO_3$
Observations:	Number of fronds and abnormal appearance of fronds were determined at days 3, 5 and 7 of the treatment phase and at days 10, 12 and 14 of the recovery phase. Dry weight was evaluated at test start and on day 7 (from 3

	out of 6 replicates per treatment level) and on day 14 (recovery phase).
Analytical measurements:	For chemical analysis of the test substance samples from all test levels were taken at test start and on days 3, 5, and 7 (fresh and old solutions). Additionally on day 10 (day 3 of recovery phase) samples of the 32 µg/L treatment level were analysed to get information on the test item concentration in recovery solutions. Analytical method: HPLC/UV
Statistical evaluation:	EC_{50}: Binomial probability method NOEC: Duncan's Multiple Range Test.
FINDINGS:	
Analytical results:	In freshly prepared test media test item concentrations were in the range of 87 – 111 % of pure nominal with two exceptions (133 % and 136 % at day 0 for the two highest test concentrations). In old test solutions test item concentrations were in the range of 81 – 98 % of nominal (corrected for purity of test substance). During the recovery phase no test item was found in the analysed nutrient solutions.
Morphological effects:	At treatment levels above 3.2 µg/L fronds formed no colonies and white-brown spots were observed on the fronds.

Table 96: Effects of tembotrione on biomass increase (dry weight) and growth rate (based on frond numbers) of Lemna Gibba after 7 days of exposure.

Tembotrione (nominal) [µg/L]	**Inhibition after 7 days of treatment [%]**		**Inhibition after 7 days of recovery [%]**	
	Biomass	**Growth rate**	**Biomass**	**Growth rate**
1	7.0	0.5	7.2	0.4
1.8	1.1	0.8	-0.9	0.5
3.2	4.4	1.4	5.5	1.9
5.6	47 *	23 *	33 *	18 *
10	77 *	61 *	24 *	6.1 *
18	81 *	76 *	38 *	18 *
32	87 *	70 *	59 *	33 *

* Denotes statistically significant difference (alpha = 0.05)

Table 97: Toxicity of tembotrione to Lemna gibba. Toxicity values are based on nominal concentrations.

Test phase	**Endpoint**	**Time scale**	**NOEC [µg/L]**	**EC_{50} [µg/L]**	**95 % CL for EC_{50} [µg/L]**
Treatment phase	Biomass (based on dry weight)	7 d	3.2	5.99	5.6 - 10
	Growth rate (based on frond numbers)	7 d	3.2	8.48	5.6 - 10
Recovery phase	Biomass (based on dry weight)	7 d	3.2	> 32	-
	Growth rate (based on frond numbers)	7 d	3.2	25.19	18 - 32

CONCLUSION:
E_bC_{50} (7 d): 5.99 µg/L
E_rC_{50} (7 d): 8.48 µg/L
NOEC (7 d): 3.2 µg/L (based on biomass increase and growth rate)
Based on nominal concentrations.

Reference:	**Lemna gibba G3 growth inhibition test with AE 0172747 (code AE 0172747 00IC950003) in a water/sediment system using spiked medium**
Author(s), year:	Dorgerloh, M. (2004a)
Report/Doc. number:	DOM 24077; M-182555-01-1
Guideline(s):	Under principal consideration of OECD 221 (proposal 2004)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: PFI 0254, purity: 95.4 % (w/w)
MATERIAL AND METHODS:	
Test species:	*Lemna gibba*
Test concentrations:	Nominal: 0 (control), 3.20, 7.04, 15.5, 31.4, 75.0 and 165 µg/L (pure a.s.) Mean measured: --, 2.4, 6.0, 13.0, 28.3, 61.5 and 141.5 µg/L (pure a.s.)
No. of replicates:	3 replicates per treatment level
Initial loading:	12 fronds per test vessel
Test type / duration:	Static water/sediment system (water spiked), 7 days
Nutrient medium:	20X-AAP medium adjusted to a pH of 7.5 ± 1
Sediment:	Artificial sediment: 74 % quartz sand, 5 % finely ground peat, 20 % kaolin, ca. 1 % calcium carbonate to adjust the pH of the sediment to 7 ± 0.5 and nutrient medium
Test conditions:	A sediment layer of approximately 0.7 cm covered by a water column of about 3 cm (200 mL test solution). Equilibration before test start: 10 days at room temperature in the dark. Continuous illumination at 6980 – 8950 lux (cool white fluorescent lamps) Temperature: 23.6 – 24.8 °C pH water phase: 7.9 – 8.5 Hardness: no information provided in the study report
Observations:	Abnormal appearance of fronds was recorded on days 2, 4 and 7. Number of fronds and dry weights were determined at test start and test end. At test start dry weight was measured of a triplicate of 12 fronds (taken from the same batch used as inoculum within this study).
Analytical measurements:	Water phase samples were analysed for the test item in all tested concentrations at test start and at test end. Analytical method: HPLC/UV
Statistical evaluation:	EC_{50}: Probit analysis NOEC: ANOVA followed by Dunnett's test (for growth rates) Welch test with Bonferroni Adjustment (for yield)
FINDINGS:	
Analytical results:	At day 0 test concentrations were in the range of 89 – 100 % of nominal. At test end measured concentrations were in the range of 61 – 73 % of nominal.
Morphological effects:	At treatment levels of 7.04 µg/L and above small fronds and chlorosis was observed. At 34.1 µg/L and above additionally necrosis was observed.

Table 98: Effects of tembotrione on growth rate and yield (based on frond number and dry weight) of Lemna Gibba after 7 days of exposure.

Tembotrione (nominal) [µg/L]	Inhibition of growth rate [%]		Inhibition of yield [%]	
	Frond number	Biomass increase	Frond number	Biomass increase
3.2	-0.2	-0.7	-0.3	-2
7.04	41 *	32 *	70 *	63 *
15.5	53 *	53 *	79 *	82 *
34.1	95 *	56 *	99 *	84 *
75.0	97 *	57 *	99 *	85 *
165	98 *	58 *	100 *	86 *

* significantly different at alpha = 0.05

Table 99: Toxicity of tembotrione to Lemna gibba. Toxicity values are based on nominal concentrations.

Test phase	Endpoint	Time scale	NOEC [µg/L]	EC_{50} [µg/L]	95 % CL for EC_{50} [µg/L]
Growth rate	Based on frond numbers	7 d	3.2	11.4	6.6 – 19.7
	Based on dry weight	7 d	3.2	28.9	n. d.
Yield	Based on frond numbers	7 d	3.2	6.0	n.d.
	Based on dry weight	7 d	3.2	6.5	n.d.

n.d. Not determined due to mathematical reasons

CONCLUSION:
E_yC_{50} (7 d): 6.0 µg/L
E_rC_{50} (7 d): 11.4 µg/L
NOEC (7 d): 3.2 µg/L (based on growth rate and yield)
Based on nominal concentrations.

E_yC_{50} (7 d): Not calculated
E_rC_{50} (7 d): Not calculated
NOEC (7 d): 2.4 µg/L (based on growth rate and yield)
Based on mean measured concentrations.

COMMENTS (RMS):
The test was conducted under consideration of the guideline OECD 221 (draft proposal 2004). In this guideline average specific growth rate is considered the principle endpoint of a Lemna toxicity test. Additionally a yield based endpoint is included in the guideline to "satisfy current regulatory requirements in some countries". The RMS holds the opinion that an EC_{50} value based on yield for frond numbers or dry weight is equivalent to E_bC_{50} estimates (calculated from the effect data from *Lemna* studies) as used prior to the development of the guideline OECD 221.

EC_{50} estimates: The fit of the probit model to the data was not good. However, from a visual inspection of the data the RMS considers the derived EC_{50} values reasonable.

The RMS re-evaluated the Lemna water/sediment study. The study authors based the EC_{50} on nominal concentrations. However, test concentrations in the water phase were between 61 and 73 % of nominal and hence below 80 % at test end. Concentrations of tembotrione in the sediment were not measured in this study.

With FOCUS modelling the shift of a portion of the active substance to the sediment is already taken into account. Therefore the RMS is of the opinion that EC_{50} values based on nominal concentrations from a study where sediment is present in the test system and the test substance

declined during the study in the water phase can not be related to FOCUS PECsw values since this would lead to a sort of double counting of the loss of active substance from the water phase to the sediment.

However, an EC_{50} based on mean measured concentrations could be used for risk assessment since here the actual exposure concentrations are used for deriving the toxicity value and this could be compared to FOCUS PECsw values which are exposure estimates. Therefore the RMS is of the opinion that only a toxicity value which is based on mean measured concentrations should be taken on board in the list of endpoints.

The RMS asked the notifier to re-calculate the EC_{50} values based on mean measured concentrations. The notifier did not submit revised EC_{50} values until the finalisation of this revised DAR. Hence the study itself can be regarded as acceptable; however, the derived EC_{50} values (based on nominal concentrations) are not valid with respect to their use in a risk assessment and hence should not be taken on board in the list of endpoints. Since a valid Lemna test according to standard procedures is available (Sowig, 2003) no data gap is given.

5.4.4 Other aquatic organisms (including sediment)

Reference:	**Chironomus riparius 28-day chronic toxicity test with AE 0172747 in a water-sediment system using spiked water**
Author(s), year:	Dorgerloh, M. (2005e)
Report/Doc. number:	DOM 24074; M-242895-01-1
Guideline(s):	OECD 219 (proposal for a new guideline, April 2003)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

Test substance:	Tembotrione (AE 0172747), batch: PFI 0254, purity: 95.4 % (w/w)
MATERIAL AND METHODS:	
Test species:	*Chironomus riparius*, first instar larvae (L1)
Test concentrations:	Nominal: 0 (control), 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32 mg/L (in overlying water) Mean measured: --, 0.604, 1.15, 2.33, 5.00 and 9.81 mg/L
No. of replicates:	Four replicates with 20 animals each per treatment level and control (total of 80 per treatment). Additional replicates with chironomids were used for chemical analysis of the test item on day 0 and day 7 for the control, the lowest, medium and highest test concentration. A further replicate of each test concentration and control was prepared with chironomids to measure temperature, pH and oxygen content in the test water during the study.
Test type / duration:	Static system, spiked water exposure, test duration: 28 days
Dilution water:	Reconstituted water (M7 – medium based on deionised water) Hardness: 267 - 320 mg/L as $CaCO_3$ measured in the control and 32 mg/L treatment level at test start and test end.
Sediment:	74 % fine quartz sand, 4 - 5 % dried, finely ground sphagnum peat, 20 % kaolin, around 1 % calcium carbonate to adjust the pH value to 7 ± 0.5. Water content, pH and organic carbon content of sediment at day –7 were 32 %, 6.6 and 2.5 %.

Size of test vessels:	0.6 L glass beakers (diameter of 9.5 cm). Test beakers were covered by clear plastic plates throughout the study to prevent evaporation.
Preparation of test vessels:	The bottom of the test vessels were covered with a 1.5 cm layer of wet sediment and filled up with M7 medium to a water column height of 6 cm 7 days before test start to allow for equilibration of the test system. One day prior to treatment (day -1) larvae were introduced into the test vessels.
Application of test substance:	Appropriate amounts of stock solution (398.5 mg/L) were inserted into the overlying water column of the vessels just below the water surface with a pipette by gently mixing of the water body ensuring homogeneous distribution of the test item without disturbance of the sediment.
Aeration:	Gentle aeration was provided through a glass Pasteur pipette situated about 2.5 cm above the sediment layer throughout the complete study.
Test conditions:	Temperature: 19.8 – 20.1 °C pH in the overlying water: 8.3 – 8.7 Dissolved oxygen: 7.0 – 8.7 mg/L (> 60 % saturation) Total ammonia: day 0: 0.6 mg/L, day 28: 36 – 49 mg/L (measured in the control and 32 mg/L treatment level) Photoperiod: 16 hours light, 8 hours darkness Feeding: About three times per week 0.5 – 1 mg /Larvae/day of a commercial ornamental fish food extract (Tetra Phyll®.)
Observations:	Behavioural differences compared to the control: 3 times per week Sex, time point of emergence and number of emerged midges: daily during the period of emergence (only fully emerged adults were taken into account).
Analytical measurements:	For chemical analysis of the test substance in the overlying water and the pore water samples were taken from additional replicates of the 0.5, 4.0 and 32.0 mg/L treatment levels 1 hour and 7 days after test substance application. The overlying water and the pore water of the sediment from the control was analysed on day 0 only. For chemical analysis on day 28 one beaker of the four replicates for biological evaluations was sampled. Analytical method: HPLC/UV
Statistical evaluation:	Differences in sensitivity between sexes: Chi^2-Contingency test

FINDINGS:

Table 100: Measured concentrations of tembotrione in the overlying water and the pore water of the test system.

Tembotrione (nominal) [mg/L]	Day 0		Day 7		Day 28	
	Overl. water	Pore water	Overl. water	Pore water	Overl. water	Pore water
Control	< 0.05	< 0.05	n.a.	n.a.	n.a.	n.a.
0.5	0.48	0.048	0.39	0.23	0.32	0.25
4.0	4.05	0.38	3.23	1.98	2.74	2.16
32	32.6	3.94	27.5	18.0	20.9	18.5
% of nominal	95 - 102		79 - 86		64 - 69	

n.a....not analysed

Sex ratio: The Chi^2-Contingency test established a statistical significant difference between sexes in numbers of emerged midges. Therefore male and female results for development rate were additionally analysed separated by sex.
$EC_{50:}$ Probit analysis
NOEC: ANOVA or Willimas multiple sequential t-test

Start of emergence: On day 15 for the control and day 14 to 16 in all treatment levels.

Table 101: Effects of tembotrione on the emergence and development rate of Chironomus riparius after 28 days of exposure

Tembotrione (nominal) [mg/L]	No. of emerged midges (out of 80)	Emergence [%]			Development rate [1/d]		
		Pooled sexes	Males	Females	Pooled sexes	Males	Females
Water control	72	90.0	47.5	42.5	0.058	0.062	0.055
0.5	74	92.5	43.8	48.8	0.059	0.063	0.056
1.0	77	96.3	41.3	55.0	0.057	0.061	0.053
2.0	70	87.5	50.0	37.5	0.059	0.062	0.055
4.0	63	78.8 *	50.0	28.8	0.060	0.063	0.055
8.0	40	50.0 *	38.8	11.3	0.061	0.061	0.057
16.0	23	28.8 *	25.0	3.8	0.059	0.059	0.051
32.0	23	28.8 *	27.0	1.3	0.059	0.059	0.061

* Denotes statistically significant difference from the control (alpha = 0.05)

CONCLUSION:
Emergence of pooled sexes:
EC_{50}: 12.5 mg/L, NOEC: 2 mg/L, LOEC: 4 mg/L
Development rate of pooled sexes, males and females:
EC_{50}: > 32 mg/L, NOEC: 32 mg/L, LOEC: > 32 mg/L

Reference:	**AE 0172747 – Acute toxicity to Mysids (*Americamysis bahia*) under flow-through conditions**
Author(s), year:	Lima, W. (2003b)
Report/Doc. number:	13798.6102; M-091558-01-1
Guideline(s):	US EPA 72-3, OPPTS 850.1035 (draft)
GLP:	Yes
Deviations:	None of relevance
Validity:	Acceptable

RANGE FINDING TESTS:

Table 102: Summary of range finding tests

Test 1 (static, 96 h) 10 Mysids / treatment, age not stated	Test conc. [mg/L]	Control	0.1	0.5	1.0	10	-
	Mortality [%]	0	80	100	100	100	-
Test 2 (static, 96 h) 10 Mysids / treatment, age not stated	Test conc. [µg/L]	Control	1	10	50	100	-
	Mortality [%]	0	0	0	0	80	-
Test 3 (flow through, 48 h) 10 Mysids (≤ 24 h old) / treatment	Test conc. [mg/L]	Control	1.6	3.1	6.3	13	25
	Mortality [%]	0	100	100	100	100	100
Test 4 (flow through, 48 h) 10 Mysids (5 – 6 d old) / treatment	Test conc. [mg/L]	Control	1.6	3.1	6.3	13	25
	Mortality [%]	0	100	100	100	100	100

Test 5 (flow through, 96 h) 10 Mysids (≤ 24 h old) / treatment	Test conc. [µg/L]	Control	17	28	47	78	130
	Mortality [%]	0	0	0	0	60	90
Test 6 (flow through, 96 h) 5 Mysids (6 d old) / treatment	Test conc. [mg/L]	Control	17	28	47	78	130
	Mortality [%]	0	0	0	0	40	40

MAIN TEST:

Test substance: Tembotrione (AE 0172747), batch: PFI 0215, purity: 94 % (w/w)

MATERIAL AND METHODS:

Test species: *Americamysis bahia*, ≤ 24 hours old

Test concentrations: Nominal: 0 (control and solvent control), 17, 28, 47, 78 and 130 µg/L (pure a.s.)
Mean measured: --, 14, 28, 46, 79 and 130 µg/L (pure a.s.)
Solvent: Dimethylformamide, 0.096 mL/L in the solvent control, this equals to the solvent concentration present in the highest treatment level.

No. of replicates: 2 replicates with 10 mysids each per treatment and control

Test type / duration: Flow-through system with approximately 9 volume replacements per test vessel every 24 hours, test duration: 96 hours

Dilution water: Natural filtered seawater

Test conditions: Temperature: 24 – 25 °C
pH: 7.6 – 8.0
Salinity: 20 – 22 ‰
Dissolved oxygen: 4.6 – 7.9 mg/L (≥ 60 % O_2 saturation)
Photoperiod: 16 hours light and 8 hours darkness with a transition period

Observations: Mortalities and sublethal effects were recorded after 24, 48, 72 and 96 hours.

Analytical measurements: For chemical analysis of the test substance samples of each treatment level were taken from replicates A at test start and from replicates B at test end (96 h).
Analytical method: HPLC/UV

Statistical evaluation: The LC_{50} was estimated by the binomial probability model.

FINDINGS:

Analytical results: Mean measured concentrations ranged from 85 – 101 % of nominal

Sublethal effects: Up to and including 46 µg/L: No effects observed
79 µg/L: Lethargy, partial loss of equilibrium

Mortality: Control, 14, 28, 46 and 79 µg/L: 0 % mortality, 130 µg/L: 95 % mortality

CONCLUSION:

LC_{50} (96 h): 100 µg/L (95 % CL: 79 - 130 µg/L)
NOEC (96 h): 46 µg/L
Based on mean measured concentrations.

COMMENT RMS:

In the study report some inconsistency between the stated measured concentrations at test start and test end and the resulting mean measured concentrations (absolute and expressed as percent of nominal) was found. However, the RMS came to the conclusion, that the mean measured values used for the calculation of the LC_{50} are worst case values (leading to a rather low LC_{50}) and hence the resulting LC_{50} is regarded acceptable.

Reference:	**AE 0172747 – Acute toxicity to eastern oysters (*Crassostrea virginica*) under flow-through conditions**
Author(s), year:	Dionne, E. (2003)
Report/Doc. number:	13798.6101; M-084955-01-1
Guideline(s):	US EPA 72-3, OPPTS 850.1025 (draft)
GLP:	Yes
Deviations:	Shell growth of control oysters was only 1.6 mm and hence below the minimum of 2.0 mm stated in the guideline. However, the value of 1.6 mm is within the 15th percentile of the historical control range of the conducting laboratory.
Validity:	Acceptable to demonstrate that oysters are not the most sensitive invertebrate species.

RANGE FINDING TESTS:

In a first test the test substance was not dissolved completely and hence results were not used.
In a second test 20 oysters per treatment level were exposed to concentrations of 0 (control), 1.9, 3.8, 7.5, 15 and 30 mg/L for 96 hours. Respective reductions in shell deposition were 8, 29, 0, 54 and 60 %.

MAIN TEST:

Test substance:	Tembotrione (AE 0172747), batch: PFI 0195 and PFI 0215 purity: 95 % and 94 % (w/w)

MATERIAL AND METHODS:

Test species:	*Crassostrea virginica* Valve height (mean ± s.d.): 32 ± 4 mm
Test concentrations:	Nominal: 0 (control and solvent control), 3.1, 6.3, 13, 25 and 50 mg/L (pure a.s.) Mean measured: --, 3.0, 5.9, 14, 25 and 50 mg/L (pure a.s.) Solvent: Dimethylformamide, 0.1 mL/L in the solvent control, this equals to the solvent concentration present in the highest treatment level.
No. of replicates:	2 replicates with 20 oysters each per treatment and control
Test type / duration:	Flow-through system with approximately 6 volume replacements per test vessel every 24 hours, test duration: 96 hours
Dilution water:	Natural unfiltered seawater
Test conditions:	Temperature: 21 - 22 °C pH: 7.6 – 8.1; Salinity: 32 ‰ Dissolved oxygen: 4.6 – 7.1 mg/L (≥ 60 % O_2 saturation) Photoperiod: 16 hours light and 8 hours darkness with a transition period Feeding: Algal suspension (*Isochrysis galbana*, approx. 10^7 cells/mL) was added to each test aquarium three times daily to maintain an average concentration of approx. 10^5 cells/mL in the test solutions during the test.
Shell preparation:	Prior to testing, 3 – 5 mm of the new peripheral shell growth of each oyster were removed by grinding the shell to a blunt edge using a fine-grit grinding wheel.
Observations:	Oysters were examined for abnormalities daily. At test end new shell growth was measured microscopically.
Analytical measurements:	For chemical analysis of the test substance replicates A of each treatment level were sampled at test initiation and replicates B of each treatment level were sampled at test termination (96 h). Analytical method: HPLC/UV
Statistical	Comparison of control and solvent control data: T-test

evaluation: EC_{50}: Linear regression of response data vs. log concentration
NOEC: Williams' test

FINDINGS:
Analytical results: Mean measured concentrations ranged from 93 – 110 % of nominal
Sublethal effects: No effects observed
Mortality: No mortalities observed

A statistically significant difference between solvent control and water control groups was found and therefore solvent control data were used for comparison of the treatment responses.

Table 103: Effects of tembotrione on shell deposition in Eastern oysters (Crassostrea virginica) after 96 hours of exposure.

Tembotrione Mean measured [mg/L]	Mean shell deposition ± s.d. [mm]	Reduction compared to solvent control [%]
Control	1.6 ± 0.9	-
Solvent control	2.5 ± 1.0	-
3.0	2.1 ± 1.2 *	17
5.9	1.4 ± 0.9 *	46
14	1.3 ± 0.9 *	49
25	0.8 ± 0.8 *	67
50	0.9 ± 0.8 *	65

* Denotes statistically significant difference from the solvent control (alpha = 0.05)

CONCLUSION:
EC_{50} (96 h): 14 mg/L, 95 % CL: 0.62 - 380 mg/L (based on mean measured concentrations)
NOEC (96 h): Could not be set because already at the lowest test concentration of 3 mg/L a statistically significant reduction of shell growth compared to the solvent control was observed.

COMMENT (RMS):
According to the guideline OPPTS 850.1025 as a validity criterion a minimum of 2.00 mm new shell growth (based on the longest finger of new growth) must be deposited in control oysters (solvent and dilution water) by the end of the test (96 h). In the submitted study the shell growth of oysters from the dilution water control was only 1.6 mm. The value of 1.6 mm equals to the 15^{th} percentile of the historical water control data from the conducting laboratory (range 0.9 – 4.5 mm). Since the shell growth was within the historical control range the study authors consider the amount of shell deposition observed during this study as representative for this species and acceptable for establishing the relative toxicity of the test substance. Shell growth in solvent control oysters was statistically significantly higher than shell growth in water control oysters. Solvent control oysters reached the validity criterion of 2.0 mm shell growth over a period of 96 hours. The EC_{50} estimate for shell growth was based on comparisons of treatment groups to solvent control groups because the solvent had obviously a positive effect on shell growth.
Due to a flat dose response curve, the EC_{50} was estimated to be 14 mg/L with extremely wide confidence limits (95 % CL: 0.62 – 380 mg/L). Therefore the value of 14 mg/L is not very reliable. However, even the lower end of the confidence limit (0.62 mg/L) is still higher than the LD_{50} from the most sensitive species *Americamysis bahia* (LD_{50} = 0.1 mg/L). The RMS is of the opinion that from these considerations the study can be regarded acceptable to demonstrate that *Crassostrea virginica* is not the most sensitive species of aquatic invertebrates although shell growth in control oysters was rather low, the solvent had obvious an effect on shell growth and no reliable EC_{50} estimate could be obtained from the results of the test.

5.4.5 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	> 100		US-EPA OPPTS 850.1075, OECD 203	y	
Lepomis acrochirus	> 100		US-EPA OPPTS 850.1075, OECD 203	y	
Cyprinodon variegatus	> 100		US-EPA OPPTS 850.1075	y	
Crustacea (48 hr EC_{50}):					
Daphnia magna	*49.8*		OECD 202, US-EPA 72-2	y	
Algae/aquatic plants (72 or 96 hr E_rC_{50}):					
Pseudokirch.subcapitata	Biomass Growth rate	0.38 0.75	OECD 201, US-EPA J § 123-2, EU C.3	y	
Anabaena flos-aquae	Biomass (96 h) Growth rate (72 h)	64 71	OECD 201, US-EPA J § 123-2, EU C.3		
Navicula pelliculosa	Biomass Growth rate	10.8 47.9	OECD 201, US-EPA J § 123-2, EU C.3		
Skeletonema costatum	Biomass Growth rate	5.1 4.5	OECD 201, OPPTS 850.5400, EU C.3	y	
Lemna gibba	Biomass Growth rate	0.00599 0.00848	OECD draft 1998, US-EPA J § 123-2	y	
Other aquatic organisms (including sediment)					
Marine *invertebrate* (96 hr E/LC_{50}):					
Americamysis bahia	**Mortality** Subl. effects	**0.1**	US EPA 72-3, OPPTS 850.1035 (draft)	Y	
Crassostrea virginica	Shell growth	14	US EPA 72-3, OPPTS 850.1025 (draft)		

Conclusion:
The 96 h LC_{50} used for classification follows from the toxicity o to the most sensitive species *Americamysis bahia* with a LC50 = 0.1 mg/L, (Lima, 2003b)

5.4.6 Summary and discussion: Chronic (long-term) aquatic toxicity

Chronic (long-term) aquatic toxicity Generally expressed in terms of NOEC (mg/L)					
	$L(E)C_{50}$ [mg/L]		Test guideline / design	GLP (y/n)	Reliability *
Fish (34 d NOEC):					
Pimephales promelas	Fry survival	0.604	OECD 210, US-EPA OPPTS 850.1400	y	
Crustacea (21 d NOEC):					
Daphnia magna	*Reproduction* *Growth*	*5*	*OECD 202, US-EPA 72-2*	y	
Algae/aquatic plants (72 or 96 hr NOEC):					
Pseudokirch.subcapitata	Biomass Growth rate	0.2 0.2	OECD 201, US-EPA J § 123-2, EU C.3	y	
Anabaena flos-aquae	Biomass (96 h) Growth rate (72 h)	16 39	OECD 201, US-EPA J § 123-2, EU C.3	y	
Navicula pelliculosa	Biomass Growth rate	5.6 5.6	OECD 201, US-EPA J § 123-2, EU C.3	y	
Skeletonema costatum	Biomass Growth rate	0.96 2.6	OECD 201, OPPTS 850.5400, EU C.3	y	
Lemna gibba	Biomass Growth rate	0.0032 0.0032	OECD draft 1998, US-EPA J § 123-2	y	
Lemna gibba	Yield **Growth rate**	0.0024 **0.0024**	OECD draft 1998, US-EPA J § 123-2	y	
Other aquatic organisms (including sediment)					
Sediment dwelling organisms (28 d, static, $NOEC_0$):					
Chironomus riparius	Emergence Develop. rate	2 32	OECD 202, US-EPA 72-2	y	

Conclusion:
The NOEC used for classification follows from the chronic toxicity to the most sensitive species *Lemna gibba* NOEC= 0.0024 mg/L, (Dogerloh, 2004a).

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

<table>
<tr><th>Endpoint</th><th colspan="3">Classifcation Criteria
(criteria in bold)</th><th>Evidence for
Tembotrione</th></tr>
<tr><td></td><td colspan="2">CLP (2nd ATP)</td><td>DSD</td><td></td></tr>
<tr><td>Degradation Tembotrione</td><td colspan="3">Tembotrione is hydrolytically stable at environmentally relevant pH values from pH 4 to pH 9.
Photodegradation of Tembotrione was moderate with an experimental half-life of 56.3 days under the test conditions.
Tembotrione is not readily biodegradable, and does not meet the criterion for rapid degradation in a water/sediment study with a DT50 whole system of 108 days.
Based on available data a non rapid degradation is proposed for tembotrione.</td><td>The classification as R53 according to Directive 67/548/EEC. is based on the fact that the active substance is not considered as ready biodegradable/rapid degradable.</td></tr>
<tr><td>Bioaccumulation Tembotrione</td><td colspan="2">Log K_{ow} is < 4
Tembotrione Log K_{ow} = 1.09
at pH 7 and 24 °C</td><td>Log K_{ow} is < 3
Tembotrione Log K_{ow} = 1.09
at pH 7 and 24 °C</td><td>The measured log POW is 1.09 (at pH 7 and 24 °C) and is below the two classification criteria of 3 and 4, therefore Tembotrione is considered to have a low bioaccumulation potential.</td></tr>
<tr><td rowspan="2">Acute aquatic toxicity Tembotrione</td><td colspan="3">0.01 < LC50 ≤ 0.1 mg/L</td><td rowspan="2">Tembotrione is of high acute toxicity to Americamysis bahia with a LC50 = 0.1 mg/l and fulfills the criteria for the proposed classification as R50 according to Directive 67/548/EEC and the criteria for the proposed classification as H400 according to Regulation EC 1272/2008. A M-factor of 10 is applicable based on 0.01 < L(E)C50 ≤ 0.1 mg/L</td></tr>
<tr><td colspan="3">Americamysis bahia LC50 = 0.1 mg/L</td></tr>
<tr><td rowspan="2">Chronic aquatic toxicity Tembotrione</td><td colspan="2">For non rapidly degradable substances:
0.001 <NOEC ≤0.01 mg/l</td><td rowspan="2"></td><td rowspan="2">Tembotrione is of high chronic toxicity to water plants (Lemna gibba) with a NOEC $_{GROWTH\ RATE}$ = 0.0024mg/L. Therefore Tembotrione fulfills the criteria for the proposed classification as H410 according to Regulation EC 1272/2008. A M-factor of 10 is applicable based on 0.001 < NOEC ≤ 0,01mg/l (no rapid degradation).</td></tr>
<tr><td>Lemna gibba</td><td>NOEC(14d) = 0.0024 mg/L</td></tr>
<tr><td rowspan="5">SUMMARY</td><td colspan="2" rowspan="5">H400 M-factor =10
H410 M-factor =10</td><td colspan="2">R50/53</td><td rowspan="5">PROPOSED CLASSIFICATION</td></tr>
<tr><td colspan="2">“SCLs based on acute toxicity data (Americamysis bahia)”</td></tr>
<tr><td>Classification</td><td>Concentration [in %]</td></tr>
<tr><td>N, R50/53</td><td>≥ 2.5</td></tr>
<tr><td>N, R51/53</td><td>≥ 0.25 - < 2.5</td></tr>
<tr><td>R52/53</td><td>≥ 0.025 - < 0.25</td></tr>
</table>

5.6 Conclusion of environmental classification and labelling according to Directive 67/548/EEC

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

N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment

SCLs

Classification	Concentration* [in %]
N, R50/53	≥ 2.5
N, R51/53	≥ 0.25 - < 2.5
R52/53	≥ 0.025 - < 0.25

*concentration of Tembotrione in the preparation

Conclusion of environmental classification according to Regulation EC 286/2011 (2nd ATP to EC 1272/2008)

Classification categories	aquatic environmental hazard **acute category 1** aquatic environmental hazard **chronic category 1**
GHS Pictogram	
Signal Word	Warning
Hazard Statement	'Very toxic to aquatic life', 'Very toxic to aquatic life with long lasting effects'EUH401
M-factor (acute)	10
M-factor (chronic)	10

6 OTHER INFORMATION

Environmental fate properties and environmental hazard assessments of this CLH report were based on studies and summaries of the Draft Assessment Report and its addenda.

RAC evaluation of environmental hazards

Summary of Dossier submitter's proposal

The DS proposed environmental hazard classification for tembotrione as Aquatic Acute 1, H400 (M=10) and Aquatic Chronic 1, H410 (M=10) according to CLP, and N, R50/53 according to DSD with SCL R50-53 ≥2,5%; R51-53 ≥0,25-<2,5; R52-53 ≥0,025-<0,25.

Degradation

Degradation was studied in a hydrolysis test, a photolysis test, a ready biodegradability test, an aerobic (water/sediment) study, three aerobic (soil) degradation laboratory studies and two anaerobic (soil) degradation laboratory studies.

The DS considered tembotrione as hydrolytically stable and moderately photodegradable with a measured half-life of 56.3 days. It degraded rapidly in air by reaction with OH radicals, although the presence of this compound in air is not expected due to its low vapour pressure.

Tembotrione is not readily biodegradable under test conditions (OECD 301D).

In a water/sediment study tembotrione showed a very slow degradation with a $DT50_{whole\ system}$ of 108 days and in aerobic soil degradation studies tembotrione degraded with a half-life from 4.3 to 56.4 days while in anaerobic conditions the DT50 was 278 days.

Based on the available data the DS considered tembotrione as not rapidly degradable.

Bioaccumulation

The log Pow of tembotrione was reported to be 2.2, at pH 2, -1.1 at pH7 and -1.4 at pH 9. Experimental bioconcentration tests are not available. Since the log Pow indicated low potential for bioaccumulation, the DS concluded that tembotrione has low potential for bioaccumulation.

Aquatic toxicity

Three acute toxicity studies in fish, one in invertebrates, three in algae, five in algae and aquatic plants, including *Lemna gibba*, and finally two more tests in marine invertebrates were reported by the DS. One long-term toxicity study in fish (34 days, *Pimephales promelas*), one in aquatic invertebrates, six in algae and aquatic plants and one more in sediment dwelling organisms (*Chironomus riparius*) were available in the CLH report.

The marine invertebrate (*Americamysis bahia*) was the most sensitive taxonomic group in acute tests, with EC50 value of 0.1 mg/l while in chronic tests the most sensitive species was *Lemna gibba,* with a NOErC value of 0.0024 mg/l. These two values were used as key studies for classification.

Comments received during public consultation

Six comments were received regarding the use of the marine invertebrate (*Americamysis bahia*) as the most sensitive taxonomic group in acute tests, with a ErC_{50} value of 0.1 mg/l, when in fact the *Lemna gibba* was the most sensitive specie for acute toxicity with an 7d ErC_{50} of = 0.00848 mg/l.

One commenter questioned the use of a *Lemna gibba* study based on OECD TG 221

performed with sediment, for aquatic chronic classification.

In their post public consultation response the DS agreed that the most sensitive species for acute classification is *Lemmna gibba*, and therefore they supported classification for Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=10) according to CLP, and N, R50/53 according to DSD with SCL R50/53 ≥0.25%; R51/53 ≥0.025-<0.25; R52/53 ≥0.0025-<0.025.

Regarding the use of studies performed with sediment for classification purposes, the DS stated that it should be discussed by the ECHA experts.

RAC assessment and comparison with criteria

Degradation

RAC agreed that tembotrione can be considered hydrolytically stable and moderately photodegradable based on the information provided in the CLH report.

RAC also agreed that tembotrione is not readily biodegradable under the reported test conditions (OECD 301D). Furthermore, in an aerobic water/sediment study tembotrione shows a very slow degradation ($DT50_{whole\ system}$ =108 days at 20ºC), therefore, based on these data, RAC agrees with the DS that tembotrione must be considered not readily biodegradable according to DSD and not rapidly degradable according to CLP.

Bioaccumulation

In the current CLP criteria (2nd ATP) bioaccumulation is relevant only if the surrogate approach is applied for assessing long-term hazards. For tembotrione, adequate chronic toxicity data is available for all trophic levels and therefore, bioaccumulation data is not used for classification according to CLP. However, under the DSD bioaccumulation should be used for assessing long-term adverse effects. In this case it does not meet the criteria for classification, since the measured log Kow = -1.09 at pH= 7 and 24ºC and therefore lower than 3.

Aquatic toxicity

Under CLP, classification for acute toxicity should be based on the most sensitive species. In the case of tembotrione, that is *Lemna gibba* (Sowig, 2003), with an ErC50 of 0.00848 mg/l. As the LC50 value is below 1 mg/l, the classification should be Acute category 1 – H400. As the value is betewwn 0.001 and 0.01 mg/l, an M factor of 100 is appropriate

According to section 4.1.3.2.3 of the Guidance on the Application of the CLP Criteria (p.409) *Lemna gibba* studies shall be considered if the test methodology is suitable. The aquatic plant growth inhibition tests are normally considered as chronic tests but the EC_{50}s are treated as acute values for classification purposes.

Regarding chronic toxicity, the lowest NOErC value is reported in a study on *Lemna gibba* based on OECD TG 221 (NOErC= 0.0024mg/l (the value based on mean measured concentration; Dorgerloh, 2004a). However the test was modified, including sediment in the test system which can modify the recoveries in water. Therefore the NOErC of 0.0032 mg/L (nominal-recoveries higher than 80%) from the study on *Lemna gibba* (Sowig, 2003) performed without sediment should be used. Nevertheless, both tests gave roughly the same NOEC value based on nominal concentrations (NOErC: 0.0032 mg/l).
Taking into account the NOEC value of 0.0032 mg/l and its persistence, tembotrione should be classified in Aquatic Chronic category 1 (H410) with an M-factor of 10, because the NOEC value is between 0.001 and 0.01 mg/l.

Under DSD, the key study for acute toxicity has an EC_{50} value of 0.0084 mg/l (*Lemna gibba*), which is below the classification criterion of 1 mg/l and therefore tembotrine should be classified as N; R50.Tembotrine is considered not rapidly degradable and it does not fulfill the criteria of ready degradability (point 5.2.1.3 of Annex 6 of 2001/59/EC). Therefore,

classification for long-term adverse effects (R53) under DSD is justified.

RAC agreed with the DS's proposal to classify tembotrione as hazardous to the aquatic environment according to the CLP criteria, however, RAC proposed higher Acute M-factor than in the original proposal by the DS. Classification as Aquatic Acute 1 (H400) with M-Factor 100 and Aquatic Chronic 1 (H410) with M-Factor 10 for tembotrione is warranted (N; R50-53 Specific concentration limits N; R50-53: C ≥ 0,25 %, N; R51-53: 0,025 % ≤ C< 0,25 % and R52-53: 0,0025 % ≤ C< 0,025 % since 0.001 < L(E)C50 ≤ 0.01).

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
Bogdoll, B.; Lemke, G.	2005	Surface Tension of AE 0172747 - Substance, Technical - Code: AE 0172747 00 1C95 0003 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: M-255557-01-1, Edition Number: M-255557-01-1 Date: 09.08.2005 GLP, unpublished	Yes	BCS
Bogdoll, B.; Strunk, B.	2005	Physical Characteristics - Color, Appearance and Odor of AE 0172747 substance technical - Code: AE 0172747 00 1C95 0003 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: M-255563-01-1, Edition Number: M-255563-01-1 Date: 09.08.2005 GLP, unpublished	Yes	BCS
Fliege, R.	2003	Abiotic hydrolysis of 14C-AE 0172747 in buffered aqueous solutions at pH 4, pH 7, and pH 9 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: MEF-083/03, Edition Number: M-107923-01-1 Date: 05.06.2003 GLP, unpublished	Yes	BCS
Franke, J.	2002	AE 0172747 - Vapour pressure Siemens Axiva GmbH & Co. KG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20020360.01, Edition Number: M-078780-01-1 Date: 23.04.2002 GLP, unpublished	No	BCS
Hellpointner, E.	2004	Phototransformation of AE 0172747 in sterile water buffered at pH 7 Bayer CropScience AG, Report No.: MEF-412/03, Edition Number: M-063564-01-1 Date: 14.04.2004 GLP, unpublished	Yes	BCS

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**
Hellpointner, E.	2003	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water: AE 0172747 Bayer CropScience AG, Report No.: MEF-154/03, Edition Number: M-105933-01-1 Date: 22.12.2003 GLP, unpublished	No	BCS
Muehlberger B.; Lemke G.	2004	Relative density AE 0172747 Code: AE 0172747 00 1B99 0001 Bayer CropScience GmbH, Frankfurt, Germany; Bayer CropScience AG, Report No.: C041074, Edition Number: M-229996-01-1 Date: 02.04.2004 GLP, unpublished	Yes	BCS
Muehlberger B.; Strunk B.	2003	AE 0172747 Physical characteristics Color, appearance and odor Code: AE 0172747 00 1B99 0001 substance, pure Bayer CropScience GmbH, Frankfurt, Germany; Bayer CropScience AG, Report No.: C037586, Edition Number: M-223199-01-1 Date: 28.10.2003 GLP, unpublished	Yes	BCS
Muehlberger, B.	2003a	Henry's law constant of AE 0172747 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: OE03/042, Edition Number: M-108444-01-1 Date: 02.04.2003 Non GLP, unpublished	Yes	BCS
Muehlberger, B.	2002a	Water solubility of AE 0172747 at pH 4, pH 7 and pH 9 (flask method) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA02/007, Edition Number: M-078747-01-1 Date: 27.11.2002 GLP, unpublished	No	BCS
Muehlberger, B.	2002b	AE 0172747 - Solubility in organic solvents Aventis Crop Science GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA02/011, Edition Number: M-078761-01-1 Date: 20.06.2002 GLP, unpublished	No	BCS

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**
Muehlberger, B.	2003b	AE 0172747 - Partition coefficient, 1-octanol/water - AE 0172747 00 1B99 0001 (substance, pure) Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA02/012, Edition Number: M-108278-01-1 Date: 07.03.2003 GLP, unpublished	Yes	BCS
Muehlberger, B.; Eyrich, U.	2005	AE 0172747 - Determination of the dissociation constant - Code: AE 0172747 00 1B99 0001 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: PA03/060, Edition Number: M-254999-01-1 Date: 26.07.2005 GLP, unpublished	Yes	BCS
Smeykal, H.	2005	AE0172747; Substance, pure; AE 0172747 00 1B99 0001 - Melting point, boiling point, thermal stability Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20050149.01, Edition Number: M-248060-01-1 Date: 15.03.2005 GLP, unpublished	Yes	BCS
Smeykal, H.	2004a	AE 0172747, substance technical, AE 0172747 00 1C96 0001 - Flammability (Solids) Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040750.02, Edition Number: M-242885-01-1 Date: 19.10.2004 GLP, unpublished	Yes	BCS
Smeykal, H.	2004b	AE 0172747, substance technical, AE 0172747 00 1C96 0001 - Auto-flammability (Solids - Determination of relative self-ignition temperature) Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040750.04, Edition Number: M-242887-01-1 Date: 19.10.2004 GLP, unpublished	Yes	BCS
Smeykal, H.	2004c	AE 0172747, substance technical, AE 0172747 00 1C96 0001 - Explosive properties Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20040750.03, Edition Number: M-242886-01-1 Date: 19.10.2004 GLP, unpublished	Yes	BCS

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**
Smeykal, H.	2005b	AE 0172747; Substance, technical AE 0172747 00 1C95 0003 - Oxidizing prpperties A.17. Siemens AG, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: 20050497.01, Edition Number: M-255234-01-1 Date: 12.07.2005 GLP, unpublished confidential	Yes	BCS
Wiche A.; Muehlberger B.	2003	Spectral data (UV / VIS, IR, 1H-NMR, 13C-NMR, MS) and molar extinction coefficient Code: AE 0172747 00 1B99 0001 Bayer CropScience GmbH, Frankfurt, DEU; Bayer CropScience AG, Report No.: C036440, Edition Number: M-220680-01-1 Date: 15.09.2003 GLP, unpublished	Yes	BCS

7.2 Toxicological 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**
Odin-Feurtet, M.	2002a	AE 0172747 - Preliminary rat A.D.M.E. study Aventis CropScience, Sophia Antipolis, France Report No.: SA 01242, Edition Number: M-078672-01-1 GLP unpublished	Y	BCS
Odin-Feurtet, M.	2002b	AE 0172747 - Preliminary rat A.D.M.E. study Bayer CropScience, Sophia Antipolis, France Report No.: SA 02060, Edition Number: M-078684-01-1 GLP unpublished	N	BCS
Odin-Feurtet, M.	2003	AE 0172747 - Single oral low and high dose - Rat A.D.M.E.study Bayer CropScience, Sophia Antipolis, France Report No.: SA 02092, Edition Number: M-103278-02-1 GLP Unpublished	Y	BCS
Koester, J.	2005a	[Cyclohexyl-UL-14C]AE 0172747: Absorption, distribution, excretion and metabolism in the rat Report No.: MEF-04/512, Edition Number: M-248187-01-1 GLP unpublished	Y	BCS
Odin-Feurtet M.	2002c	Rat blood / plasma kinetics study AE 0172747 Bayer CropScience S.A., FRANCE; Report No.: C027447, Edition Number: M-213340-01-1 GLP unpublished	Y	BCS
Koester, J.	2005b	[Cyclohexyl-UL-14C]AE 0172747: Rat blood/plasma kinetics study Report No.: MEF-04/437, Edition Number: M-247226-01-1 GLP unpublished	Y	BCS

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**
Koester, J.	2005c	[Phenyl-UL-14C]AE 0172747: Depletion of residues and metabolites in plasma, urine, liver and kidney of the rat Report No.: MEF-05/124, Edition Number: M-250472-01-1 GLP unpublished	Y	BCS
Koester, J.	2005d	[Cyclohexyl-UL-14C]AE 0172747: Depletion of residues and metabolites in plasma, urine, liver and kidney of the rat Report No.: MEF-05/123, Edition Number: M-250373-01-1 GLP unpublished	Y	BCS
Fisher, P.J.	2005a	AE 0172747: Rat bile excretion study Bayer CropScience, Sophia-Antipolis, France Report No.: M-255219-01-1, Edition Number: M-255219-01-1 GLP unpublished	Y	BCS
Odin-Feurtet, M.	2003	AE 0172747 - Single oral low and high dose - Rat A.D.M.E.study Bayer CropScience, Sophia Antipolis, France Report No.: SA 02092, Edition Number: M-103278-02-1 GLP unpublished	Y	BCS
Odin-Feurtet M.	2002c	Rat blood / plasma kinetics study AE 0172747 Bayer CropScience S.A., France Report No.: C027447, Edition Number: M-213340-01-1 GLP unpublished	Y	BCS
Koester, J.	2005b	[Cyclohexyl-UL-14C]AE 0172747: Rat blood/plasma kinetics study Report No.: MEF-04/437, Edition Number: M-247226-01-1 GLP unpublished	Y	BCS

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**
Fisher, P. J.	2005b	(14C)-AE 0172747: Repeat oral low dose A.D.M.E. study in the rat Bayer CropScience SA, Sophia Antipolis, France Report No.: M-259537-01-1, Edition Number: M-259537-01-1 GLP unpublished	Y	BCS
Fisher, P.J.	2006	Summary of Toxicokinetic Studies on the Active Substance for AE 0172747		BCS
Eigenberg, D. A.	2003a	An acute oral LD50 study in the rat with AE 0172747 Bayer CropScience, Stilwell, KS, USA Report No.: 200400, Edition Number: M-087590-01-2 GLP unpublished	Y	BCS
Eigenberg, D. A.	2003b	An acute dermal LD50 study in the rat with AE 0172747 Bayer CropScience, Stilwell, KS, USA Report No.: 200403, Edition Number: M-087592-01-2 GLP unpublished	Y	BCS
Wesson, C. M.	2003	AE 0172747 00 1C95 0002 - Acute inhalation toxicity (nose only) study in the rat Safepharm Laboratories Limited, Derby, Great Britain Report No.: 1702/005, Edition Number: M-106556-01-1 GLP unpublished	Y	BCS
Rees, P. B.	2003a	AE 0172747 - Skin irritation to the rabbit Huntingdon Life Sciences Ltd., Cambridgeshire, Great Britain Report No.: AES 114/023583, Edition Number: M-106528-01-1 GLP unpublished	Y	BCS
Rees, P. B.	2003b	AE 0172747 - Eye irritation to the rabbit Huntingdon Life Sciences Ltd., Cambridgeshire, Great Britain Report No.: AES 115/023773, Edition Number: M-106573-01-1 GLP unpublished	Y	BCS

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**
Coleman, D. G.	2003	AE 0172747 - Guinea-pig skin sensitization study (Magnusson & Kligman method) Huntingdon Life Sciences Ltd., Cambridgeshire, Great Britain Report No.: AES 116/023862, Edition Number: M-106505-01-1 GLP unpublished	Y	BCS
Kennel, P.	2002	AE 0172747 - Preliminary 28-day toxicity study in the mouse by dietary administration Aventis CropScience, Sophia Antipolis, France Report No.: SA 01218, Edition Number: M-078057-01-1 Non GLP unpublished	Y	BCS
Steiblen, G.	2002	AE 0172747 - 90-day toxicity study in the rat by dietary administration Bayer CropScience, Sophia Antipolis, France Report No.: SA 01170, Edition Number: M-078288-01-1 GLP unpublished	Y	BCS
Kennel, P.	2005a	AE 0172747 - 90-day toxicity study in the rat by dietary administration Bayer CropScience, Sophia Antipolis, France Report No.: SA05002, Edition Number: M-259687-01-1 GLP unpublished	Y	BCS
Steiblen, G.	2003	AE 0172747 - 90-day toxicity study in the mouse by dietary administration Bayer CropScience, Sophia Antipolis, France Report No.: SA01431, Edition Number: M-116043-01-1 GLP unpublished	Y	BCS
Mallyon, B.; Semino, G.	2006	Position Paper: Toxicological Significance of Findings in the Rat	Y	BCS
Kennel, P.	2004	AE 0172747 - 90-day toxicity study in the dog by dietary administration Bayer CropScience, Sophia Antipolis, France Report No.: SA 02162, Edition Number: M-108485-01-3 GLP unpublished	Y	BCS

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**
Kennel, P.	2005b	AE 0172747 - Chronic toxicity study in the dog by dietary administration Bayer CropScience SA, Sophia Antipolis, France Report No.: SA03352, Edition Number: M-257312-01-1 GLP unpublished	Y	BCS
Schorsch, F.	2009	Position paper: Tembotrione dietary toxicity studies in dogs: toxicological relevance of the 'digestion chambers' observed in the peripheral nerves	Y	BCS
Kroetlinger, F.	2005	AE 0172747 - Project: AE 0172747 - Subacute toxicity study in the rat (4 weeks dermal administration) Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02011, Edition Number: M-250635-01-1 GLP unpublished	Y	BCS
Kroetlinger, F.; Schladt, L.	2005	AE 0172747 - subacute toxicity study in the rat (4 weeks dermal administration) Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02587, Edition Number: M-260021-01-1 GLP unpublished	Y	BCS
May, K..	2003	Technical AE 0172747 - Bacterial reverse mutation test Huntingdon Life Sciences Ltd., Huntingdon, Great Britain Report No.: AES117/023631, Edition Number: M-123593-01-1 Non GLP unpublished	Y	BCS
Mason, C.	2004	Technical AE 0172747 - In vitro mammalian chromosome aberration test in human lymphocytes Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, Great Britain Report No.: AES118/024021, Edition Number: M-123707-01-1 GLP unpublished	Y	BCS

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**
May, K.	2005	Technical AE 0172747 - Mammalian cell mutation assay Huntington Life Sciences Ltd., Cambridgeshire, Great Britain Report No.: AES119/024223, Edition Number: M-248361-01-1 Date: 25.01.2005 GLP unpublished	Y	BCS
Mehmood, Z.	2003	Technical AE 0172747 - Mouse micronucleus test Huntingdon Life Sciences Ltd., Cambridgeshire, Great Britain Report No.: AES120/023519, Edition Number: M-123668-01-1 GLP unpublished	Y	BCS
Wirnitzer, U.	2005a	AE 0172747 (Project: AE 0172747) - Unscheduled DNA synthesis test with rat liver cells in vivo Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02169, Edition Number: M-257224-01-1 GLP unpublished	Y	BCS
Kennel, P.	2005c	Chronic toxicity and carcinogenicity study of AE 0172747 in the Wistar rat by dietary administration Bayer Corporation, Sophia Antipolis, France Report No.: SA02055, Edition Number: M-252181-01-1 GLP unpublished	Y	BCS
Kennel, P.	2005d	Chronic toxicity and carcinogenicity study of AE 0172747 in the male wistar rat by dietary administration Bayer CropScience SA, Sophia Antipolis, France Report No.: SA02400, Edition Number: M-260106-01-1 GLP unpublished	Y	BCS
Semino, G	2006	Position paper: Tembotrione: combined carcinogenicity and chronic toxicity study in the rat Bayer CropScience SA, Sophia Antipolis, France	Y	BCS

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**
Langrand-Lerche, C.	2005	Carcinogenicity study of AE 0172747 in the C57BL/6 mouse by dietary administration Bayer CropScience SA, Sophia Antipolis, France Report No.: SA02256, Edition Number: M-257250-01-1 GLP unpublished	Y	BCS
Young, A.D.; Fickbohm, B. L.	2005	Technical grade AE0172747: A two-generation reproductive toxicity study in the wistar rat Bayer CropScience, Stilwell, KS, USA Report No.: 201266, Edition Number: M-259850-01-1 GLP unpublished	Y	BCS
Semino, G.	2006	Position paper: Tembotrione: two-generation reproductive toxicity study in the rat Bayer CropScience, Sophia Antipolis, France	Y	BCS
Wason, S.	2003a	AE 0172747 - Developmental toxicity study in the rat by gavage Bayer CropScience, Sophia Antipolis, France Report No.: SA 02226, Edition Number: M-111508-01-1 GLP unpublished	Y	BCS
Wason, S.	2003b	AE 0172747 - Developmental toxicity study in the rabbit by gavage Bayer CropScience, Sophia Antipolis, France Report No.: SA 02056, Edition Number: M-108558-01-1 GLP unpublished	Y	BCS
Mallyon B, Semino G	2006	Position Paper Tembotrione: Rabbit teratology	Y	BCS
Semino G	2009	Tembotrione (AE 0172747): Tyrosinaemia and developmental effects	Y	BCS
Blanck, O.	2004	AE 0172747 – Effect on blood tyrosine level in pregnant rabbit after oral administration by gavage Bayer CropScience, Sophia Antipolis, France Report No.: SA 03315 GLP unpublished	Y	BCS

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**
Sheets, L. P.; Gilmore, R. G.; Elcock, L. E.	2005	An acute oral neurotoxicity screening study with technical grade AE 0172747 in Wistar rats Bayer CropScience LP, Stilwell, KS, USA Report No.: 201248, Edition Number: M-248728-01-1 GLP unpublished	Y	BCS
Gilmore, R. G.; Elcock, L. E.	2005	A subchronic neurotoxicity screening study with technical grade AE0172747 in Wistar rats Bayer CropScience LP, Stilwell, KS, USA Report No.: 201174, Edition Number: M-248275-01-1 GLP unpublished	Y	BCS
Sheets, L. P.; Gilmore, R. G.; Hoss, H. E.	2005	A developmental neurotoxicity screening study with technical grade AE0172747 in wistar rats Bayer CropScience, Stilwell, KS, USA Report No.: 201310, Edition Number: M-259074-01-1 GLP unpublished	Y	BCS
Eiben, R.	2004	AE 0456148 - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01510, Edition Number: M-091596-01-1 GLP unpublished	Y	BCS
Herbold, B.	2004a	AE 0456148 (Project: AE 0172747) - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01610, Edition Number: M-128481-01-1 GLP unpublished	Y	BCS
Herbold, B.	2005a	AE 0456148 (Project: AE 0172747) - In vitro chromosome aberration test with Chinese hamster V79 cells Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01906, Edition Number: M-247216-01-1 GLP unpublished	Y	BCS

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**
Herbold, B.	2004b	AE 0456148 (Project: AE 0172747) - V79/HPRT-test in vitro for the detection of induced forward mutations Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01738, Edition Number: M-242613-01-1 GLP unpublished	Y	BCS
Eiben, R.	2005	AE 0456148 - Study on subchronic toxicity in rats (Administration in the diet for 3 months) - Amendment 1 to report No. AT02191 of July 18, 2005 Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02191, Edition Number: M-257240-02-1 GLP unpublished	Y	BCS
Schuengel, M.	2004	AE1417268 - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01702, Edition Number: M-182231-01-1 GLP unpublished	Y	BCS
Wirnitzer, U.	2004	AE 1417268 (project: AE 0172747) - Salmonella/microsome test (plate incorporation and preincubation method) Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01713, Edition Number: M-182480-01-1 GLP unpublished	Y	BCS
Kumaravel, T. S.	2005	AE 1417268: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes Covance Laboratories Ltd., Harrogate, North Yorkshire, England Report No.: 2014/88, Report includes Trial Nos.: 2014/88 Edition Number: M-259666-01-1 GLP unpublished	Y	BCS

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**
Lloyd, M.	2005	AE 1417268 - Mutation at the Thymidine Kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique Covance Laboratories Ltd., Harrogate, North Yorkshire, England Report No.: 2014/89-D6173, Edition Number: M-259745-01-1 GLP unpublished	Y	BCS
Steiblen, G.	2005	AE 1417268 (Metabolite of AE 0172747) 90-day toxicity study in the rat by dietary administration Bayer CropScience SA, Sophia Antipolis, France Report No.: SA05004, Edition Number: M-260117-01-1 GLP unpublished	Y	BCS
Blanck, O.	2006	Effects of diets enriched with tyrosine on selected organs in rats Bayer Crop Sciene, 06903 Sophia Antipolis Cedex, France Report No.: SA05207 GLP unpublished	Y	BCS
Blanck, O.	2006	Effects of diets enriched with tyrosine on selected organs in rats Bayer Crop Sciene, 06903 Sophia Antipolis Cedex, France Report No.: SA05330 GLP unpublished	Y	BCS
Semino, G.	2006	Position paper: Tembotrione systemic toxicity Bayer Crop Sciene, 06903 Sophia Antipolis Cedex, France	Y	BCS
Repetto, M.	2008	AE 017274 Effect on blood tyrosine level in pregnant rats after oral administration by gavage Bayer Crop Sciene, 06903 Sophia Antipolis Cedex, France Report No.: SA08089 GLP unpublished	Y	BCS

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**
Repetto, M.	2009	AE 017274 Effect on blood tyrosine level in pregnant rats after oral administration by gavage Bayer Crop Sciene, 06903 Sophia Antipolis Cedex, France Report No.: SA08226 GLP unpublished	Y	BCS
Schuengel, M.	2005	AE 1392936 - Acute toxicity in the rat after oral administration Bayer HealthCare AG, Wuppertal, Germany Report No.: AT01979, Edition Number: M-250289-01-1 GLP unpublished	Y	BCS
Wirnitzer, U.	2005b	AE 1392936 (Project: AE 0172747) - Salmonella/Microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02552, Edition Number: M-259733-01-1 GLP unpublished	Y	BCS
Thum, M.	2005	AE 1392936 00 1C94 0001 (Project: AE 0172747) - In vitro chromosome aberration test with chinese hamster V79 cells Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02214, Edition Number: M-257348-01-1 GLP unpublished	Y	BCS
Herbold, B.	2005b	AE 1392936 (Project: AE 0172747) - V79/HPRT-test in vitro for the detection of induced forward mutations Bayer HealthCare AG, Wuppertal, Germany Report No.: AT02433, Edition Number: M-258495-01-1 GLP unpublished	Y	BCS
Koester, J.	2005	AE 0172747-4,6-dihydroxy (M5): Absorption, distribution, excretion and metabolism in the rat Report No.: MEF-05/170, Edition Number: M-253710-01-1 GLP unpublished	Y	BCS

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**
Spiegel, K.; Koester, J.	2005	AE 0172747 - Mouse metabolism study after subacute dietary administration Report No.: MEF-05/403, Edition Number: M-258137-01-1 GLP unpublished	Y	BCS
Blanck, O.	2005	AE 0172747 - Effects on blood coagulation parameters with and without administration of vitamin K1 Bayer CropScience, Sophia Antipolis, France Report No.: SA04296, Edition Number: M-250414-01-1 GLP unpublished	Y	BCS
Totis, M.	2005	AE 0172747: In vitro inhibition of HPPDase using liverbeads from different species Bayer CropScience SA, Sophia Antipolis, France Report No.: SA 05068, Edition Number: M-255849-01-1 GLP unpublished	Y	BCS
Esdaile D.J.	1995	Exploratory 14-day (ocular toxicity) study in the rat and mouse Tyrosine Rhone-Poulenc Agrochimie, Sophia Antipolis, FRA; Report No.: R005384, Edition Number: M-170857-01-1 GLP unpublished	Y	BCS
Kennel, P.	2006	Effect of Tyrosinaemia on pregnancy and embryo-fetal development in the rat BCS, 06903 Sophia Antipolis Cedex, France Report No.: SA 05192 GLP unpublished	Y	BCS
Leake, C. R.; Tarara, G.; Semino, G.; Glass, H.; McMillan-Staff, S.; Kley, C.	2005	The non relevance of the environmental metabolites of AE 0172747 Report No.: M-260168-01-1, Edition Number: M-260168-01-1 Non GLP unpublished	Y	BCS

7.3 Environmental effects

7.3.1 Fate and Behaviour in the environment

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
Desmarteau, D. A.	2004a	[Phenyl-UL-14C]AE 0172747 : Phototransformation on soil Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 200764, Edition Number: M-077093-01-1 Date: 16.04.2004 GLP, unpublished	Yes	BCS
Desmarteau, D. A.	2004b	[Cyclohexyl-UL-14C] AE0172747 : Phototransformation on soil Bayer CropScience AG, Report No.: 200804, Edition Number: M-077474-01-1 Date: 18.06.2004 GLP, unpublished	Yes	BCS
Desmarteau, D. A., Mathew, A. E.;	2005	[Cyclohexyl-UL-14C]AE0172747: Anaerobic soil metabolism Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 201111, Edition Number: M-251896-01-1 Date: 30.03.2005 GLP, unpublished	Yes	BCS
Dominic, A. R., Arthur, E. L., Mislankar, S. G.	2005	The route and rate of degradation of [Phenyl-U-14C]AE 0172747 in two US soils and [Cyclohexyl-U-14C]AE 0172747 in one soil under laboratory aerobic conditions at 25 °C Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 200684, Edition Number: M-255967-01-1 Date: 05.08.2005 GLP, unpublished	Yes	BCS
Fliege, R.	2003b	Route and rate of degradation of [phenyl-U-14C]-AE 0172747 and [cyclohexyl-U-14C]-AE 0172747 in one European soil under aerobic laboratory conditions at 20 °C and 10 °C Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: MEF-068/03, Edition Number: M-109255-01-2	No	BCS

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
		Date: 12.06.2003 GLP, unpublished		
Fliege, R.	2003a	Rate of degradation of [phenyl-U-14C]-AE 0172747 in three European soils under aerobic laboratory conditions at 20 °C Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: MEF-126/03, Edition Number: M-116526-01-2 Date: 24.07.2003 GLP, unpublished	Yes	BCS
Fliege, R.	2003c	Abiotic hydrolysis of 14C-AE 0172747 in buffered aqueous solutions at pH 4, pH 7, and pH 9 Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG, Report No.: MEF-083/03, Edition Number: M-107923-01-2 Date: 05.06.2003 GLP, unpublished	Yes	BCS
Heinemann, O.	2005	Determination of residues of AE 0172747 in/on soil in Germany, Great Britain, Spain, Italy and France Bayer CropScience AG, Report No.: RA-2142/04, Report includes Trial Nos.: R 2004 0862/5 R 2004 0872/2 R 2004 0873/0 R 2004 0873/0 R 2004 0874/9 R 2004 1016/6 R 2004 1017/4 Edition Number: M-253708-01-1 Date: 28.06.2005 GLP, unpublished	Yes	BCS
Hellpointner, E.	2004	Phototransformation of AE 0172747 in sterile water buffered at pH 7 Bayer CropScience AG, Report No.: MEF-412/03, Edition Number: M-063564-01-1 Date: 14.04.2004 GLP, unpublished	Yes	BCS
Hellpointner, E.	2003a	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation in water: AE 0172747	No	BCS

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 CropScience AG, Report No.: MEF-154/03, Edition Number: M-105933-01-1 Date: 22.12.2003 GLP, unpublished		
Hellpointner, E.	2003b	Calculation of the chemical lifetime of AE 0172747 in the troposphere Bayer CropScience AG, Report No.: MEF-363/03, Edition Number: M-116064-01-1 Date: 20.10.2003 Non GLP, unpublished	No	BCS
Kley, C.	2005a	Kinetic evaluation of 3 aerobic laboratory soil degradation studies with AE 0172747 and its metabolites using MATLAB Bayer CropScience AG, Report No.: MEF-05/238, Edition Number: M-255217-01-1 Date: 22.07.2005 Non GLP, unpublished	Yes	BCS
Kley, C.	2005b	Kinetic evaluation of the dissipation of AE 0172747 and its metabolite under European field conditions using MATLAB Bayer CropScience AG, Report No.: MEF-05/239, Edition Number: M-254637-01-1 Date: 19.07.2005 Non GLP, unpublished	Yes	BCS
Kley, C.	2006a	Kinetic evaluation of the aerobic aquatic metabolism of tembotrione (AE 0172747) and its metabolite in water/sediment systems by inverse modelling using TOXSWA, PEST and MatLab Bayer CropScience AG, Report No.: MEF-06/439, Date: 02.10.2006 Non GLP, unpublished	Yes	BCS
Leake, C. R.; Tarara, G.; Semino, G.; Glass, H.; McMillan-Staff, S.; Kley, C.	2005	The non relevance of the environmental metabolites of AE 0172747 Bayer CropScience AG, Report No.: M-260168-01-1, Edition Number: M-260168-01-1 Date: 08.11.2005 Non GLP, unpublished	Yes	BCS
Mathew, A. E., Desmarteau, D.	2004	[Phenyl-UL-14C]AE0172747: Anaerobic soil metabolism	Yes	BCS

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
A.		Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 200836, Edition Number: M-129068-01-1 Date: 18.11.2004 GLP, unpublished		
Mathew, A. E.; Desmarteau, D. A.	2003	Adsorption/desorption of (phenyl-UL-14C)AE 0172747 in six soils and a sediment Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG, Report No.: 200375, Edition Number: M-086484-01-1 Date: 03.10.2003 GLP, unpublished	Yes	BCS
Mills, E. A. M.	2005a	[14C]-AE 0941989: Adsorption/desorption in four soils Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/04/050, Edition Number: M-251936-01-2 Date: 16.05.2005 GLP, unpublished	Yes	BCS
Mills, E. A. M.	2005b	[14C]-AE 1392936: Adsorption to and desorption from four soils Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/04/044, Edition Number: M-250775-01-2 Date: 05.04.2005 GLP, unpublished	Yes	BCS
Nicolaus B.	2004a	Aerobic aquatic metabolism in a fine sediment system (Cyclohexyl-U-14C)- and (phenyl-U-14C)-AE 0172747 Bayer CropScience GmbH, DEU ;Metabolism & Environmental Fate Bayer CropScience AG, Report No.: C044867, Report includes Trial Nos.: CB02-008 Edition Number: M-236541-01-1 Date: 06.05.2004 GLP, unpublished	Yes	BCS
Nicolaus, B.	2004b	[Cyclohexyl-U-14C]- and [phenyl-U-14C]-AE 0172747 Aerobic aquatic metabolism in sediment system 'Rhein' Bayer CropScience GmbH, Frankfurt, Germany Bayer CropScience AG,	No	BCS

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
		Report No.: MEF-391/03, Edition Number: M-079564-01-1 Date: 27.05.2004 GLP, unpublished		
Roohi, A.	2005	[14C]-AE 0941989: Rate of degradation in three soils at 20 degrees Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/04/043, Edition Number: M-252467-01-1 Date: 26.05.2005 GLP, unpublished	Yes	BCS
Simmonds, M.	2005a	[14C]-AE 1392936: Rate of degradation in three soils at 20 degrees Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/04/046, Edition Number: M-252102-01-1 Date: 18.05.2005 GLP, unpublished	Yes	BCS
Simmonds, M.	2005b	[14C]-AE 0456148: Adsorption to and desorption from five soils Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/03/073, Edition Number: M-251815-01-2 Date: 16.05.2005 GLP, unpublished	Yes	BCS
Simmonds, M.	2005c	[14C]-AE 0968400: Adsorption/desorption to and from five soils Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/03/063, Edition Number: M-251984-01-2 Date: 18.05.2005 GLP, unpublished	Yes	BCS
Simmonds, M.	2005d	[14C]-AE 1124336: Adsorption/desorption to and from five soils Battelle AgriFood Ltd., Ongar, United Kingdom Bayer CropScience AG, Report No.: CX/03/074, Edition Number: M-252066-01-2 Date: 18.05.2005 GLP, unpublished	Yes	BCS

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**
Weyers, A.	2005	AE 0172747 – Biodegradation Bayer Industry Services, Leverkusen, Germany Bayer CropScience AG, Report No.: 1352 N/05 C, Edition Number: M-254095-01-1 Date: 14.06.2005 GLP, unpublished	Yes	BIS

7.3.2 Aquatic Toxicity

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
Dorgerloh, M.	2003a	Acute toxicity of AE 0172747 technical substance to fish (Oncorhynchus mykiss) (product code AE 0172747 00 1C97 0001) Bayer CropScience AG Report No.: DOM 23034 Edition Number: M-106511-01-1 Date: 04.06.2003 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2003b	Acute toxicity of AE 0172747 technical substance to fish (Lepomis macrochirus) (Product code AE 0172747 00 1C97 0001) Bayer CropScience AG Report No.: DOM 22065 Edition Number: M-079090-01-1 Date: 12.02.2003 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2003c	Early-life stage toxicity of AE 0172747 technical substance to fish (Pimephales promelas) (product code AE 0172747 00 1C94 0003) Bayer CropScience AG Report No.: DOM 22076 Edition Number: M-091065-01-1 Date: 08.05.2003 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2003d	Influence of AE 0172747 (tech.) on development and reproductive output of the waterflea Daphnia magna in a static renewal laboratory test system Bayer CropScience AG Report No.: DOM 23002 Edition Number: M-111125-01-1 Date: 24.10.2003 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2005a	Pseudokirchneriella subcapitata growth inhibition limit test with AE 0456148 (code: AE 0456148 00 1B99 0002) Bayer CropScience AG, Report No.: DOM 24051 Edition Number: M-244859-01-1 Date: 01.02.2005 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2005b	Pseudokirchneriella subcapitata growth inhibition limit test with AE 1392936 00 1C93 0001 Bayer CropScience AG Report No.: EBAEP012	Yes	BCS

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
		Edition Number: M-253774-01-1 Date: 27.06.2005 GLP, unpublished		
Dorgerloh, M.	2005e	Chironimus riparius 28-day chronic toxicity test with AE 0172747 (tech.) in a water-sediment system using spiked water Bayer CropScience AG Report No.: DOM 24074 Edition Number: M-242895-01-1 Date: 10.01.2005 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2005c	Lemna gibba G3: Growth inhibition test with AE 0456148 (Code: AE 0456148 00 1B99 0002) Bayer CropScience AG Report No.: EBAEX080 Edition Number: M-243948-01-1 Date: 31.01.2005 GLP, unpublished	Yes	BCS
Dorgerloh, M.	2005d	Lemna gibba G3: Growth inhibition test with AE 1392936 under static conditions (code: AE 1392936 00 1C93 0001) Bayer CropScience AG Report No.: EBAEP011 Edition Number: M-252413-01-1 Date: 07.06.2005 GLP, unpublished	Yes	BCS
Gosch, H.; Ebeling, M.	2002	Algal growth inhibition - Pseudokirchneriella subcapitata - AE 0172747; substance, technical Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG Report No.: CE02/024 Edition Number: M-078348-01-1 Date: 29.11.2002 GLP, unpublished	Yes	BCS
Hoberg, J. R.	2003a	AE 0172747 - Acute toxicity test with freshwater blue-green alga (Anabaena flos-aquae) Springborn Smithers Laboratories, Wareham, MA, USA Bayer CropScience AG Report No.: 13798.6104 Edition Number: M-091552-01-1 Date: 03.01.2003 GLP, unpublished	Yes	BCS
Kern, M. E.; Banman, C. S.; Lam, C. V.	2004	Acute toxicity of AE 0456148 to the waterflea (Daphnia magna) under static conditions Bayer CropScience, Stilwell, KS, USA	Yes	BCS

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 CropScience AG Report No.: EBAEX055 Edition Number: M-128823-01-1 Date: 15.12.2004 GLP, unpublished		
Kern, M. E.; Lam, C. V.	2004	Chronic toxicity of AE 0456148, a metabolite of AE 0172747, to the Daphnia magna under static renewal conditions Bayer CropScience, Stilwell, KS, USA Bayer CropScience AG Report No.: EBAEX059 Edition Number: M-182651-01-1 Date: 15.12.2004 GLP, unpublished	Yes	BCS
Lima, W.	2003a	AE 0172747 - Acute toxicity to sheepshead minnow (Cyprinodon variegatus) under static-renewal conditions Springborn Smithers Laboratories, Wareham, MA, USA Bayer CropScience AG Report No.: 13798.6109 Edition Number: M-086607-01-1 Date: 22.01.2003 GLP, unpublished	Yes	BCS
Lima, W.	2003b	AE 0172747 - Acute toxicity to mysids (Americamysis bahia) under flow-through conditions Springborn Smithers Laboratories, Wareham, MA, USA Bayer CropScience AG Report No.: 13798.6102 Edition Number: M-091558-01-1 Date: 22.01.2003 GLP, unpublished	Yes	BCS
Nieden, D.	2005	Acute toxicity of AE 0456148 to fish (Oncorhynchus mykiss) under static conditions (product code: AE 0456148 00 1B99 0002) Bayer CropScience AG Report No.: EBAEX092 Edition Number: M-252118-01-1 Date: 31.05.2005 GLP, unpublished	Yes	BCS
Scheerbaum, D.	2005a	AE 0968400 technical - Alga, growth inhibition test with Pseudokirchneriella subcapitata, 72 h Noack Laboratorien, Sarstedt, Germany Bayer CropScience AG Report No.: SPO99281 Edition Number: M-250025-01-1	Yes	BCS

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
		Date: 08.04.2005 GLP, unpublished		
Scheerbaum, D.	2005b	AE 0968400 technical - Aquatic plant toxicity test, Lemna gibba, static, 7 d Noack Laboratorien, Sarstedt, Germany Bayer CropScience AG Report No.: TLA9928 Edition Number: M-250021-01-1 Date: 08.04.2005 GLP, unpublished	Yes	BCS
Sowig, P.	2002a	AE 0172747 - Acute toxicity to Daphnia magna (waterflea) under static testing conditions Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG Report No.: CE02/023 Edition Number: M-078343-01-1 Date: 11.11.2002 GLP, unpublished	Yes	BCS
Sowig, P.	2003	Duckweed (Lemna gibba G3) growth inhibition test with recovery phase - AE 0172747 - substance, technical Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG Report No.: CE02/026 Edition Number: M-108152-01-1 GLP, unpublished	Yes	BCS
Sowig, P.; Gosch, H.	2003	Algal growth inhibition - Navicula pelliculosa - AE 0172747, substance technical Bayer CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience AG Report No.: CE02/025 Edition Number: M-108273-01-1 Date: 17.03.2003 GLP, unpublished	Yes	BCS

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of the triketone herbicide2-(4-methylsulfonyl-2-nitrobenzoyl)-1, 3-cyclohexandione. Crit Rev Toxicol 43(3): 185-199.
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8 ANNEXES