CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: 3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one; Flurochloridone

EC Number: 262-661-3

CAS Number: 61213-25-0

Index Number: -

Contact details for dossier submitter: Spanish Competent Authority

Ministerio de Sanidad, Servicios Sociales e Igualdad.
Dirección General de Salud Pública, Calidad e Innovación.
Subdirección General de Sanidad Ambiental y Salud Laboral.
Área de Productos Fitosanitarios.
sanidadfitos@msssi.es

Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente
Dirección General de Calidad y Evaluación Ambiental y Medio Natural
Subdirección General de Calidad del Aire y Medio Ambiente Industrial
bzn-dgcea-ppp@mapama.es

Version number: 2 **Date: 29 September 2017**

CONTENTS

1 IDENTITY OF THE SUBSTANCE 5

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 5
1.2 COMPOSITION OF THE SUBSTANCE 6

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 8

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 8

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 10

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 10

5 IDENTIFIED USES 10

6 DATA SOURCES 10

7 PHYSICOCHEMICAL PROPERTIES 11

8 EVALUATION OF PHYSICAL HAZARDS 12

8.1 EXPLOSIVES 12
8.1.1 Short summary and overall relevance of the information provided on explosive properties 12
8.1.2 Comparison with the CLP criteria 13
8.1.3 Conclusion on classification and labelling for explosive properties 13
8.2 FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES) 13
8.3 OXIDISING GASES 13
8.4 GASES UNDER PRESSURE 13
8.5 FLAMMABLE LIQUIDS 13
8.6 FLAMMABLE SOLIDS 13
8.6.1 Short summary and overall relevance of the provided information on flammable solids 13
8.6.2 Comparison with the CLP criteria 13
8.6.3 Conclusion on classification and labelling for flammable solids 13
8.7 SELF-REACTIVE SUBSTANCES 14
8.8 PYROPHORIC LIQUIDS 14
8.9 PYROPHORIC SOLIDS 14
8.10 SELF-HEATING SUBSTANCES 14
8.11 SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES 14
8.12 OXIDISING LIQUIDS 14
8.13 OXIDISING SOLIDS 14
8.13.1 Short summary and overall relevance of the provided information on oxidising solids 14
8.13.2 Comparison with the CLP criteria 14
8.13.3 Conclusion on classification and labelling for oxidising solids 14
8.14 ORGANIC PEROXIDES 15
8.15 CORROSIVE TO METALS 15
8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals 15
8.15.2 Comparison with the CLP criteria 15
8.15.3 Conclusion on classification and labelling for corrosive to metals 15

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 16

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 23

10 EVALUATION OF HEALTH HAZARDS 25

10.1 ACUTE TOXICITY - ORAL ROUTE 25
10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity 26
10.1.2 Comparison with the CLP criteria 26
10.1.3 Conclusion on classification and labelling for acute oral toxicity 26
10.2 ACUTE TOXICITY - DERMAL ROUTE 27
10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity 27
10.2.2 Comparison with the CLP criteria 27
10.2.3 Conclusion on classification and labelling for acute dermal toxicity 27
Data available indicates that flurochloridone does not require classification for acute dermal toxicity. 27
10.3 ACUTE TOXICITY - INHALATION ROUTE 27
10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity 28
10.3.2 Comparison with the CLP criteria 28
10.3.3 Conclusion on classification and labelling for acute inhalation toxicity 28
Data available indicates that flurochloridone does not require classification for acute inhalation toxicity. 28
10.4 SKIN CORROSION/IRRITATION 28
10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation 28
10.4.2 Comparison with the CLP criteria 29
10.4.3 Conclusion on classification and labelling for skin corrosion/irritation 29
Data available indicates that flurochloridone does not require classification as skin irritant. 29
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 29
10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation 29
10.5.2 Comparison with the CLP criteria 29
10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation 30
Data available indicates that flurochloridone does not require classification as eye irritant. 30
10.6 RESPIRATORY SENSITISATION 30
10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation 30
10.6.2 Comparison with the CLP criteria 30
10.6.3 Conclusion on classification and labelling for respiratory sensitisation 30
10.7 SKIN SENSITISATION 30
10.7.1 Short summary and overall relevance of the provided information on skin sensitisation 32
10.7.2 Comparison with the CLP criteria 32
10.7.3 Conclusion on classification and labelling for skin sensitisation 32
10.8 GERM CELL MUTAGENICITY 33
10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity 37
10.8.1.1 *In vitro* data 37
10.8.1.2 *In vivo* data 44
10.8.1.3 Human information 46
10.8.1.4 Other relevant information 46
10.8.1.5 Summary and discussion of mutagenicity 46
10.8.2 Comparison with the CLP criteria 47
10.8.3 Conclusion on classification and labelling for germ cell mutagenicity 48
10.9 CARCINOGENICITY 49
10.9.1 Short summary and overall relevance of the provided information on carcinogenicity 52
10.9.2 Comparison with the CLP criteria 55
10.9.3 Conclusion on classification and labelling for carcinogenicity 56
Data available indicates that flurochloridone does not require classification for its carcinogenic potential. 56
10.10 REPRODUCTIVE TOXICITY 57
10.10.1 Adverse effects on sexual function and fertility 57
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility 64
10.10.3 Comparison with the CLP criteria 68
10.10.4 Adverse effects on development 71
10.10.5 Short summary and overall relevance of the provided information on adverse effects on development 74
10.10.6 Comparison with the CLP criteria 76
10.10.7 Adverse effects on or via lactation 78
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE 79

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure ... *79*
10.11.2 Comparison with the CLP criteria ... *80*
10.11.3 Conclusion on classification and labelling for STOT SE ... *80*
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE ... 80
10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure ... *85*
10.12.2 Comparison with the CLP criteria ... *87*
10.12.3 Conclusion on classification and labelling for STOT RE ... *89*
10.13 ASPIRATION HAZARD ... 90

11 EVALUATION OF ENVIRONMENTAL HAZARDS ... 91

11.1 RAPID DEGRADABILITY OF ORGANIC SUBSTANCES ... 91
11.1.1 Ready biodegradability ... *91*
11.1.2 BOD_5/COD ... *92*
11.1.3 Hydrolysis ... *92*
11.1.4 Other convincing scientific evidence ... *93*
11.1.4.1 Field investigations and monitoring data (if relevant for C&L) ... 93
11.1.4.2 Inherent and enhanced ready biodegradability tests ... 93
11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies) ... 93
11.1.4.4 Photochemical degradation ... 100
11.2 ENVIRONMENTAL TRANSFORMATION OF METALS OR INORGANIC METALS COMPOUNDS ... 102
11.2.1 Summary of data/information on environmental transformation ... *102*
11.3 ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION ... 102
11.4 BIOACCUMULATION ... 103
11.4.1 Estimated bioaccumulation ... *103*
11.4.2 Measured partition coefficient and bioaccumulation test data ... *103*
11.5 ACUTE AQUATIC HAZARD ... 104
11.5.1 Acute (short-term) toxicity to fish ... *105*
11.5.2 Acute (short-term) toxicity to aquatic invertebrates ... *106*
11.5.3 Acute (short-term) toxicity to algae or other aquatic plants ... *107*
11.5.4 Acute (short-term) toxicity to other aquatic organisms ... *114*
11.6 LONG-TERM AQUATIC HAZARD ... 114
11.6.1 Chronic toxicity to fish ... *115*
11.6.2 Chronic toxicity to aquatic invertebrates ... *116*
11.6.3 Chronic toxicity to algae or other aquatic plants ... *118*
11.6.4 Chronic toxicity to other aquatic organisms ... *118*
11.7 COMPARISON WITH THE CLP CRITERIA ... 118
11.7.1 Acute aquatic hazard ... *118*
11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation) ... *119*
11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS ... 121

12 EVALUATION OF ADDITIONAL HAZARDS ... 121

12.1 HAZARDOUS TO THE OZONE LAYER ... 121
12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard ... *121*
12.1.2 Comparison with the CLP criteria ... *121*
12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer ... *121*

13 ADDITIONAL LABELLING ... 121

14 REFERENCES ... 122

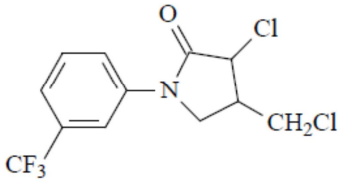
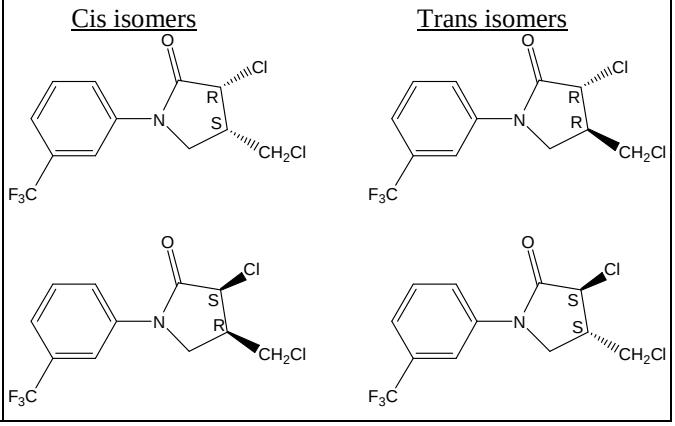
14.1 PHYSICO-CHEMICAL PROPERTIES ... 122
14.2 TOXICOLOGY AND METABOLISM ... 123
14.3 ENVIRONMENT ... 126

15 ANNEXES ... 129

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name in the IUPAC nomenclature or other international chemical name	3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one
ISO common name	*
EC number	262-661-3
EC name	3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl)phenyl]pyrrolidin-2-one
CAS number	61213-25-0
Molecular formula	$C_{12}H_{10}Cl_2F_3NO$
Structural formula	
SMILES notation	FC(F)(F)c1cccc(c1)N2CC(CCl)C(Cl)C2=O
Molecular weight or molecular weight range	312.12 g/mol
Information on optical activity and typical ratio of (stereo) isomers	Considering a minimum purity of the active substance of 940 g/kg the isomer ratio according EFSA Conclusion on the peer review (EFSA Journal 2010; 8(12):1869): (3RS,4RS)-3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl) phenyl]pyrrolidin-2-one (isomers *trans*): 720-740 g/kg (3RS,4SR)-3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl) phenyl]pyrrolidin-2-one (isomers *cis*): 220-240 g/kg 

*According to EFSA Journal 2010; 8(12):1869 "*Flurochloridone is the ISO common name for (3RS,4RS;3RS,4SR)-3-chloro-4-chloromethyl-1-(α,α,α-trifluoro-m-tolyl)-2-pyrrolidone (IUPAC) where the ratio of (1RS,2RS)(trans)- and (1RS,2SR)(cis)-isomers is 3:1. However, the compounds evaluated in the DAR and additional report, were not exactly in a 3:1 ratio.* Consequently, the ISO name flurochloridone corresponds to another isomer ratio than the one included in this report for the active substance. However the name flurochloridone is still used at European level (including EFSA).

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Flurochloridone is manufactured as a mixture of 4 isomers: a pair of enantiomeric *cis* isomers and a pair of enantiomeric *trans* isomers with an approximate ratio 1:3/cis:trans.

<table>
<tr><th>Constituent (Name and numerical identifier)</th><th>Concentration range (% w/w minimum and maximum in multi-constituent substances)</th><th>Current CLH in Annex V I Table 3.1 (CLP)</th><th>Current self- classification and labelling (CLP)</th></tr>
<tr><td>(3RS,4RS;3RS,4SR)-3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl) phenyl]pyrrolidin-2-one

CAS 61213-25-0</td><td>(≥ 94% w/w)
Isomer ratio according EFSA Conclusion on the peer review (EFSA Journal 2010; 8(12):1869):
(3RS,4RS)- 3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl) phenyl]pyrrolidin-2-one (isomers trans): 72-74% w/w
(3RS,4SR)-3-chloro-4-(chloromethyl)-1-[3-(trifluoromethyl) phenyl]pyrrolidin-2-one (isomers cis): 22-24% w/w</td><td>Not available.</td><td>According to C&L inventory (27 February 2017):
<table>
<tr><th colspan="2">Classification</th><th colspan="2">Labelling</th><th rowspan="2">Number of Notifiers</th></tr>
<tr><th>Hazard Class and Category Code(s)</th><th>Hazard Statement Code(s)</th><th>Hazard Statement Code(s)</th><th>Pictograms, Signal Word Code(s)</th></tr>
<tr><td>Repr. 2</td><td>H361</td><td>H361</td><td>GHS09</td><td rowspan="4">45</td></tr>
<tr><td>Aquatic Acute 1</td><td>H400</td><td></td><td rowspan="3">GHS08
Wng</td></tr>
<tr><td></td><td></td><td>H410</td></tr>
<tr><td>Aquatic Chronic 1</td><td>H411</td><td></td></tr>
<tr><td rowspan="2">Acute Tox. 4</td><td rowspan="2">H302</td><td>H302</td><td>GHS09</td><td rowspan="7">19</td></tr>
<tr><td>(Acute Tox. 4)</td><td>GHS08
GHS07
Wng</td></tr>
<tr><td rowspan="2">Skin Sens. 1</td><td rowspan="2">H317</td><td>H317</td></tr>
<tr><td>(Skin sens.1)</td></tr>
<tr><td>Repr. 2</td><td>H361d</td><td>H361d</td></tr>
<tr><td>Aquatic Acute 1</td><td>H400</td><td></td></tr>
<tr><td>Aquatic Chronic 1</td><td>H410</td><td>H410</td></tr>
<tr><td>Not classified</td><td></td><td></td><td></td><td>4</td></tr>
<tr><td></td><td></td><td>H361</td><td>GHS09</td><td rowspan="3">3</td></tr>
<tr><td></td><td></td><td>H400</td><td>GHS08</td></tr>
<tr><td></td><td></td><td>H410</td><td>Wng</td></tr>
<tr><td rowspan="4"></td><td rowspan="4"></td><td>H361</td><td rowspan="4"></td><td rowspan="4">1</td></tr>
<tr><td>(Repr.2)</td></tr>
<tr><td></td><td>H400</td></tr>
<tr><td></td><td>H410</td></tr>
</table></td></tr>
</table>

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

<table>
<tr><th>Impurity (Name and numerical identifier)</th><th>Concentration range (% w/w minimum and maximum)</th><th>Current CLH in Annex VI Table 3.1 (CLP)</th><th>The impurity contributes to the classification and labelling</th></tr>
<tr><td>Toluene
CAS 108-88-3</td><td>(0-0.8%)</td><td>
<table>
<tr><th colspan="2">Classification</th><th colspan="2">Labelling</th></tr>
<tr><th>Hazard Class and Category Code(s)</th><th>Hazard Statement Code(s)</th><th>Hazard Statement Code(s)</th><th>Pictograms, Signal Word Code(s)</th></tr>
<tr><td>Flam. Liq. 2</td><td>H225</td><td>H225</td><td rowspan="6">GHS02
GHS08
GHS07
Dgr</td></tr>
<tr><td>Skin Irrit. 2</td><td>H315</td><td>H315</td></tr>
<tr><td>Asp. Tox. 1</td><td>H304</td><td>H304</td></tr>
<tr><td>STOT SE 3</td><td>H336</td><td>H336</td></tr>
<tr><td>STOT RE 2</td><td>H373</td><td>H373</td></tr>
<tr><td>Repr. 2</td><td>H361d</td><td>H361d</td></tr>
</table>
</td><td></td></tr>
</table>

Further information on impurities other than toluene is included as confidential information in IUCLID 6 file. It has to be underlined that only one of these impurities exceeds 1% in the technical specification but it is not relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	-	(3RS,4RS;3RS,4SR)-3-chloro-4-chloromethyl-1-(α,α,α-trifluoro-m-tolyl)-2-pyrrolidone	262-661-3	61213-25-0	Acute Tox.4 Skin Sens. 1 Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H360Df H400 H410	GHS07 GHS08 GHS09	H302 H317 H360Df H410		Acute M factor = 100; Chronic M factor = 100	
Resulting Annex VI entry if agreed by RAC and COM	-		262-661-3	61213-25-0	Acute Tox.4 Skin Sens. 1 Repr. 1B Aquatic Acute 1 Aquatic Chronic 1	H302 H317 H360Df H400 H410	GHS07 GHS08 GHS09	H302 H317 H360Df H410		Acute M factor = 100; Chronic M factor = 100	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	-
Oxidising gases	Hazard class not applicable	-
Gases under pressure	Hazard class not applicable	-
Flammable liquids	Hazard class not applicable	-
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	-
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	-
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Hazard class not applicable	-
Corrosive to metals	Data lacking	-
Acute toxicity via oral route	**Acute Tox. 4; H302**	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Data lacking	-
Skin sensitisation	**Skin Sens. 1; H317**	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	**Repr. 1B: H360Df**	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	**Aquatic Acute 1; H400; M=100** **Aquatic Chronic 1; H410; M=100**	Yes
Hazardous to the ozone layer	Data lacking	-

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Flurochloridone is an herbicide used as an active substance in plant protection products (PPP). Flurochloridone was included in Annex I to Directive 91/414/EEC on 1 June 2011 by Commission Directive 2011/34/EU3, and has been deemed to be approved under Regulation (EC) No 1107/20094, in accordance with Commission Implementing Regulation (EU) No 540/20115, as amended by Commission Implementing Regulation (EU) No 541/20116. EFSA previously finalised a Conclusion on this active substance on 14 October 2010 in the EFSA Conclusion on Pesticide Peer Review, EFSA Journal 2010;8(12):1869 (EFSA, 2010).

EFSA proposed in the conclusion (EFSA, 2010) the following classification with regard to mammalian toxicological data, T (Toxic), R61; “May cause harm to the unborn child”, R62; “Possible risk of impaired fertility” and R43 “May cause sensitization by skin contact” and with regard to ecotoxicological data, N; R50/R53 “Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment”.

Flurochloridone is not currently listed in Annex VI of Regulation of Regulation (EC) 1272/2008. It was not previously discussed by the TC C&L (Directive 67/548/EEC).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

In accordance with article 36(2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, being flurochloridone an active substance in the meaning of PPP Regulation, it should now be considered for harmonised classification and labelling for all physico-chemical, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based on the information provided for the assessment of flurochloridone under 91/414/EEC Directive (currently repealed by Regulation (EC) 1107/2009).

5 IDENTIFIED USES

Flurochloridone is an active substance used as herbicide in plant protection products (PPP)

6 DATA SOURCES

Information on data sources used in this CLH report are included in section 14 (References) and 15 (Annexes).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Comment (e.g. measured or estimated)	Reference
Physical state at 20°C and 101,3 kPa	Light peach solid with sweetish pungent odour at room temperature. Purity: 987 g/kg (cis/trans 1:3)		**Goodmann, M., 1994b** **IIA, 2.4.1/1** **IIA, 2.4.2/1**
	Dark brown-red solid with mild disinfectant or mothball odour at room temperature. Purity: 920 g/kg (cis/trans 1:3)		**Goodmann, M., 1994b** **IIA, 2.4.1/2** **IIA, 2.4.2/2**
Melting/freezing point	Melting range 39.2°C to 78.4ºC Purity: 99.0% (83.3% trans / 16.6% cis)	OECD 102 GLP:Yes	**O'Connor, B.J., 2012**
Boiling point	The decomposition of flurochloridone was observed before boiling occurred Purity: 99.5% (isomer ratio not specified)	EEC A.2 GLP:Yes	**Tognucci, A., 2003a** **IIA, 2.1.2/1**
Relative density	1.19 g/mL at 20°C Purity: 987 g/kg (cis/trans 1:3)	EEC A.3 GLP: Yes Capillary-stoppered density bottle	**Goodmann, M., 1994a** **IIA, 2.2/1**
Vapour pressure	Flurochloridone $2.7 * 10^{-4}$ Pa at 25°C (extrapolated from the vapour pressure curve) Purity: 99.5% (ratio of isomers not specified)	EEC A.4 GLP : Yes Gas saturation method HPLC/UV for quantification	**Weissenfeld, M., 2006a** **IIA, 2.3.1/2**
	Flurochloridone (Trans Isomer) $6.0 * 10^{-5}$ Pa at 25°C $2.5 * 10^{-5}$ Pa at 20°C (extrapolated from the vapour pressure curve) Purity: 99.5%	EEC A.4 GLP : Yes Gas saturation method HPLC/UV for quantification	**Weissenfeld, M., 2008a** **IIA, 2.3.1/3**
Surface tension	54.6 mN/m at 20°C (90% of saturation concentration) Flurochloridone is a surface-active substance. Purity: 94.9% (isomer ratio not specified)	EEC A.5 GLP: Yes Ring tensiometer	**Tognucci, A., 2003b** **IIA, 2.14/1**
Water solubility	31.1 mg/L at 20°C at pH 4.0 21.9 mg/L at 20°C at pH 7.0 28.6 mg/L at 20°C at pH 9.0 Flurochloridone technical Purity: 93.8%	EEC A.6 GLP: Yes Flask shaking method with HPLC/UV	**Weissenfeld, M., 2009** **IIA, 2.6/2**
	11.0 mg/L at 20°C (pH 7) Flurochloridone (trans isomer) Purity: 99.5%	EEC A.6 GLP: Yes Flask shaking method with HPLC/UV	**Weissenfeld, M., 2008c** **IIA, 2.6/3**
	31.8 mg/L at 20°C (pH 7) Flurochloridone (cis isomer) Purity: 94.1%	EEC A.6 GLP: Yes Flask shaking method with HPLC/UV	**Weissenfeld, M., 2008d** **IIA, 2.6/4**
Partition coefficient n-octanol/water	Pow = 2280 at 25°C log Pow = 3.36 Flurochloridone Purity: 987 g/kg (cis/trans 1:3) No effect of pH in logPow	EEC A8 GLP: Yes Shake flask method with GC/NPD	**Goodmann, M., 1994a** **IIA, 2.8/1**
Flash point	Determination of flash point was not required because a test on auto-flammability was performed and flurochloridone has a melting point above 40 ºC		

Property	Value	Comment (e.g. measured or estimated)	Reference
Flammability	Not flammable Purity: 920 g/kg (cis/trans 1:3)	EEC A10 GLP: Yes	**Goodmann, M., 1994b** **IIA, 2.11.1/1**
Explosive properties	Not explosive. Assessment of chemical structure. No bond groupings known to confer explosivity. The assessment was carried out by comparing the bond groupings with those known to confer explosivility (L. Bretherick, "Handbook of Reactive Chemical Hazards", 3rd edition, Butterworths, 1985)	Expert statement	**Goodmann, M., 1994b** **IIA, 2.13/1**
Self-ignition temperature	Not auto-flammable (below the melting point) Purity: 920 g/kg (cis/trans 1:3)	EEC A.16 GLP: Yes	**Goodmann, M., 1994b** **IIA, 2.11.2/1**
Oxidising properties	Flurochloridone is found to be non-oxidising and therefore is not to be tested experimentally for the classification under division 5.1 as oxidising substances.	UN Recommendations on the Transport of Dangerous Goods (Orange Book, 3 edition, 1999) Expert Statement: On the Oxidizing Properties of Flurochloridone	**Weissenfeld, M., 2006** **IIA, 2.15/2**
Solubility in organic solvents	Solubility at ambient temperature (20ºC) of flurochloridone (purity 93.8% w/w; isomers ratio not specified) Acetone >540 g/L Acetonitrile >638 g/L Ethyl acetate >592 g/L Hexane 9.1 g/L Methanol 326 g/L Methylenchloride >508 g/L Toluene >616 g/L	CIPAC MT 181 and OECD 105 GLP: Yes	**Weissenfeld, M., 2007** **IIA, 2.7/2**
Dissociation constant	In the environmentally relevant pH range of pH 4 to 9 flurochloridone is present in its neutral form and does not dissociate or protonate. The compound has one nitrogen atom in its structure which can be protonated. The estimated pKa-value was -3.6.	Expert statement based on computer model ACD/I-Lab, version 8.03 GLP: No	**Weissenfeld, 2008e** **IIA, 2.9.4/2**
Viscosity	Substance is a solid		

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Expert statement	Not explosive. Assessment of chemical structure. No bond groupings known to confer explosivity. The assessment was carried out by comparing the bond groupings with those known to confer explosivility (L. Bretherick, "Handbook of Reactive Chemical Hazards", 3rd edition, Butterworths, 1985)		**Goodmann, M., 1994b** **IIA, 2.13/1**

8.1.1 Short summary and overall relevance of the information provided on explosive properties

Flurochloridone has no explosive properties.

8.1.2 Comparison with the CLP criteria

Flurochloridone does not meet CLP criteria to classify according to its explosive properties.

8.1.3 Conclusion on classification and labelling for explosive properties

Flurochloridone does not require classification for explosive properties.

8.2 Flammable gases (including chemically unstable gases)

Flurochloridone does not correspond to a gas having a flammable range with air at 20 ºC and a standard pressure of 101.3 kPa. Besides, it has to be noted that it decomposes before boiling according to data provided by Tognucci (2003a) on boiling point determination (see Table 7).

8.3 Oxidising gases

Flurochloridone does not correspond to a gas which may, generally by providing oxygen, cause or contribute to the combustion of other material more than air does. Besides, it has to be noted that it decomposes before boiling according to data provided by Tognucci (2003a) on boiling point determination (see Table 7).

8.4 Gases under pressure

Flurochloridone is not a gas under pressure since according to CLP criteria gases under pressure are gases which are contained in a receptacle at a pressure of 200 kPa (gauge) or more, or which are liquefied or liquefied and refrigerated. They comprise compressed gases, liquefied gases, dissolved gases and refrigerated liquefied gases. Besides, it has to be noted that it decomposes before boiling according to data provided by Tognucci (2003a) on boiling point determination (see Table 7).

8.5 Flammable liquids

Flurochloridone is a solid material.

8.6 Flammable solids

Table 9: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A10 GLP: Yes	Not flammable Purity: 920 g/kg (cis/trans 1:3)		**Goodmann, M., 1994b** **IIA, 2.11.1/1**

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Flurochloridone is not a flammable solid according to the results of the study (Goodmann, 1994b).

8.6.2 Comparison with the CLP criteria

Flurochloridone does not meet CLP criteria to be classified as flammable solid.

8.6.3 Conclusion on classification and labelling for flammable solids

Flurochloridone is not a flammable solid.

8.7 Self-reactive substances

Flurochloridone is not a self-reactive substance according to CLP criteria since it does not correspond to a thermally unstable solid liable to undergo a strongly exothermic decomposition even without participation of oxygen (air).

8.8 Pyrophoric liquids

Flurochloridone is a solid.

8.9 Pyrophoric solids

Flurochloridone is not expected to be a pyrophoric solid according to CLP criteria since it does not correspond to a material which, even in small quantities, is liable to ignite within five minutes after coming into contact with air.

8.10 Self-heating substances

Flurochloridone is not expected to be a self-heating substance since it does not correspond to a solid which, by reaction with air and without energy supply, is liable to self-heat.

8.11 Substances which in contact with water emit flammable gases

Flurochloridone does not emit flammable gases in contact with water.

8.12 Oxidising liquids

Flurochloridone is a solid material.

8.13 Oxidising solids

Table 10: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
UN Recommendations on the Transport of Dangerous Goods (Orange Book, 3 edition, 1999) Expert Statement: On the Oxidizing Properties of Flurochloridone	Flurochloridone is found to be non-oxidising and therefore is not to be tested experimentally for the classification under division 5.1 as oxidising substances.		**Weissenfeld, M., 2006** **IIA, 2.15/2**

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

Flurochloridone is not an oxidising solid according to the results of the report statement (Weissenfled, 2006).

8.13.2 Comparison with the CLP criteria

Flurochloridone does not meet CLP criteria to be classified as oxidising solid.

8.13.3 Conclusion on classification and labelling for oxidising solids

Flurochloridone is not a oxidising solid.

8.14 Organic peroxides

Flurochloridone is not an organic peroxide. It does not contain the bivalent -O-O- structure and it is not thermally unstable.

8.15 Corrosive to metals

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data available.

8.15.2 Comparison with the CLP criteria

No data available.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Data lacking.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 11: Summary table of toxicokinetic studies

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>

Blood level, distribution and metabolism after single oral administration to male and female rats

Laboratory: RCC Ltd. Laboratories

Guideline: OECD 417

GLP: Yes

Deviations: none

Study acceptable

Purity: unlabelled 99.5% and labelled with radiochemical purity of 98.8-100% containing mixture of cis 9.8-12.5% and 87.5-90.2% trans isomer

Rat strain: Wistar rats

Oral (gavage)
Vehicle: polyethylene glycol

Group 1-4: 9 animals/sex at 4 and 100 mg/kg bw. Time of observation: 0-96 h

Group 5-8: 3 animals/sex at 4 and 100 mg/kg bw. Time of observation: 0-48 h

Group 9-10: 3 animals/sex/dose level of 100 mg/kg bw at 12 h and 24 h respectively (time of termination)

</td>
<td>

Blood kinetics (96h)

Group	1		2		3		4	
Sex	**male**		**female**		**male**		**female**	
Dose [mg/kg bw]	**102.9**		**100.6**		**4.08**		**4.05**	
	bl	**pl**	**bl**	**pl**	**bl**	**pl**	**bl**	**pl**
C_{max} [ppm]	9.4	9.6	10.9	10.9	0.40	0.50	0.50	0.60
T_{Cmax} [h]	**12**	**12**	**12**	**12**	**6**	**2**	**8**	**2**
$AUC_{0\text{-}96\,h}$ [µg·h/g]	315	237	385	308	11.9	9.1	15.5	12.4
$AUC_{0\text{-}\infty}$ [µg·h/g]	458	253	514	337	(14.9)	9.6	19.4	13.3
$T_{1/2}$ (initial) [h]	16.9	11.6	19.5	14.5	12.2	9.3	11.3	10.1
$T_{1/2}$ (terminal) [h]	75	31	56	32	(44)	25	45	28

bl = blood, pl = plasma
( ) values less reliable based on a correlation coefficient below 0.9

The higher AUC values and longer half times for blood in comparison to plasma indicate some binding of radioactivity to blood cells. It has to be noted that often two maximum peaks were observed in blood and plasma.

Excretion after 48 h

Group	**5**	**6**	**7**	**8**
Sex	**male**	**female**	**male**	**female**
Dose [mg/kg bw]	100.4	99.9	4.07	4.05
Urine	41.2	45.3	48.1	49.6
Faeces	47.8	41.7	47.1	40.0
Cage wash	3.2	3.5	1.5	2.4
Total excretion (48 h)	92.3	90.4	96.7	91.9

Tissues residues in ppm after oral application of 100 mg/kg bw (groups 9-10)

Sex	**male**		**female**	
Dose (mg/kg bw)	**100.6**		**100.3**	
Sacrifice [h]	**12**	**24**	**12**	**24**
Blood	7.60	3.78	7.98	5.13
Brain	5.86	1.66	7.99	4.20
Epididymes	35.7	12.8	-	-
Fat	103	41.0	143	89.1
Heart	10.1	3.53	13.4	7.20
Kidney	26.0	9.7	31.9	17.6
Liver	36.6	14.8	39.8	22.0
Lung	10.5	4.65	14.0	7.93
Muscle	6.07	2.84	7.28	3.82
Ovaries	-	-	34.8	19.1
Pancreas	15.1	4.40	19.9	9.36
Plasma	8.52	3.18	9.39	4.92
Testicles	6.89	3.13	-	-
Thyroid glands	9.70	3.17	12.7	5.87
Uterus	-	-	10.7	6.76

At 12 hours after application (TC_{max}) of the dose of 100 mg/kg bw tissue residues were between 5.9-7.3 ppm (brain, muscle) and 103-143 ppm (fat). Relatively high levels were seen in liver and kidney (tissue/plasma ratio 4-5) and in the epididymis

</td>
<td>Kunz, Ch. (2006a)</td>
</tr>
</table>

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>(tissue/plasma ratio 4.2) and ovaries (tissue/plasma ratio 3.7). The tissue/plasma ratio in other sexual organs was 0.8 in testes and 1.1 in uterus.
At 24 hours after application of the dose of 100 mg/kg bw tissue residues were about 2.7 times (2.0-3.5) and 1.8 (1.6-2.1) times lower than the 12 hour values for males and females, respectively. The tissue/plasma ratio in gonads was 4.0 in epididymis, 3.9 in ovaries, 1.0 in testes and 1.4 in uterus.
In parallel to the marginally higher blood levels and slower depletion from blood, tissue levels were slightly higher for females than for males (mean 1.2 and 1.8 times at 12 and 24 hours, respectively).
<u>Metabolism</u>
Large numbers of no or barely discernible fractions were seen in the analysis of metabolite pattern after oral administration of 100 and 4 mg/kg bw in urine and faeces and 100 mg/kg bw in plasma.
<u>Urine</u>: > 30 fractions most of them not clearly discernible. Major metabolites were acetylcysteine conjugates of the parent identified as U1 in both sexes (10-16% of applied dose) and U2 (1.4% of applied dose) only in females. U1 and U2 may be either position isomers (acetylcysteine residue at either of the two possible pyrrolidone ring positions) or geometric isomers (cis/trans, acetylcysteine residue at the same pyrrolidone ring position). Traces of another acetylcysteine conjugate of flurochloridone were detected (U3), with the acetylcysteine residue linked to the trifluoromethyl-phenyl ring. Rat metabolite U1 was shown to be identical to rabbit metabolite U1. Unchanged flurochloridone was not detected.
<u>Faeces</u>: > 30 fractions most of them not clearly discernible. Unchanged flurochloridone was detected at the high dose (0.4-0.7% of applied dose) in both sexes. One metabolite (F5) was identified as U1. After low dose administration no unchanged flurochloridone was found.
<u>Plasm</u>: ≥ 19 mostly undiscernible fractions. Unchanged flurochloridone was detected at 12 h (0.06 ppm) in females.</td><td></td></tr>
<tr><td>Blood level, distribution and metabolism after single oral administration to male and female rabbits
Laboratory: RCC Ltd. Laboratories
Guideline: OECD 417
GLP: Yes
Deviations: none
Study acceptable
Purity: unlabelled 99.5% and labelled with radiochemical purity of 98.8-100% containing mixture of 9.8-12.5% cis and 87.5-90.2% trans isomer
Rabbit strain: New Zealand White
Oral (gavage).
Vehicle: polyethylene glycol.
Group 1-4: 2 animals/sex at 100 and 4 mg/kg bw. Time of observation: 0-96 h
Group 5-6: 2 animals/sex at 100 mg/kg bw at 5 h and 12 h respectively (time of termination)</td><td><u>Blood kinetics (96h)</u>
<table><tr><th>Group</th><th colspan="2">1</th><th colspan="2">2</th><th colspan="2">3</th><th colspan="2">4</th></tr><tr><th>Sex</th><th colspan="2">male</th><th colspan="2">female</th><th colspan="2">male</th><th colspan="2">female</th></tr><tr><th>Dose [mg/kg bw]</th><th colspan="2">100.7</th><th colspan="2">100.4</th><th colspan="2">4.07</th><th colspan="2">4.07</th></tr><tr><th></th><th>bl</th><th>pl</th><th>bl</th><th>pl</th><th>bl</th><th>pl</th><th>bl</th><th>pl</th></tr><tr><td>C_{max} [ppm]</td><td>11.27</td><td>14.07</td><td>10.98</td><td>13.47</td><td>0.486</td><td>0.710</td><td>0.582</td><td>0.990</td></tr><tr><td>T_{max} [h]</td><td>5</td><td>5</td><td>5</td><td>7</td><td>3</td><td>3</td><td>2.5</td><td>0.8</td></tr><tr><td>$AUC_{0\text{-}96\,h}$ [µg·h/g]</td><td>293</td><td>353</td><td>285</td><td>324</td><td>8.81</td><td>(-)</td><td>8.84</td><td>(-)</td></tr><tr><td>$AUC_{0\text{-}\infty}$ [µg·h/g]</td><td>434</td><td>413</td><td>407</td><td>373</td><td>10.30</td><td>8.48</td><td>10.44</td><td>9.37</td></tr><tr><td>$T_{1/2}$ (initial) [h]</td><td>11.1</td><td>11.3</td><td>10.8</td><td>8.9</td><td>4.2</td><td>3.5</td><td>4.4</td><td>3.1</td></tr><tr><td>$T_{1/2}$ (terminal) [h]</td><td>78</td><td>40</td><td>74</td><td>41</td><td>43</td><td>34</td><td>44</td><td>34</td></tr></table>bl = blood, pl = plasma
(-) value at 96 h was below the LOQ
<u>Excretion after 96 h</u>
<table><tr><th>Group</th><th>1</th><th>2</th><th>3</th><th>4</th></tr><tr><th>Sex</th><th>male</th><th>female</th><th>male</th><th>female</th></tr><tr><td>Dose [mg/kg bw]</td><td>100.7</td><td>100.4</td><td>4.07</td><td>4.07</td></tr><tr><td>Urine</td><td>69.2</td><td>72.0</td><td>66.8</td><td>62.1</td></tr><tr><td>Faeces</td><td>15.6</td><td>16.8</td><td>23.8</td><td>21.9</td></tr><tr><td>Cage wash</td><td>5.6</td><td>1.3</td><td>0.5</td><td>3.1</td></tr><tr><td>Total excretion (96h)</td><td>90.4</td><td>90.1</td><td>91.1</td><td>87.1</td></tr><tr><td>Excretion (48 h)</td><td>69.8</td><td>78.9</td><td>81.6</td><td>69.0</td></tr></table></td><td>Kunz, Ch. (2006b)</td></tr>
</table>

Method	Results	Reference
	Tissues residues in ppm after oral application of 100 mg/kg bw (groups 5-6)	

Sex	male		female	
Dose (mg/kg bw)	100.0		100.4	
Sacrifice [h]	5	12	5	12
Blood	8.83	4.19	7.07	4.53
Brain	10.4	3.42	6.66	2.04
Epididymes	29.1	15.6	-	-
Fat	104	82.1	83.8	58.6
Heart	17.0	12.1	15.2	6.18
Kidney	67.4	48.4	65.5	52.0
Liver	89.6	43.4	67.0	42.8
Lung	15.7	7.76	12.8	9.11
Muscle	9.12	3.95	5.47	3.67
Ovaries	-	-	27.3	7.78
Pancreas	46.9	19.0	20.9	10.2
Plasma	10.1	5.90	8.93	6.29
Testicles	14.2	6.03	-	-
Thyroid glands	31.2	19.6	24.4	6.84
Uterus	-	-	30.3	24.8

At 5 hours after application (TC_{max}) of the dose tissue residues were between 5.5-6.6 ppm (brain, muscle) and 84-104 ppm (fat). Relatively high levels were seen in liver and kidney (tissue/plasma ratio 7-9). The tissue/plasma ratio in gonads was 2.9 in the epididymis, 3.1 in ovaries, 3.4 in uterus and 1.4 in testes.

At 12 hours tissue residues after application were about 2.0 times (1.3-3.0) and 2.0 (1.2-3.6) times lower than the 5 hour values for males and females, respectively. The tissue/plasma ratio in gonads was 2.6 in epididymis, 1.2 in ovaries, 3.9 in uterus and 1.0 in testes.

Tissue residues were marginally lower (by a factor of about 1.4) for females in comparison to males for most tissues.

Metabolism

Large numbers of no or barely discernible fractions tissue residues were seen in the analysis of metabolite pattern after oral administration of 100 and 4 mg/kg bw in urine and faeces and 100 mg/kg bw in plasma.

Urine: most of the fractions not clearly discernible. 3 major metabolites U1 (5.5-10.6% of applied dose), U2 (3.2-4.6% of applied dose but only seen at high dose) and U3 (4.2-6.1% of applied dose) and a minor metabolite U4 were identified (0.1-2.4%) in both sexes. Major metabolites were identified as acetylcysteine conjugates of the parent where the conjugated residue replaced one of the two chlorine atoms at the pyrrolidone ring. They may be either position isomers (acetylcysteine residue at either of the two possible ring positions) or geometric isomers (cis/trans, acetylcysteine residue at the same ring position). U4 was tentatively identified as an acetylcysteine conjugate with the acetylcysteine residue linked to the trifluoromethyl-phenyl ring. Rat metabolite U1 was shown to be identical to rabbit metabolite U1. Unchanged flurochloridone was not detected.

Faeces: > 30 fractions most of them not clearly discernible. Unchanged flurochloridone was detected (0.2% of applied dose) in both sexes.

Plasm: ≥ 20 mostly undiscernible fractions. Unchanged flurochloridone was detected after 5 h (0.7-1.1 ppm in both sexes) and after 12 h (0.05 ppm males only).

Method	Results	Reference
Metabolism of flurochloridone by rats Laboratory: Stauffer Chemical Company Guideline: performed previous guidelines but equivalent to B.36 GLP: No Deviations: at the low dose level, groups consisted of only 2 (instead of 4) males; at the high dose level groups consisted of 2 males and 2 females. Content of radioactivity in blood was not determined. Multiple dose levels were not applied. The temperature and humidity of the animal experimental room is not given **Study acceptable** Purity: 95% (70% *trans* and 30% *cis* isomers) Vehicle: 1,2-propanediol: acetone = 6:1 (v/v) (low dose) 1,2-propanediol:ethanol: acetone = 2:1:1 (v/v) (high dose) In vivo experiment Rat strain: HSD:Sprague Dawley Route of administration: oral intubation and intraperitoneal Group A: 2 males at 192 mg/kg bw and 2 females at 236 mg/kg bw by oral intubation. Time of observation: 90 h. Group B: 2 males at 20.2 mg/kg bw by oral intubation and 2 males at 22.6 mg/kg bw by intraperitoneal route. Time of observation: 90 h. Group C: 4 males at 201 mg/kg bw by oral route. Time of observation: 72 h. In vitro experiments of flurochloridone cis, trans and mixture of both using rat liver enzyme systems containing MFO (mixed function oxidase) and glutathione-S-transferase (GSH-S-transferase) plus different cofactors.	Absorption and excretion Absorption was rapid >91% after 90 hours (37-48% by urine and 46-62% by faeces). Excretion after oral and i.p. application of flurochloridone in rats: **Flurochloridone equivalents [% of applied dose]** 	**Group**	**A**		**B**	**B**
---	---	---	---	---
Route	**Single oral**		**Single oral**	**Single i.p.**
Sex	**male**	**female**	**male**	**male**
Dose [mg/kg bw]	**192**	**236**	**20.2**	**22.6**
Urine				
0 - 18 hours	20.5	18.0	27.5	27.9
18 - 42 hours	21.4	24.2	11.1	7.3
42 - 90 hours	1.62	6.0	2.7	1.7
subtotal	*43.5*	*48.1*	*41.1*	*36.9*
Faeces				
0 - 18 hours	14.3	13.8	17.6	23.7
18 - 42 hours	33.1	24.9	30.5	24.2
42 - 90 hours	4.7	6.9	13.8	5.2
subtotal	*52.1*	*45.7*	*61.9*	*53.1*
Expired air	0.4	0.3	1.2	
Cage wash	1.4	2.8	0.3	0.3
Tissues	0.6	0.7	-	-
Total recovery	98	97.6	104.5	91.5 | Comparison of oral and i.p. application revealed that flurochloridone equivalents are extensively excreted via bile. Therefore the amount detected in faeces after oral application was considered to be absorbed and then excreted into the gastro-intestinal tract via bile. Tissue distribution after 90 h | **Sex**	**males**		**females**	
---	---	---	---	---
Dose (mg/kg bw)	**192**		**236**	
	ppm	**% dose**	**ppm**	**% dose**
Blood	2.10	-	3.81	-
Brain	0.40	<0.01	0.69	<0.01
Fat	0.95	-	2.01	-
Stomach	1.26	<0.01	1.79	0.01
Small intestine	1.89	0.02	4.01	0.02
Caecum	0.90	<0.01	3.31	0.01
Colon	0.91	<0.01	1.88	0.01
Rectum	0.89	<0.01	2.33	<0.01
Gonads	1.42	0.01	1.30	<0.01
Hair	15.76	-	15.26	-
Heart	1.55	<0.01	2.17	<0.01
Hide	2.22	0.17	2.64	0.17
Kidney	3.32	0.02	4.40	0.02
Liver	7.16	0.20	6.54	0.17
Lung	1.77	0.01	2.63	0.01
Muscle	0.51	-	0.99	-
Spleen	1.18	0.01	1.85	<0.01
Thymus	0.75	<0.01	1.12	<0.01
Carcass	0.72	0.18	1.03	0.22	 No signs of accumulation at 90 h. The highest residues were found in blood, hide, kidney, liver and gastrointestinal tract. Radioactivity appeared to be slightly higher in females but it has to be pointed out that tested dose level was 236 mg/kg bw in females and 192 mg/kg bw in males.	**Mcbain, J.B. (1985) (IIA. 5.1/1)**

<table>
<tr><th>Method</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td>Metabolism
In vivo
No sex-difference was observed in urine and faeces metabolite pattern. Large number of metabolite fractions in urine and faeces were seen but not identified at the high dose level tested of 201 mg/kg bw. Unchanged flurochloridone corresponded <0.2% of applied dose in urine and approximately 0.6-1.5% of applied dose in faeces.
In vitro
No metabolism of flurochloridone was seen when the compound was incubated in absence of the enzyme system. Incubation with liver enzymes in absence of co-factors leads to polar metabolites probably due to the presence of endogenous GSH and NADPH. Incubation with cofactors NADPH and GSH indicated both oxidative processes and conjugation with glutathione (GSH pathway). Trans isomer degraded faster via oxidation and cis isomer appeared to be equally metabolised by both pathways.</td><td></td></tr>
<tr><td>Excretion and tissue distribution in rats after single oral dose of 4 mg/kg bw
Laboratory: Central Toxicology Laboratory
Guideline: OECD 417 and B.36
GLP: Yes
Deviations: only a single oral application of a low dose level was performed in this study. Further requested applications (single oral high dose, multiple) are described in the studies by Silcock 2001b and 2002. Area under blood curve was not determined. No identification of metabolites was performed. Batch number of the test substance is not given
Study acceptable
Purity: radiolabelled flurochloridone (purity >97.5%; cis:trans ratio of 26.96:73.04) diluted with unlabelled flurochloridone (purity of 99.1% of cis isomer and purity of 99.7% of trans isomer with a final ratio cis:trans 28.3:71.7)
Vehicle: polyethylene glycol 600
Rat strain: Alpk:ApfSD (Wistar-derived)
Oral gavage
4 animals/sex at 4 mg/kg bw. Time of observation: 0-72 h.</td><td>Absorption and excretion
Rapidly absorbed and almost completely excreted (independently of sex) via urine (38-47% of applied dose) and faeces (49-58% of applied dose) within 72 hours after application. Based on the results of McBain 1985, the amount of flurochloridone equivalents detected in faeces is considered to be bio-available as extensive excretion into the gastro-intestinal tract via bile occurs.
<table>
<tr><th colspan="3">Flurochloridone equivalents [% of applied dose]</th><th>male</th><th>female</th></tr>
<tr><td colspan="2">Sex</td><td></td><td>male</td><td>female</td></tr>
<tr><td colspan="2">Dose [mg/kg bw]</td><td></td><td>3.91</td><td>3.91</td></tr>
<tr><td rowspan="6">Urine</td><td rowspan="4">0 - 12 h
12 - 24 h
24 - 36 h
36 - 72 h</td><td></td><td>26.5
6.8
2.8
2.3</td><td>34.4
7.2
2.8
3.1</td></tr>
<tr><td>subtotal</td><td>38.4</td><td>47.4</td></tr>
<tr><td rowspan="6">Faeces</td><td rowspan="4">0 - 12 h
12 - 24 h
24 - 36 h
36 - 72 h</td><td></td><td>10.7
24.7
12.7
10.1</td><td>15.4
17.6
8.3
7.7</td></tr>
<tr><td>subtotal</td><td>58.1</td><td>49.0</td></tr>
<tr><td colspan="3">Cage wash</td><td>1.3</td><td>1.3</td></tr>
<tr><td colspan="3">Total excretion</td><td>97.8</td><td>97.7</td></tr>
<tr><td colspan="3">Tissues
Gastro-intestinal tract</td><td>1.5
0.4</td><td>1.9
0.4</td></tr>
<tr><td colspan="3">Total recovery</td><td>99.7</td><td>100.1</td></tr>
</table>
Tissue distribution at 72 h
<table>
<tr><th>Sex</th><th colspan="2">males</th><th colspan="2">females</th></tr>
<tr><th>Dose (mg/kg bw)</th><th colspan="2">3.91</th><th colspan="2">3.91</th></tr>
<tr><th></th><th>ppm</th><th>% dose</th><th>ppm</th><th>% dose</th></tr>
<tr><td>Brain</td><td>0.020</td><td><0.01</td><td>0.035</td><td>0.01</td></tr>
<tr><td>Kidney</td><td>0.188</td><td>0.04</td><td>0.229</td><td>0.05</td></tr>
<tr><td>Liver</td><td>0.507</td><td>0.72</td><td>0.540</td><td>0.62</td></tr>
<tr><td>Spleen</td><td>0.035</td><td><0.01</td><td>0.055</td><td><0.01</td></tr>
<tr><td>Heart</td><td>0.071</td><td>0.01</td><td>0.097</td><td>0.01</td></tr>
<tr><td>Lung</td><td>0.136</td><td>0.02</td><td>0.175</td><td>0.02</td></tr>
<tr><td>Testes</td><td>0.083</td><td>0.02</td><td>-</td><td>-</td></tr>
<tr><td>Epididymis</td><td>0.113</td><td>0.01</td><td>-</td><td>-</td></tr>
<tr><td>Ovaries</td><td>-</td><td>-</td><td>0.082</td><td><0.01</td></tr>
<tr><td>Abdominal fat</td><td>0.032</td><td>-</td><td>0.100</td><td>-</td></tr>
</table></td><td>Silcock, R.C. (2001a) (IIA. 5.1/2)</td></tr>
</table>

Method	Results	Reference

Bone	0.016	-	0.020	-
Muscle	0.017	-	0.035	-
Blood	0.074	-	0.089	-
Plasma	0.017	-	0.026	-
Partial tissues	-	0.10	-	0.13
Shaved skin	0.024	-	0.051	-
Hair	0.055	-	0.171	-
Residual carcass	0.031	0.60	0.056	1.08
Total	-	1.53	-	1.92

After 72 h slightly lower in males than females. The liver and kidneys showed the highest radioactivity concentrations. All tissue concentrations were slightly lower in males than in females. Radioactivity was also found in bone (0.016 ppm in males and 0.020 ppm in females). Residual radioactivity in tissues and carcass was ≤ 1.9% of applied dose. Due to the rapid excretion (69-75% within 24 hours), no relevant potential for accumulation was considered for flurochloridone.

Excretion and tissue distribution in rats after single oral dose of 200 mg/kg bw

Laboratory: Central Toxicology Laboratory

Guideline: OECD 417 and B.36.

GLP: Yes.

Deviations: only a single oral application of a low dose level was performed in this study. Further requested applications (single oral low dose, multiple) are described in the studies by Silcock 2001a and 2002. Area under blood curve was not determined. No identification of metabolites was performed.

Study acceptable

Purity: radiolabelled flurochloridone (purity >97.5%; cis:trans ratio of 26.96:73.04) diluted with unlabelled flurochloridone (purity of 99.1% of cis isomer and purity of 99.7% of trans isomer with a final ratio cis:trans 28.3:71.7)

Vehicle: polyethylene glycol 600

Rat strain: Alpk:ApfSD (Wistar-derived)

Oral gavage

4 animals/sex at 200 mg/kg bw. Time of observation: 0-72 h.

Absorption and excretion

Rapidly absorbed and almost completely excreted (independently of sex) via urine (34% of applied dose) and faeces (51-55% of applied dose) within 72 hours after application. If the results by McBain 1985 are considered, absorption was at least 91%.

Flurochloridone equivalents [% of applied dose]			
Sex		**male**	**female**
Dose [mg/kg bw]		**202**	**202**
Urine	0 - 12 h	7.3	6.8
	12 - 24 h	11.1	11.3
	24 - 36 h	10.2	10.3
	36 - 72 h	5.3	5.8
	subtotal	***33.9***	***34.3***
Faeces	0 - 12 h	6.2	2.1
	12 - 24 h	15.9	18.5
	24 - 36 h	20.1	21.7
	36 - 72 h	12.9	8.8
	subtotal	***54.9***	***51.1***
Cage wash		2.0	3.7
Total excretion		90.8	89.0
Tissues		0.8	1.5
Gastro-intestinal tract		1.0	0.8
Total recovery		**92.6**	**91.3**

Tissue distribution at 72 h

Sex	**males**		**females**	
Dose (mg/kg bw)	**202**		**202**	
	ppm	**% dose**	**ppm**	**% dose**
Brain	0.463	<0.01	0.936	<0.01
Kidney	4.726	0.02	7.240	0.03
Liver	8.440	0.23	10.181	0.21
Spleen	1.368	<0.01	2.328	<0.01
Heart	1.489	<0.01	2.348	<0.01
Lung	2.000	0.01	2.843	0.01
Testes	1.403	0.01	-	-
Epididymis	3.942	<0.01	-	-
Ovaries	-	-	2.431	<0.01
Abdominal fat	8.228	-	10.580	-

Silcock, R.C. (2001b) (IIA. 5.1/3)

Method	Results	Reference
	<table><tr><td>Bone</td><td>0.683</td><td>-</td><td>0.872</td><td>-</td></tr><tr><td>Muscle</td><td>0.616</td><td>-</td><td>1.107</td><td>-</td></tr><tr><td>Blood</td><td>3.540</td><td>-</td><td>4.847</td><td>-</td></tr><tr><td>Plasma</td><td>0.778</td><td>-</td><td>0.862</td><td>-</td></tr><tr><td>Partial tissues</td><td>-</td><td>0.10</td><td>-</td><td>0.14</td></tr><tr><td>Shaved skin</td><td>1.503</td><td>-</td><td>1.698</td><td>-</td></tr><tr><td>Hair</td><td>6.529</td><td>-</td><td>1.663</td><td>-</td></tr><tr><td>Residual carcass</td><td>1.235</td><td>0.45</td><td>2.950</td><td>1.09</td></tr><tr><td>Total</td><td>-</td><td>0.82</td><td>-</td><td>1.49</td></tr></table> After 72 h there were no pronounced sex differences in the tissue distribution of radioactivity. The highest values were found in liver, abdominal fat, hair, kidney, epididymis (males) and blood. The author attributes the high levels found in hairs to contamination with urine and /or faeces. Tissue residues including residual carcass at 72 hours after application were ≤ 1.5% of applied dose. Radioactivity was also found in bone (0.683 ppm in males and 0.872 ppm in females). Due to the rapid excretion, no relevant potential for accumulation was considered for flurochloridone.	
Excretion and tissue distribution in rats after repeated oral dose of 4 mg/kg bw Laboratory: Central Toxicology Laboratory Guideline: OECD 417 and B.36. GLP: Yes. Deviations: only multiple oral application of a low dose level was performed in this study. Further requested applications (single oral low and high dose) are described in the studies by Silcock 2001a/b. Area under blood curve was not determined. **Study acceptable** Purity: radiolabelled flurochloridone (purity >97.5%; cis:trans ratio of 26.96:73.04) diluted with unlabelled flurochloridone (purity of 99.1% of cis isomer and purity of 99.7% of trans isomer with a final ratio cis:trans 28.3:71.7) Vehicle: polyethylene glycol 600 Rat strain: Alpk:ApfSD (Wistar-derived) Oral gavage 30 animals/sex at 4 mg/kg bw during 14 days divided in 10 groups of 3 animals/sex Time of observation: 24 hours after last dose for excretion and 37 days for tissue distribution.	<u>Absorption and excretion</u> Rapidly absorbed after multiple application of 4 mg/kg bw for 14 days and almost completely excreted via urine (42-47% of applied dose) and faeces (53-58% of applied dose). No great differences compared to values after single oral administration of 4 mg/kg bw (Silcock, 2001a). <table><tr><td></td><td>males</td><td>females</td></tr><tr><td></td><td colspan="2">% of excreted ^{14}C</td></tr><tr><td colspan="3">Within 24 hours after multiple application</td></tr><tr><td>Urine</td><td>41.60</td><td>47.04</td></tr><tr><td>Faeces</td><td>58.40</td><td>52.96</td></tr><tr><td>% applied dose#</td><td>93.93</td><td>87.87</td></tr><tr><td colspan="3">Within 72 hours after multiple application</td></tr><tr><td>Urine</td><td>39.79</td><td>49.34</td></tr><tr><td>Faeces</td><td>60.21</td><td>50.66</td></tr><tr><td>% applied dose#</td><td>96.53</td><td>96.4</td></tr></table># % of the last of 14 daily doses <u>Tissue distribution</u> Highest residues were seen in blood and highly perfused organs like liver and kidney. Comparison of residue values in whole blood and plasma revealed that the radioactivity in blood was associated with the cellular components of blood. Whole blood also had with 14-18 days the longest terminal half-life time. After reaching a peak concentration at 15 days (1 day after the last of 14 applications), residues in liver and kidney declined with half-life times of 4-6 days. Radioactivity was also found in bone. Accumulation and elimination profiles in the remaining tissues were similar to plasma with a half-life time of 6-7 days.	**Silcock, R.C. (2002) (IIA. 5.1/3)**

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Absorption

Flurochloridone was found to be rapidly absorbed from the gastro intestinal tract considering the available data in rat studies with >90% of applied dose within 42-90 h after administration based on the comparison of excretion patterns after oral and intraperitoneal administrations. It has to be noted that flurochloridone absorption was essentially independent of sex and dose level.

Absorption in rabbits was also rapid with 87-91% after 96 h considering urine and faeces excretion. As it occurred in rats absorption was independent of sex and dose level.

Maximum blood and plasma levels were reached much earlier in the rabbit than in the rat. The values of these maximums were slightly higher in plasma in rabbits as compared to rats. The two maximum values observed in blood and plasma kinetics in rats along with higher faecal excretion may indicate more marked excretion from the liver via bile into the gastrointestinal tract in the rat as compared to rabbits, with at least partly reabsorption from the gastrointestinal tract into the systemic circulation.

Distribution

No evidence of accumulation was observed in rats and rabbits.

In Kunz (2006a) rat study observations of tissue residues after 12 h (TC_{max}) revealed relatively high levels in kidney and liver. At 24 h tissue residues were reduced. It has to be noted that depletion in blood was slower than plasma indicating some binding of flurochloridone equivalents to blood cells. This fact was confirmed in other rat studies.

In Mcbain (1985) rat study there were no signs of accumulation after 90 h. The highest residues were found in blood, hide, kidney, liver and gastrointestinal tract. In Silcock (2001a) and Silcock (2001b) studies in rats after single oral doses the residual radioactivity in tissues after 72 h was found to be ≤1.9% and ≤1.5% respectively. It has to be noted that high level of flurochloridone equivalents were found in liver and kidney in Silcock (2001a) study and in abdominal fat, kidney, liver, blood and epididymis in Silcock (2001b) study. After repeated dose exposure of 14 days in rats (Silcock, 2002) the highest levels were found in liver and kidney.

In Kunz (2006b) rabbit study, observations of tissue residues after 5 h (TC_{max}) revealed relatively high levels in kidney and liver. At 12 h tissue residues were reduced. It has to be noted that depletion in blood was slightly slower than plasma. The blood/plasma ratio was not as high as those observed in rats.

Regarding tissue residues in gonads, absolute values were similar for the epididymis in both species and slightly higher in rabbits for testes. For females, absolute ovary residues were lower and residues in uterus were higher in rabbits as compared to rats. Concerning tissue/plasma ratio values in the epididymides of rats (4.0-4.2) were higher than those for rabbits (2.6-2.9). The same applies to ovaries in females (ovary/plasma ratio was 1.2-3.1 in rabbits and 3.7-3.9 in rats). No relevant species difference was seen for testes/plasma ratio and uterus/plasma ratios were lower in rats (1.1-1.4) than in rabbits (3.4-3.9).

Metabolism

Metabolite pattern in urine, faeces and plasma in Kunz in vivo studies (2006a and 2006b) in both rats and rabbits revealed large number of not or barely discernible fractions. This had also been observed in Mcbain (1985) study in rats. Metabolism was extensive since very minor amounts of unchanged parent flurochloridone were seen in the faeces and plasma of both species. Metabolites that could be tentatively identified were all acetylcysteine conjugates. Either one of the chlorine atoms at the

pyrrolidone ring was substituted by acetylcysteine (or their cis/trans isomers; metabolites U1 and U2 in the rat and U1, U2 and U3 in the rabbit) or the acetylcysteine residue was linked to the trifluoromethyl-phenyl ring (rat metabolite U3, rabbit metabolite U4). Rat metabolite U1 was shown to be identical to rabbit metabolite U1. No sex differences were observed in metabolism.

In vitro experiment performed in Mcbain (1985) study revealed that incubation of flurochloridone with active enzymes and cofactors NADPH and GSH indicated both oxidative and GSH pathways. Three oxidative metabolites were identified.

Excretion

Excretion in rats was > 97 % of applied dose within 72 h after administration with of 38 – 47 % of applied dose in urine and 49 – 58 % of applied dose in faeces according to Silcock (2001a) study after single oral doses. Other studies in rats showed similar pattern of excretion even after repeated exposure.

Excretion in rabbits was 87-91 % of applied dose within 96 h after administration with of 62-72 % of applied dose in urine and 16-24 % of applied dose in faeces according to Kunz (2006b) study after single oral doses.

Lower initial half-life times ($T_{1/2}$) in rabbits compared to rats indicated faster depletion in rabbits. However, the area under curve (AUC) values was only slightly lower in rabbits. Elimination by urine in rabbits was higher than rats in which the amount of flurochloridone equivalents in faeces was elevated. This fact may indicate a more marked excretion in rats via bile into the gastrointestinal tract compared to rabbits. Two maximum values often observed in blood and plasma kinetics in rats could be related with at least partly reabsorption from the gastrointestinal tract into the systemic circulation.

<u>Comparison of toxicokinetics between rats and rabbits</u>

In conclusion, minor variations in toxicokinetic parameters were observed between rats and rabbits. The species difference observed in reproductive toxicity endpoints (rats affected but not rabbits at similar or higher dose levels) included in section 10.10 does not appear to be caused by differences in toxicokinetic parameters between the two species. The faster uptake into blood together with slightly higher maximum values (at least for plasma) in rabbits as compared to rats may indicate a slightly higher sensitivity of rabbits concerning acute toxicity. The fact that in rats often two maximum values are seen in blood and plasma kinetics along with the higher faecal excretion may indicate more marked excretion from the liver via bile into the gastrointestinal tract in the rat as compared to rabbits, with at least partly reabsorption from the gastrointestinal tract into the systemic circulation.

Despite some quantitative differences (U1 in slightly higher amounts in rats than in rabbits), there appeared to be no marked species differences in metabolism of flurochloridone.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 12: Summary table of animal studies on acute oral toxicity

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no./group, test substance, dose levels, duration of exposure</th><th>Value
LD_{50}</th><th>Reference</th></tr>
<tr>
<td>Toxicology laboratory report: acute oral toxicity study in rats
Guideline: US EPA (1978). Checked for compliance with OECD 401.
GLP: No.
Deviations: bodyweights after the observation period and individual toxicity were not reported.
Study acceptable</td>
<td>Purity: 89.7% (w/w) Proportion of isomers not indicated.
Rat strain: Sprague-Dawley albino rats.
Oral (gavage).
Vehicle: corn oil.
10 rats minimum/sex/dose
Doses: 0, 2000, 2500, 3200, 3600 (only males), 4000, 4500 and 5000 mg/kg bw
14-day observation period</td>
<td>Mortality:
<table>
<tr><th rowspan="2">Dose (mg/kg bw)</th><th rowspan="2">Sex</th><th rowspan="2">Mortality</th><th colspan="4">Time of death (days)</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th></tr>
<tr><td rowspan="2">5000</td><td>m (♂)</td><td>20/20</td><td>1</td><td>18</td><td>-</td><td>1</td></tr>
<tr><td>f (♀)</td><td>17/20</td><td>-</td><td>16</td><td>1</td><td>-</td></tr>
<tr><td rowspan="2">4500</td><td>m (♂)</td><td>15/20</td><td>-</td><td>13</td><td>2</td><td>-</td></tr>
<tr><td>f (♀)</td><td>8/10</td><td>-</td><td>7</td><td>1</td><td>-</td></tr>
<tr><td rowspan="2">4000</td><td>m (♂)</td><td>11/20</td><td>3</td><td>7</td><td>-</td><td>1</td></tr>
<tr><td>f (♀)</td><td>27/45</td><td>-</td><td>21</td><td>5</td><td>1</td></tr>
<tr><td>3600</td><td>m (♂)</td><td>3/10</td><td>1</td><td>2</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">3200</td><td>m (♂)</td><td>2/15</td><td>1</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td>f (♀)</td><td>21/35</td><td>2</td><td>16</td><td>3</td><td>-</td></tr>
<tr><td rowspan="2">2500</td><td>m (♂)</td><td>1/10</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td>f (♀)</td><td>1/10</td><td>-</td><td>1</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">2000</td><td>m (♂)</td><td>0/10</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>f (♀)</td><td>0/10</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>0</td><td>m (♂)</td><td>0/25</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td colspan="7">LD_{50} males: 4000 mg/kg bw (3701-4323 mg/kg bw)
LD_{50} females: 3650 mg/kg bw (3349-3979 mg/kg bw)</td></tr>
</table>
Clinical signs: toxic signs were observed in all treated groups and included mild to severe depression, salivation, diarrhoea, blood-like stains around eyes, nose and mouth, yellow stains around the ano-genital area, ruffled fur and alopecia. Severe diarrhoea also occurred in controls during the first 24 hours.
All toxicity signs were reversible in six days for the survivors treated with 2000 and 2500 mg/kg. Clinical signs of surviving rats treated from 3200 mg/kg returned to normal in twelve days, although they remained depressed with staining and alopecia until the end of the test.
Necropsy: findings of animals that died during the study included dark red lungs, pale and mottled liver, bloated gastrointestinal tract with were filled with a gelatinous material. In addition one rat that died after treatment with 5000 mg/kg had a dark spotted thymus and unusually small testes.</td>
<td>Howell, A.M. (1979) (IIA. 5.2.1/01)</td>
</tr>
<tr>
<td>Acute oral toxicity study in mice
Laboratory: Research &Consulting Company AG
Guideline: OECD 401.
GLP: Yes.
Deviations: bodyweights of test animals at start of treatment exceed 20% of the mean weight. LD_{50} was</td>
<td>Purity: 90% (w/w) Proportion of isomers not indicated.
Mice strain: KFM-NMRIS
Oral (gavage)
Vehicle: polyethylene glycol (PEG 400)
5 animals/sex/dose
Doses: 0, 1000, 3000 and 5000</td>
<td>Mortality
<table>
<tr><th rowspan="2">Dose (mg/kg bw)</th><th colspan="2">Males</th><th colspan="2">Females</th></tr>
<tr><th>Mortality</th><th>Time of death</th><th>Mortality</th><th>Time of death</th></tr>
<tr><td>5000</td><td>2/5</td><td>1 (day 1)
1 (day 2)</td><td>0/5</td><td>-</td></tr>
<tr><td>3000</td><td>2/5</td><td>2 (day 2)</td><td>1/5</td><td>1 (day 2)</td></tr>
<tr><td>1000</td><td>0/5</td><td>-</td><td>0/5</td><td>-</td></tr>
<tr><td>0</td><td>0/5</td><td>-</td><td>1/5</td><td>1 (day 2)</td></tr>
<tr><td colspan="5">LD_{50}: > 5000 mg/kg bw for both sexes</td></tr>
</table>
</td>
<td>Ullmann, L. (1985) (IIA. 5.2.1/02)</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no./group, test substance, dose levels, duration of exposure</th><th>Value
LD_{50}</th><th>Reference</th></tr>
<tr><td>estimated without use of a statistical model.

Study acceptable</td><td>mg/kg bw

14-day observation period</td><td>Clinical signs: sedation, dyspnoea, ataxia, curved body position and ruffled fur in all animals including controls. From 3000 mg/kg bw also ventral position and lateral abdominal position were also noted. Effect were reversible on day 3 (controls and 1000 mg/kg bw), day 4 (3000 mg/kg bw) and day 6 (5000 mg/kg bw) after administration.

Necropsy: No pathologic changes in survivors. Mottled lunges and reddened stomach and/or intestines in dead animals.</td><td></td></tr>
<tr><td>Acute oral toxicity study in rats – Acute toxic class method

Laboratory: Harlan Laboratories Ltd

Guideline: B.1 tris.

GLP: Yes.

Deviations: none.

This study was included in an addendum of May/September 2012 prepared in order to evaluate the confirmatory data required in Part B of the specific provisions under inclusion Directive 2011/34/EU

Study acceptable</td><td>Purity: 95.5% (w/w) (73.5% trans-isomer, 22.7% cis-isomer)

Rat strain: RccHan:WIST (SPF)

Oral (gavage)
Vehicle: corn oil

3 females/dose

Doses: 2000 and 300 mg/kg bw. This last dose was administered twice.

14-day observation period</td><td>Mortality:
<table><tr><th>Dose (mg/kg bw)</th><th>Mortality</th><th>Time of death</th></tr><tr><td>2000</td><td>2/3</td><td>1 (day 1)
1 (day 2)</td></tr><tr><td>300</td><td>0/0</td><td>-</td></tr><tr><td>300</td><td>0/0</td><td>-</td></tr></table>300 mg/kg bw < LD_{50} (female rat) < 2000 mg/kg bw

Clinical signs:
<u>2000 mg/kg bw</u>: swaying gait, dragging of fore and rear limbs, decreased activity, prostration, hunched posture and ruffled fur were observed after treatment in all animals until test day 2 (day of sacrifice of 2/3 animals). The surviving still showed decreased activity and ruffled fur on test day 3, but no clinical signs thereafter until the end of the study.
<u>300 mg/kg bw</u>: 3/6 animals treated showed swaying gait, dragging of fore and rear limbs and decreased activity after treatment on test day 1. No clinical signs from test day 2 until the end of the observation period were observed.

Necropsy: the two sacrificed animals at 2000 mg/kg bw showed yellowish discoloured and distended stomach. One of them additionally showed kidneys reduced in size. One animal treated with 300 mg/kg bw showed haemorrhagic lungs upon scheduled necropsy.</td><td>Sieber, M. (2011)</td></tr>
</table>

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In Howell, A.M. (1979) acute oral toxicity in rats, a LD_{50} males of 4000 mg/kg bw (3701-4323 mg/kg bw) and a LD_{50} females of 3650 mg/kg bw (3349-3979 mg/kg bw) were seen.

In Ullmann, L. (1985) acute oral toxicity in mice LD_{50} is considered greater than 5000 mg/kg bw for both sexes.

In Sieber, M. (2011) acute oral toxicity study in rats, LD_{50} was observed to be in the interval of 300 mg/kg bw < LD_{50} (female rat) < 2000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

The LD_{50} observed in rats in Sieber study (300 mg/kg bw < LD_{50} (female rat) < 2000 mg/kg bw) meets with the criteria for classification as Acute Tox. 4 (300 mg/kg bw < LD_{50} < 2000 mg/kg bw) after oral exposure according to CLP Regulation.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Acute Tox. 4 – H302: Harmful if swallowed.

ATE: 500 mg/kg bw (estimation for 300 mg/kg bw < LD_{50} < 2000 mg/kg bw)

10.2 Acute toxicity - dermal route

Table 13: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no./group, test substance, dose levels, duration of exposure	Value LD_{50}	Reference
Toxicology laboratory report: acute dermal toxicity study in rats Guideline: US EPA (1978). Check for compliance with OECD 402. GLP: No Deviations: four instead five animals per group were used and on half of them the skin was abraded. Bodyweights and individual toxicity data were not reported. **Study acceptable**	Purity: 89.7% (w/w) Proportion of isomers not indicated. Rabbit strain: New Zealand albino Vehicle: not used. Treated group: 4 animals/sex (skin was abraded in half animals and intact on the others) Controls: 1 animal/sex Doses: 5000 mg/kg bw 24 h of exposition. Material was removed after exposition. 14-day observation period	No mortality occurred. No indication of skin irritation though staining properties of the substance made difficult the erythema evaluation. No abnormalities in necropsy. **LD_{50} > 5000** mg/kg bw	**Howell, A.M. (1979) (IIA. 5.2.2/01)**

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In Howell, A.M. (1979) acute dermal toxicity study in rabbits LD_{50} was observed to be higher than 5000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

LD_{50} of 5000 mg/kg bw is above the threshold value of 2000 mg/kg bw for triggering acute dermal toxicity classification.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that flurochloridone does not require classification for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Table 14: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no./group Dose levels, duration of exposure	Test substance, , form and particle size (MMAD)	Value LC_{50}	Reference
4-hour acute inhalation toxicity study in rats Laboratory: RCC Ltd Guideline: OECD 403. GLP: Yes. Deviations: none **Study acceptable**	Rat strain: HanBrl:WIST (SPF) Nose only exposure to an aerosol. 4 hour exposure and thereafter 14-day observation. 5 animals/sex/dose Analytical concentration: 4.821 mg/L (attempt of 5 mg/L)	Purity: 94.9% (w/w) (79.9% trans-isomer, 23.04% cis-isomer). Flurochloridone aerosol was generated using a polyethylene glycol bath. Aerosol properties: MMAD: 2.70 / 2.75 µm GSD: 2.98 / 2.90 µm Particles ≤ 4.6 µm (72-73% w/w)	Mortality did not occur and no clinical signs were observed. **LC_{50} > 4.821 mg/L**	**Decker, U., Knuppe, C., Ullrich, A. (2004) (IIA. 5.2.3/01)**

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In Decker et al. (2004) acute inhalation toxicity study in rats the LC_{50} was found to be greater than 4.821 mg/l after 4-hour exposition.

10.3.2 Comparison with the CLP criteria

The tested concentration of 4.821 mg/L was obtained after an attempt to achieve 5 mg/L. In the original study it is not stated that this concentration corresponds to the maximum attainable concentration. Consequently, considering that the cut-off value for classification after inhalation exposure is 5 mg/L, there are no data available for the interval (4.821-5] mg/l. However, no mortality and clinical signs occurred at the tested dose level and accordingly it is not plausible to find mortality in this exiguous interval. Besides, the MMAD and GSD of the generated atmosphere indicate that a high respirable aerosol was achieved. Taking into account the whole available data, the MSCA is of the opinion that no classification for inhalation is required for flurochloridone.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Data available indicates that flurochloridone does not require classification for acute inhalation toxicity.

10.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no./group
Test substance,
Dose levels
duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr>
<td>Toxicology laboratory report: skin irritation study in rabbits
Guideline: US EPA (1978). Checked for compliance with OECD 404.
GLP: No.
Deviations: Exposure period was 24 hours instead of 4 hours. Test material was applied to both intact and abraded skin. Skin reactions were recorded only directly after exposure and at 48 hours. Body weights after the observation period were not reported.
Study acceptable</td>
<td>Purity: 89.7% (w/w) Proportion of isomers not indicated.
Rabbit strain: New Zealand albino
0.5 g of undiluted test material (vehicle not used)
6 animals (sex not specified): intact and abraded skin in each rabbit.
24 h of exposition
Observations at 0 and 48 h after patch removal.</td>
<td>
<table>
<tr><th>Time</th><th colspan="6">Erythema</th><th colspan="6">Oedema</th></tr>
<tr><th>Animal number</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr>
<tr><td>After patch removal</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>48 hours after patch removal</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
Conclusion: not skin irritant.
</td>
<td>Howell, A.M. (1979)
(IIA. 5.2.4/01)</td>
</tr>
</table>

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In Howell, A.M. (1979) skin irritation study in rabbits the results of erythema and oedema were 0 in the six animals treated after 48 h. The test was carried out with 24 h of exposition and using intact

and abraded skin. There is no data for the reactions after 24 and 72 h. Despite these deviations the skin irritation test was considered acceptable to evaluate the irritancy potential to skin of flurochloridone.

10.4.2 Comparison with the CLP criteria

CLP criteria for skin irritation are based on the average results of erythema and oedema after 24, 48 and 72 h. Results in the available study were observed 48 h after the patch removal. However, considering the absence of lesions on intact and abraded skin and the prolonged exposition (24 h) to the active substance, the MSCA is of the opinion that flurochloridone is not irritant to the skin.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Data available indicates that flurochloridone does not require classification as skin irritant.

10.5 Serious eye damage/eye irritation

Table 16: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no./group, test substance, dose levels duration of exposure</th><th>Results
-Observations and time point of onset
-Mean scores/animal
-Reversibility</th><th>Reference</th></tr>
<tr>
<td>Toxicology laboratory: eye irritation study in rabbits
Guideline: US EPA (1978). Checked for compliance with OECD 405.
GLP: No.
Deviations: Eye reactions were not recorded 1 hour after treatment.
Study acceptable</td>
<td>Purity: 89.7% (w/w) Proportion of isomers not indicated.
Rabbit strain: New Zealand albino
9 animals (sex not specified)
0.1 g of undiluted test material (vehicle not used) placed into the conjunctival sac of the left eye of the animals.
Eyes were rinsed after instillation in 3 animals and remained unwashed in 6 animals.
Observations after 24, 48 and 72 h and day 4, 1 and 14 (end of the study)</td>
<td>Results of animals with unwashed eyes after instillation:
<table>
<tr><th></th><th colspan="6">Cornea</th><th colspan="6">Iris</th><th colspan="12">Conjunctiva</th></tr>
<tr><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th colspan="6">Redness</th><th colspan="6">Chemosis</th></tr>
<tr><td>After 24 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 48 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 72 hours</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean scores 24-72 h</td><td colspan="6">0</td><td colspan="6">0</td><td colspan="6">0</td><td colspan="6">0</td></tr>
</table>
Conclusion: not eye irritant.</td>
<td>Howell, A.M. (1979)
(IIA. 5.2.5/01)</td>
</tr>
</table>

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In Howell, A.M. (1979) eye irritation study in rabbits no lesions were observed in the eye of rabbits. Results of conjunctival erythema and edema, iritis or corneal opacity were 0 in the six animals treated after 48 h.

10.5.2 Comparison with the CLP criteria

The individual and group mean eye irritation scores do not meet the criteria for classification as irritating to the eyes according to CLP.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Data available indicates that flurochloridone does not require classification as eye irritant.

10.6 Respiratory sensitisation

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data available.

10.6.2 Comparison with the CLP criteria

No data available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

Data lacking.

10.7 Skin sensitisation

Table 17: Summary table of animal studies on skin sensitisation

Type of study/data	Test substance, species, strain, sex, no./group	Dose levels duration of exposure and results	Reference
Open Epicutaneous Test Laboratory: Richmond Toxicology Laboratory Guideline: Open Epicutaneous Test Protocol GLP: Yes. Deviations: the purity of test substance was not reported. The highest concentration of test substance used in induction phase (30%) was not the highest well-tolerated systemically to cause mild-to-moderate skin irritation. Test material was applied 20 times (instead of 3) to an area of 2 cm^2 (instead of 4-6 cm^2) and the test sites left uncovered. **Study not acceptable** since it did not show that active substance penetrates the skin and was systemically available for contact with the immune system. Dose levels of flurochloridone used in induction and challenge phases were not selected appropriately.	Purity: unknown Proportion of isomers not indicated Hartley male guinea pigs 10 animals in preliminary test 10 animals/group main test Vehicle: ethanol and acetone in induction and challenge phases	Preliminary test: Epidermal applications of 25 µl at active substance concentrations of 1%, 3%, 10% and 30% in 70% aqueous ethanol for a 24-h exposure period did not cause any skin reactions. Brown-stained fur was observed at 30%. Although there was no skin reactions at any dose level up to 30% this concentration was used for induction and challenge. Main test: *(Time of exposure was not shown in any group in the main test)* - Induction phase (day 1-26): five groups of 10 guinea pigs each were applied topically to the right flank with 100 µl on 2 cm^2 of 0 (vehicle), 1%, 3%, 10% or 30% daily on 5 days/week during four weeks (20 applications). 70% ethanol was the vehicle. The 30% solution was prepared with pure ethanol after week 1 due to solubility problems. The treated skin area was left uncovered. - Challenge phase (day 29-32): three days after the last induction, each animal received on the left flank epidermal administration of 25 µl on 1 cm^2 at 0% (vehicle), 3%, 10% and 30% of the test substance. Vehicle was acetone. Treated areas were left uncovered. - Rechallenge (day 43-46): 2 weeks after the first challenge using the same application scheme. Sensitivity of the system was confirmed with a test using dinitro-chlorobenzene (DNCB) 0.4% in 70% ethanol during induction and acetone during challenge. Results: No skin reactions were observed after challenge and rechallenge in treated groups at any dose level after 24, 48 and 72 h. Positive group revealed skin sensitisation at challenge (9/10) and rechallenge (10/10) after 24 h.	**Mutter, L.C. (1985) (IIA. 5.2.6/01)**

<table>
<tr><th>Type of study/data</th><th>Test substance, species, strain, sex, no./group</th><th>Dose levels
duration of exposure and results</th><th>Reference</th></tr>
<tr>
<td>Guinea pig maximisation test (GPMT)
Laboratory: RCC Ltd
Guideline: OECD 406.
GLP: Yes.
Deviations: none
Study acceptable</td>
<td>Purity: 93.1% (w/w)
Female Alpk Dunkin Hartley guinea pigs
10 animals for main tested group and 5 for control
Vehicle: polyethylene 300 (PEG 300)</td>
<td>Preliminary test:
3 animals received four intradermal (0.1 ml) injections of a solution 1:1 of FCA/saline. 6 days later:
- 1 animal received 3 intradermal injections (0.1 ml) of 25%, 15% and 10% of flurochloridone in the vehicle.
- 2 animals received epidermal applications (0.2 ml) of 75% (maximum attainable concentration), 50%, 25% and 15% in the vehicle by occlusive dressing for 24 h.
In a second test two additional animals were treated dermally (0.2 ml) with 15%, 10%, 5% and 1% flurochloridone in the vehicle by occlusive dressing for 24 h.
Results: after the intradermal injections erythema of grade 2 was recorded of all tested concentrations. After epidermal applications erythema of grade 1 was seen at concentrations ≥ 10%. The selected concentrations were:
- 25% for intradermal induction
- 75% for epidermal induction.
- 5% for epidermal challenge
Main test:
<table>
<tr><th>Induction intradermal injection (0.1 ml) Day 1</th><th>Test</th></tr>
<tr><td>1</td><td>FCA/physiological saline 1:1</td></tr>
<tr><td>2</td><td>25% test sample in PEG 300</td></tr>
<tr><td>3</td><td>Test sample at 25% in a 1:1 prep. of FCA/ physiological saline</td></tr>
<tr><td>Induction topical application (0.3 ml) Day 8</td><td>75% in PEG 300 under occlusive dressing for 48 h</td></tr>
<tr><td>Challenge (0.2 ml) Day 22</td><td>Test sample at 5% in PEG 300 under occlusive dressing for 24 h</td></tr>
</table>
Control group had the same treatment on day 1 and 8 using PEG 300 instead of active substance.
Sensitivity of the system was confirmed 2 months prior to the study using α-hexylcinnamaldehyde as positive control group.
Results
No skin reactions were observed in the control group (0/5).
The sensitisation rate was 100%. All animals (10/10) after challenge at 5% of concentration showed discrete to intense erythema with swelling in the 24 and 48 hour readings. Individually skin responses according to Magnusson and Kligman scale are in the following table.
<table>
<tr><th>Animal no.</th><th>24 hours</th><th>48 hours</th></tr>
<tr><td>835</td><td>2</td><td>2</td></tr>
<tr><td>836</td><td>2</td><td>2</td></tr>
<tr><td>837</td><td>2</td><td>3</td></tr>
<tr><td>838</td><td>2</td><td>2</td></tr>
<tr><td>839</td><td>1</td><td>2</td></tr>
<tr><td>840</td><td>1</td><td>2</td></tr>
<tr><td>841</td><td>2</td><td>2</td></tr>
<tr><td>842</td><td>2</td><td>2</td></tr>
<tr><td>843</td><td>2</td><td>2</td></tr>
<tr><td>844</td><td>2</td><td>2</td></tr>
</table>
Flurochoridone elicited a sensitisation response in all animals and consequently should be classified as skin sensitiser.</td>
<td>Arcellin, G. (2006) (IIA. 5.2.6/02)</td>
</tr>
</table>

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

An open epicutaneous test carried out by Mutter, L.C. (1985) was not considered acceptable due to relevant deviations. Purity of the active substance was not reported and the concentration used in the induction was not the highest well-tolerated systemically to cause mild-to-moderate skin irritation. Besides, the study did not show that active substance penetrates the skin and was systemically available for contact with the immune system due to low concentration used, small area of application, as long as the test sites remained uncovered during the application. According to the results of the study flurochloridone did not induced skin sensitisation but taking into account the deficiencies of the study, sensitisation potential of the active substance cannot be determined with the available information.

A guinea pig maximization study was performed (Arcellin, G., 2006) in order to elucidate the sensitization potential of flurochloridone. In this study, sensitization response was seen in 10/10 animals (100% of response) following challenge with 5% diluted flurochoridone in polyethylene 300 (PEG 300). Concentration of intradermal injection was 25% in PEG 300 and topical induction concentration was 75% in PEG 300. According to the results of the study, flurochloridone showed skin sensitization potential.

10.7.2 Comparison with the CLP criteria

The only acceptable information was provided by a guinea pig maximisation study (Arcellin, G., 2006). According to CLP criteria sub-categories in guinea pig maximisation studies depend on the grade of response and the concentration of the intradermal injection.

- Sub-category 1A is required when positive response of ≥ 30% at ≤ 0.1 % of concentration of the intradermal induction dose or positive response of ≥ 60% at > 0.1% to ≤ 1% concentration of the intradermal induction dose.
- Sub-category 1B is required when positive response of ≥ 30 % to < 60 % at 0.1% to ≤ 1% of concentration of the intradermal induction dose or positive response of ≥ 30 % at > 1% of concentration of the intradermal induction dose.

In the guinea pig maximisation study it was observed a positive response of 100% with an induction intradermal injection concentration of 25%. In absence of results at lower concentrations than 25% it is not possible to rule out responses that would lead to a sub-category 1A. According to CLP criteria, when data is not sufficient for sub-categorisation category 1 is required. Taking into account the available data, category 1 is required for the active substance flurochloridone.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Skin Sens. 1 – H317: May cause an allergic reaction.

10.8 Germ cell mutagenicity

Table 18: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
***In vitro* gene mutation in bacterial**					
Bacterial reverse mutation assay GLP compliant (OECD 471) *Flurochloridone (Batch no. 11083467 and purity 95.5%, 73.5%%trans-isomer, 22.7% cis-isomer).* Solvent: DMSO.	*S. typhimurium* TA98, TA100, TA1535 and TA 1537 E Coli WP uvrA S9-mix from livers of rats induced with phenobarbital and β-naphtoflavone.	Experiment I and II: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate (±S9)	Negative in all strains (±S9)	1st experiment: Cytotoxicity from dose level of 5000 µg/plate: (-S9) in strain TA1537 and (+S9) in strain TA 1537, TA98 and WP2 uvrA 2nd experiment: Cytotoxicity : (-S9) strains TA 1535, T1537 and WP2uvrA (+S9) strains TA1535, TA 1537, TA98 and WP2 uvrA.	Sokolowski A., 2011 (Report No. 11450002). **Acceptable**
Bacterial reverse mutation assay GLP compliant (OECD 471) *Flurochloridone (Batch no. D-FI29 and purity 96.2%).* Solvent: DMSO.	*S. typhimurium* TA98, TA100, TA1535 and TA 1537 E Coli WP uvrA S9-mix from livers of rats induced with phenobarbital and β-naphtoflavone.	Experiment I and II: 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate (±S9)	Negative in all strains (±S9)	1st experiment: Cytotoxicity from dose level of 5000 µg/plate: (-S9): in TA100 (+S9): in all strains except E. Coli WP2 uvrA 2500 µg/plate: TA 1535, T1537, TA98 2nd experiment: Cytotoxicity: (-S9) TA1535 2500 µg/plate TA1537 at 333 µg/plate an above TA98 at 5000 µg/plate (+S9): in all strains except E. Coli WP2 uvrA at 5000 µg/plate	Sokolowski A., 2008 (Report No. 1182503). **Acceptable**
Bacterial reverse mutation assay Not guideline and GLP *Flurochloridone (batch RRC 5276-20-1 and purity 89.7%)* Solvent: DMSO.	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 *Saccharomyces cerevisae* D4 S9 from livers of rats and mouse induced with aroclor 1254 or with phenopharbital	One experiment: 0.1, 1, 10, 100, and 500 µg/plate (±S9)	Negative in all strains in the absence or presence of metabolic activation.	No repeat experiments. No positive control for yeast. One plate only per concentration. No statistics.	Jagannath R., 1978a. T-6350 Only Supplementary information

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
Bacterial reverse mutation assay Not guideline and GLP *Flurochloridone (batch RRC 5276-20-1 and purity 89.7%)* Solvent: DMSO.	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 *Saccharomyces cerevisae* D4 S9 from livers of rats and mouse induced with aroclor 1254 or with phenopharbital	<u>One experiment:</u> 0.1, 1, 10, 100, and 500 µg/plate (±S9)	Negative in all strains in the absence or presence of metabolic activation.	No repeat experiments. No positive control for yeast. One plate only per concentration. No statistics.	Jagannath R., 1978b. T-6153 **Only Supplementary information**
***In vitro* gene mutation in mammalian cells**					
In vitro mammalian cell gene mutation assay GLP compliant (OECD 476) *Flurochloridone (Batch no. D-FI29 and purity 96.2%).* Solvent: DMSO	L5178Y (tk^+/tk^-) mouse lymphoma cells without/with metabolic activation S9 mix from liver of rats induced with phenobarbital and β-naphtoflavone <u>1st experiment:</u> (±S9): Treatment 4 h <u>2nd experiment:</u> (-S9): treatment 24h (+S9): treatment 4 h	<u>1st experiment:</u> (-S9): 6.3, 12.5, 25, 50, 75 and 100 µg/ml (+S9): 6.3, 12.5, 25, 50, 75 and 100 µg/ml <u>2nd experiment:</u> (-S9): 5, 10, 20, 40, 60, 80 and 100 µg/ml (+S9): 10, 20, 40, 60, 80 and 100 µg/ml	-S9: Negative +S9: Negative	<u>1st experiment:</u> Cytotoxicity (±S9)at 100 µg/ml <u>2nd experiment:</u> Cytotoxicity (-S9) at 80 µg/ml and above	Wollny H., 2008 (Report No. 1182502). **Acceptable**
In vitro mammalian cell gene mutation assay Not guideline and GLP *Flurochloridone (purity 89.7% and batch not reported).* *Solvent: DMSO*	L5178Y (tk^+/tk^-) mouse lymphoma cells S9 from livers of males rats with aroclor 1254 <u>Treatment</u>: 4 h	(-S9): 40, 60, 120 and 160 µg/ml (+S9): 20, 40, 60 and 80 µg/ml	(-S9): Negative (+S9): Increase in the number of mutant colonies at 60 µg/ml (increase was by less than factor 2.5 and there was no dose-relationship).	(+S9): Cytotoxicity at 160 µg/ml. (+S9): Cytotoxicity from dose level of 60 µg/ml and above.	Matheson W., 1978 T-6348 **Only supplementary information**

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
***In vitro* chromosome aberrations in mammalian cells**					
In vitro mammalian chromosome aberration assay GLP compliant (OCDE 473) *8 Flurochloridone (Batch no. D-FI29 and purity 96,2%).* Solvent: DMSO	V79 cells of Chinese hamster without/with metabolic activation S9-mix from liver of rats induced with phenobarbital and β-naphtoflavone. 1st experiment: (±S9): Treatment 4 h 2nd experiment: (-S9): treatment 18h (+S9): treatment 4 h Observation: all 18 h	1st experiment: Preliminary (±S9): 12.7-3245 µg/ml Main (±S9): 12.7, 25.4 and 50.7µg/ml 2nd experiment: Preliminary (-S9): 0.8 -202.8 µg/ml (+S9): 3.2- 202.8 µg/ml Main (±S9): 12.7, 25.4 and 50.7µg/ml	Chromosome aberration: EQUIVOCAL -Experiment I (-S9): increase in the number of aberrant cells at 25.4 and 50.7 µg/ml (s.s.) Experiment II: (-S9): increment in the number of aberrant cell at 12.7 µg/ml (s.s.) (+S9): increment in the number of aberrant cell at 12.7 µg/ml (not dose-dependency) Polyploid cells -Experiment I (±S9): increase in the number polyploidy cells at 12.7, 25.4 and 50.7 µg/ml. Not reconfirmed in the second experiment.	Precipitation at (-S9): 202.8 µg/ml and above. (+S9): 101.4 µg/ml and above Toxicity: No toxicity was observed except at 50.7 µg/ml in 2nd (-S9) experiment relative MI decreased to 43.1%. Experiment II (+S9) 5.6% poliploid cells were found in the solvent control	Hoffmann H., 2008 (Report 1182501) Acceptable
In vitro micronucleus test in Chinese hamster V79 cells GLP compliant (OCDE 487) *8 Flurochloridone (Batch no. 11083467 and purity 95.5% (73.5% of trans isomer and 22.7% cis isomer).* Solvent: DMSO	V79 cells of Chinese hamster without / with metabolic activation S9-mix from liver of rats induced with phenobarbital and β-naphtoflavone. 1st experiment: (±S9): Treatment 4 h Observation: 24h 2nd experiment: (-S9): treatment 24h (+S9): treatment 4h Observation: 24h	1st experiment: Preliminary (-S9): 2.5-200 µg/ml (+S9): 1.6-3268 µg/ml Main (-S9): 80, 100 and 200 µg/ml (+S9): 6.4, 12.8, 51.1 and 102.1 µg/ml 2nd experiment: Preliminary (-S9): 0.1-200 µg/ml (+S9): 2.5-200µg/ml Main (-S9): 6.3, 12.5 and 25 µg/ml (+S9): 10, 20 and 120 µg/ml	Chromosome aberration: QUESTIONABLE -Experiment I (-S9): increase in the number of micronuclei at 100 and 200 µg/ml (s.s.) Not dose-dependent Reduced proliferation index and precipitation at these concentrations. Experiment II: (-S9): increase in the number of micronuclei at 25 µg/ml (s.s.)	Experiment I: Precipitation at (-S9): 100 µg/ml and above. (+S9): 12.8 µg/ml and above (-S9): 100 and 200 µg/ml: reduced proliferation index. Experiment II Precipitation +S9: 20µg/ml and above	Bohnenberger S., 2012 (Report 1450001) Acceptable

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
DNA Damage					
Unscheduled DNA synthesis in cultured Not guideline and GLP Flurochloridone (90% purity and batch nº L-SC-0402	*In vitro* culture HeLa cells S9 from livers of rats induced with aroclor 1254	1st experiment (±S9): 1, 10, 100, 500 and 1000 µg/ml 2nd experiment (-S9):1, 5, 10, 50, and 100 µg/ml (+S9):1, 10, 50, 100 and 200 µg/ml	1st experiment (-S9): slight increases of means cpm at 10 µg/ml. (+S9): slight increases of means cpm at 1 µg/ml. No doses dependent 2nd experiment: NEGATIVE	Cytotoxicity (-S9): at 50 µg/ml and above. (+S9): at 500 µg/ml and above.	Pirovano R., 1986 T-M971-/I-2 Only supplementary information
Repair DNA strand breaks and DNA repair activity Not guideline and GLP Flurochloridone (86.3% purity and batch nº 7253-12-1 (EHC-0525-42)	Human foreskin fibroblasts Alkaline sucrose velocity sedimentation analysis and Nick translation assay	1mg/ml (30 minutes) With or without subsequent incubation for 2h	NEGATIVE	Higher concentration than 1 mg/mL cause precipitation and detachment of cells from the dishes.	Snyder, Ph. D., 1985 T-12058 Only supplementary information
Transformation of cells					
Transformation of cells Not guideline and GLP Flurochloridone (purity and batch not reported). Solvent: DMSO	BALB/3T3 Cells	0.625, 1.25, 2.50, 5 and 10 µg/ml	Negative	Preliminary study: 10 µg/ml, the highest non-toxic concentration.	Matheson, P.D., 1978 T-6424 Only supplementary information

In vivo, somatic cells					
Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
In vivo mammalian erythrocyte micronucleus test GLP compliant (OECD 474) *8 Flurochloridone (Batch no. D-157 and purity 95.0%).* *Vehicle: 30% DMSO- 70% PEG 400*	Bone marrow cells from male rat Wistar (7 animals per dose group)	Single dose (oral) levels of 0, 500, 1000 and 2000 mg/kg bw by (oral) (samples at 24 h) and 2000 mg/kg bw (samples 48 h)	Negative	No toxic reaction was observed	Roth M., 2012 (Report 1464700) Acceptable
In vivo mammalian bone marrow micronucleus test Not guideline and GLP Flurochloridone (purity 86.3% batch WRC 7253-12-1, EHC-0525-42). Vehicle: 10% ethanol in corn oil (v/v)	Bone marrow cells from male and female CD1 mice. (5 animals/sex/ dose/sacrifice time)	Experiment I: 0, 1250, 2500 and 5000 mg /kg bw by oral gavage. Experiment I: 0, 1250, 2500 and 3500 mg/kg bw by oral gavage in males. Bone marrow samples at 24, 48 and 72h.	Experiment I males: Incremented micronuclei (s.s.) at 2500 mg/kg bw and sacrificed at 24 and 48 h (Not confirmed in the second experiment).	Positive control group gave negative results in females in the experiment I.	Majeska J., 1985. (Report Nº T-11916) Only supplementary information

s.s.: statistically significant

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Flurochloridone was tested for its genotoxic potential using a battery of *in vitro* (bacterial assay for gene mutation, chromosome aberrations in mammalian cells, gene mutation in mammalian cells, DNA damage and transformation of cell) and *in vivo* tests (bone marrow micronucleus assay). The weight of the evaluation fell on the six well conducted studies with test substance well characterized (known purity and batch), performed according to OECD guidelines and GLP compliant. Additionally, other genotoxicity studies are available. These studies were non-GLP compliant and pre-guideline and were evaluated and included in DAR but considered only as supplementary information.

Flurochloridone genotoxicity *in vitro* and *in vivo* studies are summarised Table 18.

10.8.1.1 *In vitro* data

***In vitro* genotoxicity testing - Bacterial assay for gene mutation**

Title	***Salmonella Typhimurium* and *Escherichia Coli*. Reverse mutations assay with Flurochloridone Technical**
Author (s) (year):	Sokolowski A. (2011)
Guideline	OECD guideline 471 "Bacterial Reverse Mutation test" adopted July 21, 1997.
System	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia Coli* strain WP2 uvrA. S9-mix from livers of rats induced with phenobarbital and β-naphtoflavone
GLP	Yes
Purity:	95.5% (73.5% trans-isomer, 22.7% cis-isomer)
Dose levels	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg /plate in all strains (±S9).
Study acceptable	

Executive Summary

Flurochloridone was evaluated for its ability to induce mutations in the histidine operon of *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *Escherichia Coli* strain WP2 uvrA. Dimethylsulphoxide (DMSO) was used to solve the test substance. The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration (3, 10, 33, 100, 333, 1000, 2500 and 5000 µg /plate), including the controls, was tested in triplicate. Appropriate reference mutagens were used as positive control.

Cytotoxicity: Toxic effects (below the indication factor of 0.5), were observed at the following concentrations (µg/plate) (Table 19).

Table 19: Concentration (µg/plate) which cytotoxicity.

Strain	Experiment I		Experiment II	
	-S9	+S9	-S9	+S9
TA 1535	/	/	5000	5000
TA 1537	5000	2500, 5000	2500, 5000	2500,5000
TA 98	/	5000	/	5000
TA 100	/	/	/	/
WP2uvrA	/	5000	/	5000

/: no toxic effects.

Precipitation was noted at ≥1000 µg/plate in all strains in presence and in absence of S9 (both experiments).

No substantial increase in revertant colony numbers in any of the five tested strains was observed with flurochloridone technical at any concentration, neither in the presence nor in the absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates increasing concentration in the range below the generally acknowledged border of biological relevance. The positive controls resulted in clearly increases values in all experiments.

Conclusion: Under the experimental conditions reported, flurochloridone did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

***In vitro* genotoxicity testing - Bacterial assay for gene mutation**

Title	***Salmonella Typhimurium* and *Escherichia Coli*. Reverse mutations assay with Flurochloridone Technical**
Author (s) (year):	Sokolowski A. (2008)
Guideline	OECD guideline 471 "Bacterial Reverse Mutation test" adopted July 21, 1997.
System	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia Coli* strain WP2 uvrA. S9-mix from livers of rats induced with phenobarbital and β-naphtoflavone
GLP	Yes
Purity:	96.2%
Dose levels	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg /plate in all strains (±S9).
Study acceptable	

Executive Summary

Flurochloridone was evaluated for its ability to induce mutations in the histidine operon of *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 and *Escherichia Coli* strain WP2 uvrA. Dimethylsulphoxide (DMSO) was used to solve the test substance. The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration (3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate), including the controls, was tested in triplicate with and without liver microsomal activation. Appropriate reference mutagens were used as positive control.

The plates incubated with the test item showed reduced background growth at higher concentrations in nearly all strains.

Cytotoxicity: Toxic effects (below the indication factor of 0.5), were observed at the following concentrations (µg/plate) (Table 20).

Table 20: Concentration (µg/plate) which cytotoxicity.

Strain	Experiment I		Experiment II	
	-S9	+S9	-S9	+S9
TA 1535	/	2500, 5000	2500	5000
TA 1537	/	2500, 5000	333-5000	5000
TA 98	/	2500, 5000	5000	5000
TA 100	5000	5000	/	5000
WP2uvrA	/	/	/	/

/: no toxic effects.

Precipitation was noted at ≥2500 µg/plate in all strains in presence and in absence of S9 (both experiments).

No substantial increase in revertant colony numbers in any of the five tester strains was observed with flurochloridone Technical at any concentration, neither in the presence nor in the absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates increasing concentration in the range below the generally acknowledged border of biological relevance. The positive controls resulted in clearly increases values in all experiments.

Conclusion: Under the experimental conditions reported, flurochloridone did no induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Additional studies on genotoxicity in bacterial and yeast

Other two studies on bacterial mutagenicity were available (Jagannath R., 1978a; Jagannath R., 1978b). The assays were non-GLP compliant and pre-guideline. Purity of flurochloridone was 89.7%.

The two studies had several deficiencies: one instead of triplicate planting was performed for each experiment; each experiment was not repeated; no range-finding toxicity study was performed; no historical control data was presented; no statistical analysis was performed; no positive control was used for S*accharomyces cerevisae* D4 strain.

In two studies no mutagenic activity was reported both in the presence or in the absence of metabolic activation in tested *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and D4 of *Saccharomyces cerevisae* at dose levels from 0.1 to 500 µg flurochloridone per plate.

These studies are considered as supplementary information.

***In vitro* gene mutation in mammalian cells**

Title	**Cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells with flurochloridone technical**
Author (s) (year):	Wollny H., 2008
Guideline	OECD guideline 476 "In vitro mammalian cell gene mutation test" adopted May 19, 2000
System	L5178Y (TK^{+}/TK^{-}) mouse lymphoma cells S9 from livers of rats induced with phenobarbital and β-naphthoflavone
GLP	Yes
Purity:	96,2% (proportion of isomers is not indicated in the report)
Dose levels	1st exp : Without S9: 6.3, 12.5, 25, 50, 75 and 100 µg/ml. With S9: 6.3, 12.5, 25, 50, 75 and 100 µg/ml. 2nd exp.: Without S9: 5, 10, 20, 40, 60, 80 and 100 µg/ml With S9: 10, 20, 40, 60, 80 and 100 µg/ml
Study acceptable	

Flurochloridone was examined for genetic activity in the L5178Y $TK^{+/-}$ mouse lymphoma multiple endpoint test in the absence and presence of metabolic activation. DMSO (dimethylsulfoxide) was used as solvent. Point mutation is measured in this test system by examining the induction of trifluorothymidine (TFT) resistance by forward gene mutation at the thymidine kinase (TK) locus. The test was performed with and without metabolic activation (S9 mix from the liver of phenobarbital and β-naphthoflavone induced male Wistar HsdCpb:WU rats). Two independent mutagenicity experiments were carried out.

Cytotoxicity: relevant toxic effects indicated by a relative cloning efficiency 1 or a relative total growth of less than 50% of survival in both parallel cultures were observed at the maximum concentration of 100 µg/ml in experiment I (with and without metabolic activation). In experiment II toxic effects occurred at 80 µg/ml and above without metabolic activation (24h treatment).

No increase in mutant frequency was found in both experiments with and without metabolic activation. The historical range of solvent controls was occasionally exceeded but without statistical significance. The linear regression analysis did not indicate a significant dose dependent trend of the mutation frequency ($p<0.05$) in any of the experimental groups.

The positive controls demonstrated in both assays sensibility of the test system.

Conclusion: Under the experimental conditions reported flurochloridone did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y (TK^+/TK^-) in the absence and presence of metabolic activation. Therefore, flurochoridone technical is considered to be non-mutagenic in this mouse lymphoma assay.

Additional study on mutation in mammalian cells

In other study (Matheson W., 1978) flurochloridone was assayed for the potential to induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y (TK^+/TK^-) in the absence and presence of metabolic activation. The assays were non-GLP compliant and pre-guideline. Purity of flurochloridone was 89.7%. The concentrations used for flurochloridone were 40, 60, 80, 120 and 160 µg/ml in absence of metabolic activation and 40, 60 and 80 µg/ml in presence of metabolic activation.

In the main study marked cytotoxicity was observed at 160 µg/ml and at 60 µg/ml and above in absence and presence of S9, respectively. Slight increment in the number of mutant colonies was found in presence of S9 at 60 µg/ml. However this increase did not exceed 2.5 times the spontaneous control values and there was no dose-relationship. Besides, this was observed only in areas of less than two relative growths. Positive controls induced a relevant increase in mutant frequencies

Flurochloridone did not induce gene mutations at the mouse lymphoma cells L5178Y (TK^+/TK^-) under the conditions of this study.

This study is considered as supplementary information.

Chromosome aberration *in vitro*

Title	**In vitro chromosome aberration test in Chinese hamster V79 cells with flurochloridone technical**
Author (s) (year):	Hoffmann H., 2008
Guideline	OECD guideline 473 "In vitro mammalian chromosome aberration test" adopted 21 July 1997.
System	V79 cells of Chinese hamster S9 from livers of rats induced with phenobarbital and β-naphthoflavone
GLP	Yes
Purity:	96.2 %
Dose levels	Experiment I: Preliminary: (-/+S9) 12.7, 25.4, 50.7, 101.4, 202.8, 405.6, 811.3, 1622.5 and 3245 µg/ml. Main: (±S9) 12.7, 25.4 and 50.7µg/ml Experiment II: (-S9): 0.8, 1.6, 3.2, 6.4, 12.7, 25.4, 50.7, 101.4, and 202.8 µg/ml (+S9): 3.2, 6.4, 12.7, 25.4, 50.7, 101.4, and 202.8 µg/ml Main: (±S9) 12.7, 25.4 and 50.7µg/ml
	Study acceptable

Executive Summary

Flurochloridone was assessed for its potential to induce structural chromosome aberrations in V79 cells of Chinese hamster *in vitro* in the absence and the presence of metabolic activation by S9 mix.

In Experiment I test substance concentrations between 12.7 and 3245.0 µg/mL (approx. 10 mM) (±S9) were chosen for the evaluation of cytotoxicity. Using reduced cell numbers as an indicator for toxicity, strong toxic effects were observed after treatment with 101.4 µg/mL and above with and without metabolic activation. In Experiment II, concentrations between 0.8 and 202.8 µg/mL (–S9) as well as 3.2 and 202.8µg/mL (+S9) were applied.

A chromosome damaging effect was examined by staining the cells and evaluating the metaphases (100 metaphases from each duplicate culture). Cytotoxicity was determined by means of relative cell suspension growth and by means of mitotic index (number of mitosis per 500 cells).

Cytotoxicity: Test item precipitation was observed at concentration of 202.8µg/ml and above in the absence of S9 mix in Experiment I and II as well as at 101.4µg/mL and above in the presence of S9 mix in Experiment I. Concentrations showing clear cytotoxicity were not evaluated for cytogenetic damage, thus no cytotoxicity was observed in the evaluated concentrations, except in experiment II (-S9) after continuous treatment with 50.7 µg/ml where a reduced mitotic index of 43.1% of control was observed indicating cytotoxicity.

Table 21: Summary of results of the chromosome aberration study with flurochloridone

Flurochloridone [µg/mL]	**Meta-phases per culture**	**Cell No [% control]**	**Mitotic index [% control]**	**Aberrant metaphases [%]**			**Endomi-totic cells [%]**	**Poly-ploid cells [%]**
				+ gaps	**- gaps**	**with exchanges**		
-S9, 1st experiment (4 h exposure)								
Solvent control	100	100	100	0.5	0.0	0.0	0.0	2.9
12.7	100	106	100	1.0	0.5	0.0	0.0	3.9
25.4	100	95	97	3.0	**2.5***	1.5	0.2	**4.8***
50.7	100	82	108	3.0	**2.5***	0.0	**1.9***	**5.0***
Positive control	100	-	86	16.0	14.5*	3.5	0.0	4.0
-S9, 2nd experiment (18 h exposure)								
Solvent control	100	100	100	1.5	1.0	0.0	0.0	2.3
12.7	200	115	93	3.5	**3.3***	0.3	0.0	2.1
25.4	100	98	74	3.5	3.0	0.0	0.0	1.6
50.7	100	57	43	2.5	2.5	0.5	0.0	1.8
Positive control	100	-	73	12.5	11.5*	3.0	0.0	2.1
+S9, 1st experiment (4 h exposure)								
Solvent control	100	100	100	3.0	3.0	0.0	0.2	3.5
12.7	100	102	105	3.5	3.0	0.5	0.1	3.3
25.4	100	102	96	1.5	1.5	0.0	**2.1***	4.0
50.7	100	95	90	5.0	**4.0**	1.5	**1.4***	4.7
Positive control	100	-	43	12.0	11.5*	5.5	0.0	4.1
+S9, 2nd experiment (4 h exposure)								
Solvent control	100	100	100	2.0	2.0	0.5	0.0	5.6
12.7	200	67	100	6.3	**5.5***	1.0	0.3	4.3
25.4	100	85	98	5.5	3.5	1.0	**0.4***	6.4
50.7	100	71	127	5.5	4.0	0.0	**0.4***	5.9
Positive control	50	-	90	40.0	38.0*	7.0	0.0	5.1

Experiment I: Concentrations of 25.4 and 50.7µg/mL (–S9) induced statistically significant increases in the number of aberrant cells excluding gaps (2.5% each). However these values lay within the laboratory's historical control data range (0.0-4.0% aberrant cells excluding gaps).

Experiment II: two statistically significant increases in the number of aberrant cells were observed. At concentration of 12.7 µg/mL (-S9) a value of 3.3% aberrant cells excluding gaps were observed. This value is within the laboratory's historical control data range (0.0-3.5%). In the presence of S9 mix the concentration of 12.7 µg/mL induced 5.5% aberrant cells excluding gaps. This value is out the laboratory's historical control data range (0.0-4.0%). However, the next two higher concentrations of 25.4 and 50.7µg/mL did not increase the aberration frequencies and no dose-dependency was observed.

In Experiment I Flurochloridone induced a dose-dependent increase in the number of polyploid cells (-S9: 3.9, 4.8, 5.0%; +S9: 3.3, 4.0, 4.7%). This observation was not reconfirmed in the second experiment in the absence of S9. With S9, 5.6% polyploid cells were found in the solvent control. This value was higher than the laboratory's historical solvent control data (0.6-4.3% polyploidy cells).

Conclusion

Under the experimental conditions reported, the results of this study are not clearly negative and they might be considered as equivocal.

***In vitro* mammalian erythrocyte micronucleus test**

Title	***In vitro* micronucleus in Chinese hamster V79 cells with flurochloridone technical**
Author (s) (year):	Bohnenberger S., 2012
Guideline	OECD guideline 487 "*In vitro* mammalian cell micronucleus test (Mnvit)" adopted 21 July 1997.
System	V79 cells of Chinese hamster S9 from livers of rats induced with phenobarbital and β-naphthoflavone
GLP	Yes
Purity:	95.5 % (w/w) 73.5% trans-isomer, 22.7% cis-isomer
Dose levels	Experiment I: Preliminary: (-S9): 2.5, 5, 10, 20, 40, 50, 60, 80, 100 and 200 µg/ml (+S9): 1.6, 3.2, 6.4, 12.8, 25.5, 51.1, 102.1, 204.1, 408.5, 817, 1634 and 3268 µg/ml Main: (S9): 80, 100 and 200 µg/ml; (+S9): 6.4, 12.8, 51.1 and 102.1 µg/ml 2nd experiment: Preliminary: (-S9): 0.1, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5, 25, 50, 100 and 200 µg/ml (+S9): 2.5, 5, 10, 20, 40, 80, 100, 120, 140, 160, 180 and 200 µg/ml Main: (-S9): 6.3, 12.5 and 25 µg/ml; (+S9): 10, 20 and 120 µg/ml
	Study acceptable

Executive Summary

Flurochloridone was assessed for its potential to induce structural micronuclei in V79 cells of Chinese hamster *in vitro* in the absence and the presence of metabolic activation by S9 mix.

In Experiment I test substance concentrations between 1.6 and 3268 µg/ml (approx. 10mM) (±S9) were applied for the evaluation of cytotoxicity. Cytotoxic effects were observed in the absence of S9 mix at 200 µg/ml and above and in the presence of S9 mix at 204.1 µg/mL and above. In Experiment II, concentrations between 0.1 and 200 µg/ml (-S9) as well as 2.5 and 200 µg/mL (+S9) were applied.

In the Experiment I, visible precipitation of the test item in the culture medium was observed at 100 µg/ml and above in the absence of S9 and at 12.8 µg/ml and above in the presence of S9 mix. In the experiment II, precipitation occurred in the presence of S9 mix at 20 µg/ml and above.

In Experiment I in the presence of S9 mix and in Experiment II in the absence and presence of S9 mix, concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage. In the Experiment I in the absence of S9 mix clear cytotoxicity of approx. 55% of control was observed at the highest evaluated concentration.

Table 22: Summary of results of the micronucleus test with flurochloridone

Experiment	Exposure (h)	Concentration (µg/ml)	S9	Proliferation index	Precipitation	Micronucleus cell (%)
II	**4**	Solvent control	-	2.65		0.80
		80	-	2.19	-	0.90
		100	-	**1.72**	+	**3.60***
		200	-	**1.45**	+	**2.15***
		Positive control	-	2.65	-	13.30*
	4	Solvent control	+	2.58		1.15
		6.4	+	2.60	-	0.85
		12.8	+	2.31	+	100
		51.1	+	2.65	+	0.90
		102.1	+	2.19	+	0.55
		Positive control	+	2.09	-	17.85*
II	**24**	Solvent control	-	2.56		0.20
		6.3	-	2.43	-	0.55
		12.5	-	2.29	-	0.55
		25	-	2.13	-	**0.70***
		Positive control	-	2.35	-	12.35*
	4	Solvent control	+	1.79		0.60
		10	+	1.86	-	0.70
		20	+	1.85	+	0.15
		120	+	1.70	+	1.05
		Positive control	+	1.54	-	20.75*

*Number of micronucleated cells statistically significantly higher than corresponding control values (Chi square test).

In the Experiment I without S9 mix, the two highest concentrations were statistically significant (3.6% and 2.15% micronucleated cells). However, this finding is not dose-dependent and reduced proliferation index and precipitation of the test item was observed at these doses. In Experiment II without S9 mix one single statistical significant value was observed with 25 µg/ml (0.7% micronucleated cells). In the presence of S9 mix no relevant mutagenic effects were observed.

Conclusion

Under the experimental conditions reported, Flurochloridone technical induced micronuclei in V79 cells (Chinese hamster cell line) *in vitro* in the absence of metabolic activation. This finding was observed at doses with cytotoxicity. Therefore the results are not clearly positive and might be considered questionable.

DNA Damage

The DNA damage was tested in two *in vitro* studies:

In one study (Pirovano R., 1986), flurochloridone was assessed to induce DNA damage and unscheduled DNA synthesis in cultured Hela cells in the absence and the presence of metabolic activation by S9 mix at dose levels of up to 1-1000 µg/ml of flurochloridone per plate (triplicates for each concentration with and without hydroxyurea). There is no available OECD Guideline. The purity of the test compound was 90%. Markedly reduced replicative DNA synthesis as indicator for cytotoxicity was seen at flurochloridone concentrations ≥50 µg/ml and ≥500 µg/ml in the HeLa cells in absence and presence of metabolic activation, respectively. In the 1st experiment slight increases of means cpm (+HU) not statistically significant was observed at dose levels of 10 µg/ml in absence of metabolic activation and 1 µg/ml in presence of metabolic activation. No dose-dependency was observed. This observation was not reconfirmed in the second experiment.

In the other study (Snyder Ph.D., 1985), the objective was to assess to potential of flurochloridone to induce DNA stand beak in human foreskin fibroblast (HSBP) *in vitro.* There is no available OECD Guideline. The purity of the test compound was 86.3%. The human fibroblasts were exposed 30 minutes to the test substance in the absence of metabolic activation. The dose level selected was 1 mg/mL. Higher concentrations than 1 mg/ml caused precipitation and detachment of cells from the dishes.

No indication for induction of DNA damage (breaks) or DNA repair by flurochloridone treatment was detected in this *in vitro* assay with human fibroblasts.

These studies are considered as supplementary information.

Cell transformation

Flurochloridone was assessed for its potential to induce malignant transformation of BALB/3T3 cells *in vitro* in the absence of metabolic activation by S9 mix (S9 from induced rat liver appears to be toxic to BALB/3T3 cells). There is no available OECD Guideline. The purity and batch specifications were unknown. The doses tested were 0.625, 1.25, 2.50, 5 and 10 µg/ml.

Under the conditions of this assay, Flurochloridone did not induce a significant increase in morphological transformation in BALB/3T3 cells.

This study is considered as supplementary information.

10.8.1.2 *In vivo* data

***In vivo* studies in mammalian somatic cells**

***In vivo* mammalian erythrocyte micronucleus test**

Title	**Micronucleus assay in bone marrow cells of the rat with flurochloridone tech.**
Author (s) (year):	Roth M. (2012)
Guideline	OECD guideline 474 "Mammalian erythrocyte micronucleus test", adopted July 21, 1997
System	Bone marrow cells from males rat Wistar.
GLP	Yes
Purity:	95.0%
Dose levels	Single dose by oral route 500, 1000 and 2000 mg/kg p.c. (sampling of bone marrow: 24h) 2000 mg/kg p.c. (sampling of bone marrow: 48h)
Study acceptable	

Executive summary

In a bone marrow micronucleus assay using Wistar rat, flurochloridone technical was administered perorally to groups of seven males per dose and sampling time. Male animals only were used in the main experiments since no substantial gender specific differences in toxicity were observed in the pre-experiment or preceding toxicity studies. Negative control group was treated with the vehicle only (30% DMSO/ 70% PEG 400) and positive control groups were treated with cyclophosphamide (20 mg/kg b.w).

Bone marrow cells were collected for micronuclei analysis 24 h and 48 h after a single administration of flurochloridone.

In order to describe a cytotoxic effect due to the treatment with flurochloridone the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample. Polychromatic erythrocytes (PCEs) (at least 2000 per animal) were scored for micronuclei.

After treatment with the test item the number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the mean value of PCEs per 2000 erythrocytes of the vehicle control, thus indicating that flurochloridone did not exert any significant cytotoxic effects in the bone marrow. Exposure of the bone marrow was demonstrated in toxicokinetic studies in rats.

Proportion of polychromatic erythrocytes (PCE): the PCE values observed in the groups treated were comparable to negative control. The mean values of micronuclei observed after treatment with flurochloridone were below or near to the value of the vehicle control group and within the historical vehicle control range. Additionally no dose-dependency was observed.

Positive control treatment showed a statistically significant increase of induced micronucleus frequency.

Conclusion

Under the experimental conditions reported, flurochloridone did not induce micronuclei in the bone marrow cells of the rat.

Additional studies mammalian erythrocyte micronucleus test

In the other study (Majeska J., 1985) flurochloridone was assayed for the potential to induce micronuclei in bone marrow cells in CD1 mice *in vivo*. There is no available OECD Guideline. The purity of the test compound was 86.3%. Flurochloridone was administered per oral gavage to groups of males and females animals at target doses of 1250, 2500 and 5000 mg /kg in the Experiment I and 1500, 2500 and 3500 mg/kg in the Experiment II. Sampling of the bone marrow was done 24, 48 and 72 hours after treatment.

There were statistically significant increases in micronucleated PCEs in males at 1250 mg/kg after 48 hours and at 2500 mg/kg after 24 and 48 hours. This observation was not reproducible in the Experiment II. There was no significant increase in micronucleated PCEs in female mice.

Conclusion

Under the experimental conditions reported, flurochloridone did not induce micronuclei in the bone marrow cells when administered by oral gavage to mice.

This study is considered as supplementary information.

10.8.1.3 Human information

No data available.

10.8.1.4 Other relevant information

No data available.

10.8.1.5 Summary and discussion of mutagenicity

Flurochloridone was tested for its genotoxic potential using a battery of *in vitro* (bacterial assay for gene mutation, chromosome aberrations in mammalian cells, gene mutation in mammalian cells, DNA damage and transformation of cell) and *in vivo* tests (bone marrow micronucleus assay). The weight of the evaluation fell on the six well conducted studies with test substance well characterized (known purity and batch), performed according to OECD guidelines and GLP compliant. Additionally, other genotoxicity studies are available. These studies were non-GLP compliant and pre-guideline and were evaluated and included in DAR but considered only as supplementary information.

Flurochloridone was tested in four bacterial assays for gene mutation. No mutagenic activity was noted in the strains tested in the absence or presence of a metabolic activation system.

Similarly, no increases in gene mutagenic were seen in the mouse lymphoma L5178Y (TK^+/TK^-) in the absence or presence of a metabolic activation system.

Assays on chromosomal aberrations *in vitro* showed equivocal results. In a study (Hoffman H., 2008) with flurochloridone (batch and purity known and considered appropriate) performed according to OECD guideline 473, test substance induced structural chromosomal aberrations in the V79 Chinese hamster cells.

In the experiment I, statistically significant increases in the number of aberrant cells were observed at two higher doses in the absence of metabolic activation. However these values lay within the laboratory's historical control date range. In the experiment II, statistically significant increases in the number of aberrant cells were observed at 12.7 µg/ml without and with metabolic activation. However, the next two higher concentrations did not increase the aberration frequencies and no dose-dependency was observed. Furthermore, in the experiment I, Flurochloridone induced a dose-dependent increase in the number of polyploidy cells. This observation was not reconfirmed in the experiment II. Therefore, the results of this study are not clearly negative and might be considered equivocal.

A recent study (Bohnenberger S., 2012) with Flurochloridone (batch and purity known) performed according OECD guideline 487, revealed questionable results in the micronucleus test in Chinese hamster V79 cells in the absence of metabolic activation in the experiment I. However this finding is not dose-dependent and precipitation and reduced and proliferation index of the test item was observed at these doses. In the experiment II, statistical significant value was observed at the highest dose in the absence of metabolic activation. Therefore, the results of this study are not clearly positive and might be considered questionable.

In relation to DNA damage *in vitro,* negative results were obtained in DNA breaks study in diploid human fibroblast cells and in a UDS assay in HeLa cells.

An *in vitro* mammalian transformation assay was performed in BALB/3T3 cells in the absence of S9 where Flurochloridone gave negative results.

Two *in vivo* genotoxicity assays in mammalian somatic cells are available.

A new study (Roth M., 2012) with flurochloridone (batch and purity known and considered appropriate) performed according to OECD guideline 474, showed negative results in the micronucleus test in the bone marrow cells of rat at dose levels ranged from 500 to 2000 mg/kg bw.

Negative results were also observed in other *in vivo* bone marrow micronucleus test (Majeska, J.B., 1985).

Conclusion

Flurochloridone did not induce mutation in bacterial and mammalian cell (mouse lymphoma cells L5178Y TK^+/TK^-) in the absence and presence of metabolic activity. Furthermore, equivocal result was observed *in vitro* chromosome aberrations test in V79 cells of Chinese hamster. *In vitro* well conducted test for micronuclei in V79 cells of Chinese hamster showed positive results in the absence of metabolic activation, though this finding was observed at doses with cytotoxicity and not dose-dependent (experiment I). Therefore the result of this study is considered questionable. Moreover, negative results were found in two *in vivo* studies on micronucleus test in bone marrow cells of the rat and the mouse.

Based on the results of all studies provided, the weight of evidence suggests no *in vivo* genotoxic potential of flurochloridone. Therefore flurochloridone does not warrant classification for mutagenicity according to CLP criteria.

10.8.2 Comparison with the CLP criteria

According to CLP classification of a substance as mutagen Category 1B is based on the following criteria.

- Positive result (s) from *in vivo* heritable germ cell mutagenicity test in a mammals; or
- Positive result (s) for *in vivo* somatic cell mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or
- Positive result from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cell of exposed people.

Classification into category 2 according to CLP is required for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

  Somatic cell mutagenicity tests in vivo, in mammals.

  Other *in vivo* somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive *in vitro* mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

In conclusion: Flurochloridone did not induce mutation in bacterial and mammalian cell (mouse lymphoma cells L5178Y TK^+/TK^-) in the absence and presence of metabolic activity. Furthermore, equivocal result was observed *in vitro* chromosome aberrations test in V79 cells of Chinese hamster. *In vitro* well conducted test for micronuclei in V79 cells of Chinese hamster showed positive results in the absence of metabolic activation, though this finding was observed at doses with cytotoxicity

and not dose-dependent (experiment I). Therefore the result of this study is considered questionable. Moreover, negative results were found in two *in vivo* studies on micronucleus test in bone marrow cells of the rat and the mouse.

Based on the results of all studies provided, the weight of evidence suggests no *in vivo* genotoxic potential of flurochloridone. Therefore flurochloridone does not warrant classification for mutagenicity according to CLP criteria.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

CLP: A classification is not required

10.9 Carcinogenicity

Table 23: Summary table of animal studies on carcinogenicity

NOAELS have been copied from the DAR for information only

† *Increase/decrease relative to controls denoted by ↑/↓; *p<0.05; **p<0.01; n.s.: non-significant*

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no./group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Long-term toxicity and carcinogenicity study in rats (2-years)

Laboratory: Stauffer Enviromental Health Center

B.33

GLP: Yes

Rat strain: Sprague Dawley

60 rats/sex/dose except for dose of 400 ppm with 70 rats/sex.
Interim sacrifice at 12 months was performed in 10 male/sex/dose except for dose of 400 ppm with 20 rats/sex.

Study acceptable

Deviations: none</td>
<td>Purity: 86.3%
Proportion of isomers not indicated.

Oral (diet): test item was dissolved in acetone (0.05% w/w) and mixed with diet.

Doses: 0, 40, 100 and 400 ppm equivalent to: Males: 1.5, 3.9 and 15.7 mg/kg bw/day. Females: 2.0, 4.8 and 19.3 mg/kg bw/day.</td>
<td>Mortality: not affected by treatment.
<table>
<tr><th>Sex</th><th>Dose level</th><th>N</th><th>Found dead</th><th>Human or Moribund sacrifice</th><th>Incidence of unscheduled deaths</th></tr>
<tr><td rowspan="4">Male</td><td>0</td><td>60</td><td>13</td><td>14</td><td>45%</td></tr>
<tr><td>40</td><td>60</td><td>17</td><td>11</td><td>47%</td></tr>
<tr><td>100</td><td>60</td><td>21</td><td>8</td><td>48%</td></tr>
<tr><td>400</td><td>70</td><td>13</td><td>15</td><td>40%</td></tr>
<tr><td rowspan="4">Female</td><td>0</td><td>60</td><td>12</td><td>18</td><td>50%</td></tr>
<tr><td>40</td><td>60</td><td>9</td><td>19</td><td>47%</td></tr>
<tr><td>100</td><td>60</td><td>7</td><td>22</td><td>48%</td></tr>
<tr><td>400</td><td>70</td><td>7</td><td>25</td><td>41%</td></tr>
</table>
Clinical signs: no treatment related clinical signs.
<u>400 ppm</u>
<u>Bodyweight and food consumption</u>:
▪ (↓*) Bodyweight in males on weeks 11, 13, 15 and during the period on week 19-41 and on week 55 but not greater that 10% in any case.
▪ (↓) Bodyweight gain in males and females on week 0-7 (15%/16%), week 0-13 (12%/15%) and week 0-53 (14%/10%).
▪ (↓*) Food consumption was reduced for most of the weekly values during the first 25 weeks in males and 23 weeks on females. Onwards there were only occasionally significant reductions.
<u>Haematology</u>:
▪ (↓*)White blood cells (WBC) in males and females on month 6 (27%/43%) and month 12 (31%/22%) but within the historical control values and with no clear dose-dependency. It has to be noted a high mean white cell counts for male and female controls.
<u>Blood chemistry</u>:
▪ (↓*)Serum cholesterol in males on month 6 (26%). Control values were unusually high.
▪ (↓*)Serum potassium (9%) in males on month 18 but not dose-related.
▪ (↓*)Alanine transaminase (ALAT) in females on month 12 (36%).
<u>Organ weights and gross pathology:</u>
▪ Heart: (↓**) absolute (21%) and (↓*) relative (22%) weight in males at interim sacrifice but comparable to controls at terminal sacrifice.
▪ Testes: (↓n.s) of absolute and relative weight but ≥ 10% at both interim (12% of absolute and 13% of relative) and terminal sacrifice (22% of absolute and 10% of relative). Small size (31/70), enlargement (12/70), discoloration (37/70) and fluid content (21/70) were observed.
▪ Epididymides: small size (15/70) and discoloration (10/70).
<u>Microscopic findings</u>:
<u>Non-neoplastic changes:</u>
▪ Testes: tubular atrophy (66/70), interstitial cell hyperplasia (37/70), edema (21/70) and vessel degeneration (27/70), tubule-dyspermatogenesis (10/70).
▪ Epididymides: spermatic degeneration (48/70) and tubular hyperplasia (49/70).
<u>Neoplastic changes:</u> no evidence of treatment effect on the development of tumours.</td>
<td>Sprague, G.L. (1985a)

(IIA/5.5/01)</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no./group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td><u>100 ppm</u>
<u>Haematology</u>:
▪ (↓*)White blood cells (WBC) in males and females on month 6 (29%/32%) and month 12 (35%/28%) but within the historical control values and with no clear dose-dependency. It has to be noted a high mean white cell counts for male and female controls.
<u>Blood chemistry</u>:
▪ (↓*)Cholesterol in males on month 6 (24%). Control values were unusually high.
<u>Organ weights and gross pathology:</u>
▪ Heart: (↓*) absolute (21%) and (↓*) relative (19%) weight in males at interim sacrifice. Values were comparable to controls at terminal sacrifice.
<u>Microscopic findings</u>:
<u>Non-neoplastic changes:</u>
▪ Testes: edema (11/60).
<u>Neoplastic changes:</u> No evidence of treatment effect on the development of tumours.
<u>40 ppm</u>
<u>Haematology</u>:
▪ (↓*)White blood cells (WBC) in males and females on month 6 (23%/34%) and month 12 (22%/27%) but within the historical control values and no clear dose-dependency. It has to be noted a high mean white cell counts for male and female controls.
<u>Organ weights and gross pathology:</u>
▪ Heart: (↓*) absolute (20%) and (↓*) relative (21%) weight in males at interim sacrifice. Values were comparable to controls at terminal sacrifice.
<u>Microscopic findings</u>:
<u>Neoplastic changes:</u> No evidence of treatment effect on the development of tumours.
NOAEL: 100 ppm corresponding to 3.9 and 4.8 mg/kg bw/day for males and females respectively.</td><td></td></tr>
<tr><td>Carcinogenicity study in mice (2-years)
Laboratory: Stauffer Enviromental Health Center
B.32
GLP: Yes
Mice strain: B6C3F1
60 mice/sex/dose
Interim sacrifice at 12 months was performed in 10 mice/sex/dose
Study acceptable
Deviations: Biochemical parameters were not performed in this study. Haematology examinations were not performed on month 18. Dosing</td><td>Purity: 86.3% Proportion of isomers not indicated.
Oral (diet): test item was dissolved in acetone (0.06% w/w) and mixed with diet.
Doses: 0, 50, 200 and 800 ppm equivalent to: Males: 0, 6.3, 25.7 and 100.1 mg/kg bw/day. Females: 0, 9.1, 32.1 and 143.5 mg/kg bw/day.</td><td>Mortality: not affected by treatment. It was only observed a slightly lower survival or younger mean age at death at 200 ppm but in absence of dose-relationship could be considered not relevant.
Survival (%):
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><th>ppm</th><th>0</th><th>50</th><th>200</th><th>800</th><th>0</th><th>50</th><th>200</th><th>800</th></tr>
<tr><td>week 52</td><td>96</td><td>98</td><td>92</td><td>94</td><td>96</td><td>96</td><td>98</td><td>98</td></tr>
<tr><td>week 78</td><td>92</td><td>92</td><td>78</td><td>90</td><td>90</td><td>90</td><td>90</td><td>90</td></tr>
<tr><td>week 104</td><td>64</td><td>76</td><td>37</td><td>42</td><td>60</td><td>58</td><td>68</td><td>50</td></tr>
<tr><td>Age at death [days]</td><td>603</td><td>645</td><td>533</td><td>623</td><td>676</td><td>675</td><td>563</td><td>639</td></tr>
</table>
Clinical signs: no treatment related clinical signs.
<u>800 ppm</u>
<u>Bodyweight gain</u>
▪ (↓) Bodyweight gain on week 53-79 in males (-2.9 g vs. -1 g of controls) and females (-0.3 g vs. 1.9 g of controls) and on week 0-79 in males (7%) and females (16%).
<u>Haematology</u>:
▪ (↓*) Red blood cells (RBC) in females on month 12 (8%) not dose-related.
▪ (↓*) Hematocrit in females on month 12 (7%) not dose-related.
<u>Organ weights and gross pathology:</u>
▪ Adrenals: at interim sacrifice (↑**) absolute weight in males (136%) and (↑*) in females (43%) and (↑**) relative weight in males (103%) and females (50%). Values were comparable to controls at terminal sacrifice.
▪ Liver: at interim sacrifice (↑*) absolute weight in males (21%) and (↑*)</td><td>Sprague, G.L. (1985b)
(IIA/5.5/02)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
error was documented during 3 days on week 8 at 800 ppm when variable amounts of flurochloridone and another test material were mixed in diet. Concentration of flurochloridone was from <5 to 740 ppm while the range for the other compound was from <3 to 190 ppm. Duration of the study was 24 month instead of 18 month recommended for mice.		relative weight in females (14%). Values were comparable to controls at terminal sacrifice. ▪ Ovaries: at interim sacrifice (↑*) absolute weight (48%) and (↑*) relative weight (61%) but not observed at terminal sacrifice. Microscopic findings (non-neoplastic findings): ▪ Liver: necrosis in males (7/60 *vs*. 1/60 of controls). In most cases the foci of necrosis were secondary to neoplastic or inflammatory processes. Biochemical parameters that could discard liver damage were not performed in this study. ▪ Bone marrow: haemosiderosis in females (7/60 *vs*. 1/60 of controls). Neoplastic findings (*trend analysis using Matel-Hanzel 2X4 analysis)* ▪ Increase of hepatocellular carcinoma in males (26.6% *vs.* 16.6% of controls) not significant and not clearly dose-dependent. The incidence of this carcinoma in males was 21.6% in a study conducted at similar time and 21.3% in historical control data (Haseman et al., 1984) cited in the study. The incidence was within the range of one of the historical controls provided (4%-38.8%) and slightly above the other one (4.2%-24.6%). ▪ The incidence of adrenocortical adenomas in males (higher than values of the historical control data provided) was comparable to controls (31/60 *vs.* 27/60 of controls), not significant and not clearly dose-related. **200 ppm** Haematology: ▪ (↓*) platelets (27%) but not dose related. Organ weights and gross pathology ▪ Adrenals: at interim sacrifice (↑**) absolute weight in males (100%) and (↑**) relative weight in males (78%) and females (42%). Values were comparable to controls at terminal sacrifice except for relative weight in females in which a significant not dose-dependent reduction was observed in contrast to increases seen at interim sacrifice. ▪ Liver: at interim sacrifice (↑*) relative weight in females (12%). ▪ Ovaries: at interim sacrifice (↑*) absolute weight (41%) and relative weight (59%) but not at terminal sacrifice. Neoplastic findings (*trend analysis using Matel-Hanzel 2X4 analysis)* ▪ The incidence of adrenocortical adenomas in males (higher than values of the historical control data provided) was comparable to controls (26/58 *vs.* 27/60 of controls), not significant and not clearly dose-related. **50 ppm** Haematology ▪ (↓*) platelets (19%) but not dose-related. Neoplastic findings (*trend analysis using Matel-Hanzel 2X4 analysis)* ▪ The incidence of adrenocortical adenomas in males (higher than values of the historical control data provided) was slightly above to controls (35/60 *vs.* 27/60 of controls) but not significant and not clearly dose-related. NOAEL: **50 ppm** corresponding to **6.3** and **9.1 mg/kg bw/day** for males and females respectively.	

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a 2-year long-term toxicity and carcinogenicity study in rats (Sprague, 1985a) tested dose levels were 0, 40, 100 and 400 ppm equivalent to 0, 1.5, 3.9 and 15.7 mg/kg bw/day for males and 0, 2.0, 4.8 and 19.3 mg/kg bw/day for females.

No mortality or clinical signs were associated to treatment. There was no evidence of development of tumours due to treatment at any dose level. Bodyweight and food consumption decreases were observed only at the top dose level of 400 ppm. Bodyweight was significantly reduced in males on weeks 11, 13, 15, on week 19-41 and on week 55 but decreases were not greater that 10% in any case. Bodyweight in females remained comparable to controls during treatment. With respect to bodyweight gain there were reductions in males and females on weeks 0-7, 0-13 and 0-53. Food consumption was significantly reduced for most of the weekly values during the first 25 weeks in males and the first 23 weeks on females. Onwards there were only occasionally significant reductions.

Statistically significant decreases in white blood cells (WBC) were registered from 40 ppm on months 6 and 12 for both sexes. The statistical significance of this finding is related to the relatively high mean white cell counts for male and females controls. Besides it has to be pointed out that there was not a clear dose-relationship and the values were within the historical control range. Blood chemistry revealed random significant variations of doubtful toxicological relevance. Cholesterol was significantly reduced in males on month 6 from 100 ppm but this was not considered treatment-related due to the unusually high control values. Alanine transaminase (ALAT) was significantly reduced in females on month 12 at 400 ppm. Significant decrease at 400 ppm of serum potassium was observed on month 18 in males but not dose-dependent.

Absolute and relative weight of heart was significantly reduced in males from 40 ppm at interim sacrifice. The values remained comparable to controls at terminal sacrifice and no microscopic findings were observed in this organ to suggest a treatment relationship for this effect. At 400 ppm testes absolute and relative weights were not statistically different from controls but the observed reduction followed a trend throughout the study and was greater than 10% at both interim and terminal sacrifice at this dose level. Alterations in testes at this dose level were general discoloration, enlargement, fluid content or reduction of size and in epididymides small size and discoloration.

Microscopic findings at 400 ppm in testes were atrophy of the seminiferous tubule with a variable interstitial cell hyperplasia, tubule-dyspermatogenesis, interstitial edema (observed from 100 ppm) and medial hypertrophy/degeneration of small muscular arteries. At this same dose level, epididymal changes were characterized by extensive degeneration of spermatic elements and microtubular hyperplasia. The microtubular hyperplasia may be secondary to alterations in epididymal epithelial “clear cells”. The distribution and occurrence of other non-neoplastic alterations were similar to those expected for a study in this strain of rat. No evidence of neoplastic changes was observed.

In conclusion there was no evidence of carcinogenicity at tested dose levels. **NOAEL** was established at **100 ppm** equivalent to **3.9** and **4.8 mg/kg bw/day** for males and females respectively.

In a 2-year carcinogenicity study in mice (Sprague, 1985b) tested dose levels were 0, 50, 200 and 800 ppm equivalent to 0, 6.3, 25.7 and 100.1 mg/kg bw/day for males and 0, 9.1, 32.1 and 143.5 mg/kg bw/day for females. It has to be noted that duration of the study was 24 month instead of 18 month recommended for mice according to B.32 method.

No mortality or clinical signs were associated to treatment. Bodyweight and food consumption were not affected by treatment. Only bodyweight gain at 800 ppm was reduced in both sexes mainly during the second half of the study and on week 0-79 in females.

The only significant effects on haematology were observed at 800 ppm in females on month 12 (interim sacrifice) with decrease in red blood cells and hematocrit with no clear dose-relationship and no occurrence at terminal sacrifice.

At interim sacrifice the absolute weight of adrenals was significantly increased from 200 ppm in males and at 800 ppm in females and the relative weight of adrenals was also significantly increased from 200 ppm in both sexes. Values were comparable to controls at terminal sacrifice except for relative weight in females at 200 ppm in which a significant not dose-dependent reduction was observed in contrast to increases seen at interim sacrifice. It was also observed at interim sacrifice at 800 ppm significant increase of the absolute weight of liver in males and from 200 ppm significant increase in the relative weight in females. Values were comparable to controls at terminal sacrifice. It has to be noted a significant trend in liver necrosis in males at 800 ppm. In most cases the foci of necrosis were secondary to neoplastic or inflammatory processes. Biochemical parameters that could discard liver damage were not performed in this study. It was also observed a significant trend in haemosiderosis in bone marrow in females at 800 ppm. It was seen at interim sacrifice significant increase in the absolute and relative weight of ovaries from 200 ppm though not observed at terminal sacrifice. No changes in the male reproductive system, similar to those observed in the rat study, were seen in male mice.

There were no relevant changes in the number or incidence of tumors. The most relevant findings at the end of treatment were an increase in the hepatocellular carcinomas in males with respect to controls at 800 ppm and an increase in the adrenocortical adenomas in males at all dose levels including controls. However, no significant relationship between incidence and flurochloridone concentration was observed for both tumors and dose-dependency along tested dose levels was unclear.

Table 24: Incidence of neoplasms

		Males				**Females**			
ppm		**0**	**50**	**200**	**800**	**0**	**50**	**200**	**800**
Hepatocell. adenoma Total (NS)		25/60 (41%)	23/60 (38.4%)	27/59 (45%)	24/60 (40%)	9/60 (15%)	9/59 (15.25%)	10/60 (16.6%)	11/60 (18.3%)
Hepatocell. carcinoma Total (NS)		10/60 (16.6%)	7/60 (11.6%)	11/59 (18.3%)	16/60 (26.6%)	3/60 (5%)	2/59 (3.3%)	0/60 (0%)	4/60 (6.9%)
Adrenal cort. adenoma Total (NS)		27/60 (45.0%)	35/60 (58.3%)	26/58 (44.8%)	31/60 (51.7%)	3/60 (5%)	2/59 (3.4%)	1/60 (3.4%)	0/60 (0%)
Animals examined	IS	10	10	10	10	10	10	10	10
	UD	17	11	22	21	20	18	13	17
	TS	33	39	27	28°	30	39	37	33
	total	60	60	59	59	60	59	60	60
Benign neoplasms	IS	6	5	10	5	1	0	2	2
	UD	13	8	20	16	6	6	3	10
	TS	54	62	40	41	28	30	41	36
	total (pa)	73 (1.2)	75 (1.3)	70 (1.2)	62 (1.0)	35 (0.6)	36 (0.6)	46 (0.8)	48 (0.8)
Malign neoplasms	IS	0	0	2	0	2	1	0	1
	UD	10	3	13	17	14	6	6	7
	TS	5	12	7	11	17	25	20	13
	total (pa)	15 (0.3)	15 (0.3)	22 (0.4)	28 (0.5)	33 (0.6)	32 (0.5)	26 (0.4)	21 (0.4)
All neoplasms	IS	6	5	12	5	3	1	2	3
	UD	23	11	33	33	20	12	9	17
	TS	59	74	47	52	45	55	61	49
	total (pa)	88 (1.5)	90 (1.5)	92 (1.5)	90 (1.5)	68 (1.1)	68 (1.1)	72 (1.2)	69 (1.2)
Animals with neopl.	IS	5	5	9	5	2	1	2	2
	UD	13	9	17	18	16	10	8	12
	TS	28	38	23	25	25	28	34	31
	total (%)	46 (77)	52 (87)	49 (83)	48 (81)	43 (72)	39 (66)	44 (73)	45 (75)

IS = interim sacrifice; UD = unscheduled deaths; TS = terminal sacrifice; pa = mean per animal; hepatocell. = hepatocellular; cort. = cortex NS: absence of a significant relationship between incidence and flurochloridone concentration (using Matel-Haenszel 2X4 trend analysis

The following information includes historical control data available of the most relevant neoplastic findings observed in Sprague (1985b) mice study:

Table 25: Spontaneous neoplastic lesions in B6C3F1/CrlBR Mouse (sex not specified) obtained from 19 studies (1979-86) (Charles River Laboratories).

Location & Lesion	Lesions	Percent	Range
Liver (1294 tissues examined)			
Nodular hyperplasia	6	0.5	0-20.0
Hepatocellular adenoma	222	17.2	0-41.3
Hepatocellular carcinoma	171	**13.2**	**4.2-24.6**
Hepatocellular carcinosarcoma	1	0.1	0-1.4
Hemangioma	9	0.7	0-3.3
Hemangiosarcoma	6	0.5	0-3.3

Table 26: Spontaneous neoplastic lesions in B6C3F1/CrlBR Mouse (sex not specified) obtained during the period 1985-2000 (Historical Histopathology Data, 2005; Huntington Life Sciences, UK)

Location & Lesion	Lesions	Percent	Range
Liver (749 tissues examined)			
Hepatocellular adenoma	148	19.76	1.9-32.0
Hepatocellular carcinoma	150	**20.03**	**4.0-38.8**
Hemangioma	4	0.53	0.0-2.0
Hemangiosarcoma	9	1.20	0.0-5.8
Cholangiocellular Carcinoma	2	0.27	0.0-1.9
Hepatoblastoma	1	0.13	0.0-1.8
Sarcoma	1	0.13	0.0-1.4

Information extracted from the publication *Use of Historical Control Data in Carcinogenicity Studies in Rodents* (Haseman, J.K., Huff, J. and Boorman, G.A. Toxicol. Pathol. 12:126-135, 1984) was cited in the original study to rule out relevancy of the observed tumours. This historical control data provide the incidences of the more frequently-occurring tumours in the National Toxicology Program (NTP) historical control database from 51 B6C3F1 mice studies until March 1983.

Table 27: Haseman et al. (1984) historical control data

Location & Lesion	Male		Female	
	No. of tumors / animals examined	(%) tumors	No. of tumors / animals examined	(%) tumors
Hepatocellular carcinoma	498/2334	21.3%	101/ 2469	4.1%
Adrenocortical adenoma	53/2240	2.4%	7/2306	0.3%

Besides it has to be noted that the Draft Assessment Report (DAR) on the active substance flurochloridone included in the study summary information on the incidence of hepatocellular carcinoma and adrenocortical adenomas from a study conducted at a similar time than Sprague (1985b) mice study shown in the following table.

Table 28:

Location & Lesion	Male		Female	
	No. of tumors / animals examined	(%) tumors	No. of tumors / animals examined	(%) tumors
Hepatocellular carcinoma	13/60	21.7%	5/60	5%
Adrenocortical adenoma	3/60	5%	2/59	3.4%

The following table summarizes data of hepatocellular carcinoma and adrenocortical adenomas comparing incidence obtained in male mice from Sprague (1985b) mice study with historical control data provided.

Table 29: Hepatocellular carcinoma and adrenocortical adenomas incidence in males in Sprague (1985b) mice study compared to available historical controls

	Hepatocellular carcinoma	Adrenocortical adenoma
Sprague (1985b) (males)	16/60 (26.6%) at 800 ppm	27/60 (45%) at 0 ppm 35/60 (58.3%) at 50 ppm 26/58 (44.8%) at 200 ppm 31/60 (51.7%) at 800 ppm
Charles River Laboratories (1979-86)	4.2-24.6%	-
Huntington Life Sciences (1985-2000)	4.0-38.8%	-
Haseman et al. (1984) (males)	498/2334 (21.3%)	53/2240 (2.4%)
Study conducted at similar time (DAR) (males)	13/60 (21.6%)	3/60 (5%)

The incidence of hepatocellular carcinomas in males (26.6%) at the top dose of 800 ppm was within the range of one of the historical controls provided (4%-38.8 from studies conducted during the period 1985-2000) and slightly above another one (4.2%-24.6% from 19 studies conducted during the period 1979-86). It was also comparable to that observed in a study conducted at similar time (21.6%) and to the incidence of historical control data (21.3%) of Haseman et al. (1984).

The incidence of adrenocortical adenomas incidence in males, including controls, were higher than those from a study conducted at similar time (5%) and also from historical control data (2.4%) of Haseman et al. (1984). No explanation was provided for this fact.

In conclusion, flurochloridone was considered not carcinogenic for mice and besides no incidence in reproductive system was seen in contrast to male reproductive toxicity observed in rats. **NOAEL** was established at **50 ppm** equivalent to **6.3** and **9.1 mg/kg bw/day** for males and females respectively.

10.9.2 Comparison with the CLP criteria

Relevant information on carcinogenicity

In the 2-year long-term toxicity and carcinogenicity study in rats (Sprague, 1985a) no evidence of carcinogenicity was observed at tested dose levels.

In a 2-year long-term toxicity and carcinogenicity study in mice (Sprague, 1985b) the main incidence of neoplasms is compiled in Table 24. There were no relevant changes in the number or incidence of tumors. The most relevant findings were:

- Increase in the hepatocellular carcinomas in males with respect to controls at 800 ppm with absence of significant relationship between incidence and flurochloridone concentration and unclear dose-dependency along tested dose levels. The incidence (26.6%) was within the range of one of the historical controls provided (4%-38.8 from studies conducted during the period 1985-2000) and slightly above another one (4.2%-24.6% from 19 studies conducted during the period 1979-86). It was also comparable to that observed in a study conducted at

similar time (21.6%) and to the incidence (21.3%) of historical control data of Haseman et al. (1984). In consequence hepatic carcinomas in males are not considered related to treatment.

- Increase in the adrenocortical adenomas in males at all dose levels including controls with absence of significant relationship between incidence and flurochloridone concentration, unclear dose-dependency along tested dose levels and comparable incidence to controls. Consequently they were not considered related to treatment. It has to be noted that the incidence of these tumors in male mice, including controls, was much higher than that from a study conducted at similar time (5%) and also from historical control data (2.4%) of Haseman et al., (1984). This high incidence at all dose levels was inexplicable but it has to be noted that duration of the study was 24 month instead of 18 month recommended for mice.

In conclusion, taking into account the whole available data, flurochloridone was not considered carcinogenic to both rat and mice.

Conclusions

As there is no epidemiological evidence of carcinogenicity to humans with flurochloridone, given that no human data is available, a classification in Category 1A does not apply.

The available information does not provide evidence to support a classification of flurocholoridone either in Category 1B or Category 2 according results obtained in animal studies. No incidence was observed in rat studies and the only results relevant in mice studies was considered not related to treatment. Overall, the weight and strength of the evidence is considered to be insufficient to justify a classification for carcinogenicity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Data available indicates that flurochloridone does not require classification for its carcinogenic potential.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 30: Summary table of animal studies on adverse effects on sexual function and fertility

† *Increase/decrease relative to controls denoted by ↑/↓; *p<0.05; **p<0.01; n.s.: non-significant*

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
Multigeneration reproductive study in rats Laboratory: Stauffer Enviromental Health Center OECD 416 GLP: Yes **Study acceptable** Rat strain: Crl CD (SD) BR 80 males and 80 females in 20 rats/sex/dose for P0 and also for P1 and P2 There was also a cross mating of P0 animals of different groups after 1st mating Deviations: evaluation of the no. of cycles, cycle length and pre-coital interval in females not available. No examination was performed on thymus and spleen of adults and thymus of pup. The uterus weight was not recorded and a quantitative evaluation for the follicles in the ovary was not performed. The developmental landmarks of preputial separation, vaginal opening and anogenital distance and the bodyweight at	Purity: 86.3%. Proportion of isomers not indicated Oral (diet) Test substance was premixed with acetone before blended with rodent meal. Doses: 0, 40, 400 and 1000 ppm equivalent to: Pre-mating (males-females): P0: 2.8-3.2, 27.7-31.6 and 70.0-78.1 mg/kg bw/day. P1: 2.8-3.3, 29.3-33.1 and 76.2-83.3 mg/kg bw/day. P2: 2.8-3.2, 28.7-31.3 and 75.8-83.5 mg/kg bw/day. Average values of the chemical intakes through pre-pairing period used for NOAEL: 2.8, 27.7 and 70.0 mg/kg bw/day. Gestation: P0: 3.2, 31.6 and 78.1 mg/kg bw/day. P1: 3.3, 33.1 and 83.3 mg/kg bw/day. P2: 3.2, 31.3, 83.5 mg/kg bw/day. Lactation: P0: 6.7, 69.0 and 154 mg/kg bw/day. P1: 69.7, 68.9 and 163.0 mg/kg bw/day. P2: 6.4, 62.7 and 157.9 mg/kg bw/day. Time of exposure: test substance administered weekly. P0: mating on day 70. Males treated on day 7-63 and 77-133 and females on day 7-63 and 119-140. Cross	**PARENTAL ANIMALS** ***Parental toxicity*** *Mortality:* 3 females in P0 (two with renal dysfunction at necropsy and one by acute blood loss during parturition) at 40 ppm and 2 other females of P0 at 400 ppm (one with renal disease and the other sacrificed following 9 weeks of treatment after developing two palpable masses in the jaw regions). *Clinical signs:* alopecia, reddened ears and dehydration observed in all parental groups. It was only significant the alopecia in P1 males from 400 ppm and the reddened ears in P1 females from 40 ppm. ***P0*** 1000 ppm ▪ (↓*) Bodyweight on day 28 (7%) and 63 (8%) in males. ▪ (↓) Bodyweight gain on day 0-28 in males (13%) and on day 0-63 in males (12%) and females (11%). ▪ (↓*) Terminal bodyweights in males (8%). ▪ (↓*) Absolute weight of kidney in males (8.5%) and (↑*) liver in females (16.8%). ▪ (↑*) Relative weight of liver in females (23.9%). 400 ppm ▪ (↓) Bodyweight gain on day 0-63 in females (15%). ▪ (↑*) Absolute weight of liver in females (14.6%). ▪ (↑*) Relative weight of liver (14.4%) in females. 40 ppm ▪ (↑*) Absolute weight of liver (9.7%) and kidney (10.3%) in females. ▪ (↑*) Liver relative weight in females (8.4%) ***P1*** 1000 ppm ▪ (↓*) Bodyweight in males on day 0 (12%), 28 (14%), 63 (15%) and 133 (16%) and in females on day 0 (12%), 28 (11%), 63 (12%). ▪ (↓) Bodyweight gain on day 0-28 in males (15%) and females (11%) and on day 0-63 in males (16%) and females (12%). ▪ (↓) Food consumption on day (0-63) in males (14%) and during lactation (11%) in females. ▪ (↓*) Terminal bodyweights in males (17%) and females (10%). ▪ (↓*) Absolute weight in males/females of kidney (15.1%/10.3%) and liver (19.1%/9.9%), brain (3.7%) in males and heart (14.2%) in females. ▪ (↑*) Relative weight of brain (15.4%) and heart (17.2%) in males. 400 ppm ▪ (↑*) Relative weight of heart (10.3%) in males. 40 ppm ▪ (↑*) Relative weight of heart (10.3%) in males. ***P2*** 1000 ppm ▪ (↓*) Bodyweight in males on day 0 (20%), 28 (15%) and 63 (14%) and in females on day 0 (18%), 28 (21%), 63 (17%) and 140 (16%). There was also significant decreases in females during gestation on day 0 (21%) and 20 (18%) and lactation on day 0 (19%) and 21 (15%). ▪ (↓) Bodyweight gain on day 0-28 in males (10%) and females (24%)	**Downs, J.R., Minor, J.L. (1983)** **(IIA. 5.6.1)**

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
landmark would have been desirable.	mating on day 119. P1: mating on day 77. Males treated on day 7-70 and 84-126 and females on day 7-69 and 126-133. P2: mating on day 77. Males treated on day 7-63 and 84-113 and females on day 7-63 and 126-140.	and on day 0-63 in males (10%) and females (16%). There were also decreases during gestation on day 0-20 (10%). ▪ (↓) Food consumption on day (0-63) in males (12%) and females (15%) and also during gestation (13%) and lactation (20%). ▪ (↓*) Terminal bodyweights in males (14%) and females (16%). ▪ (↓*) Absolute weight in males/females of kidney (11.3%/12.0%), liver (13.0%/11.8%) and heart (13.0%/18.3%). ▪ (↑*) Relative weight of brain in males (14.6%) and females (14.1%). ***Reproductive parameters in parental animals*** ***P0*** <u>1000 ppm</u> ▪ (↓*) Mating index in females (85% *vs*. 100% of controls) and males (80% *vs*. 95% of controls). ▪ (↓*) Delivered after mating (5 *vs*. 18 of controls). ▪ (↓*) Gestation index (29.4% *vs*. 90% of controls). ▪ (↓*) Fertility index in females (25% *vs*. 90% of controls) and males (25% *vs*. 90% of controls). ▪ Testes: (↓*) absolute (49%) and relative weight (45%). (↑*) Incidence of small size and atrophy. ▪ Epididymides: (↑*) incidence of small size, sperm degeneration and tubular epithelial hyperplasia. ▪ (↓*) Sperm motility (94%). ▪ (↑*) Abnormal sperm (8.1 times than control). <u>400 ppm</u> ▪ (↑*) Abnormal sperm (4.4 times than control). ***P1*** <u>1000 ppm</u> ▪ (↓*) Mating index in females (75% *vs*. 100% of controls) and males (65% *vs*. 90% of controls). ▪ (↓*) Delivered after mating (6 *vs*. 18 of controls) ▪ (↓*) Gestation index (40% *vs*. 90% of controls). ▪ (↓*) Fertility index in females (30.3% *vs*. 90% of controls) and males (30% *vs*. 90% of controls). ▪ (↓*) Pups born/litter, live birth index and live pups per litter on day 0. ▪ Testes: (↓*) absolute (57%) and relative weight (49%). (↑*) Incidence of small size, atrophy, dyspermatogenesis, interstitial cell hyperplasia and vascular degeneration. ▪ Epididymides: (↑*) incidence in small size, sperm degeneration, tubular epithelial hyperplasia. ▪ Uterus: (↓*) number of females with implant sites. ▪ (↓*) Sperm motility (74%). ▪ (↑*) Abnormal sperm (6.4 times than control). <u>400 ppm</u> ▪ (↑*) Abnormal sperm (1.9 times than control). ***P2*** *(no sperm analysis was performed for these animals)* <u>1000 ppm</u> ▪ (↓*) Delivered after mating (4 *vs*. 18 of controls). ▪ (↓*) Gestation index (22.2% *vs*. 94.7% of controls). ▪ (↓*) Fertility index in females (20% *vs*. 90% of controls) and males (21.1% *vs*. 90% of controls). ▪ Testes: (↓*) absolute (51%) and relative weight (43%). (↑*) Incidence of small size, atrophy, dyspermatogenesis, interstitial cell hyperplasia and non-significant vascular degeneration. ▪ Epididymes: (↑*) incidence in small size, sperm degeneration, tubular epithelial hyperplasia and prominent clear cells. ▪ Uterus: (↓*) number of females with implant sites. <u>400 ppm</u> Small testes and epidydimides, atrophy in testes and sperm degeneration and tubular cell hyperplasia in epidydimides were cases without	

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
		statistical significance. **LITTER DATA** *No data for absolute and relative organ weights for males at 1000 ppm for F1 and F2.* ***F1*** <u>1000 ppm</u> ▪ (↓*) Bodyweight on day 21 (10%). ▪ (↓*) Bodyweight gain on days 14-21 (12%) and (↓) 0-21 (12%). ▪ (↓*) Absolute weight of kidneys (21.3%) in females. ***F2*** <u>1000 ppm</u> ▪ (↓*) Viability index (live day 4/total born), pups born/litter and live birth index. ▪ (↓*) Survival day 0-4 and live pups/litter on day 0 and 4 (pre-culled). ▪ (↓*) Bodyweight on day 14 (11%) and 21 (22%). ▪ (↓*) Bodyweight gain on days 4-7 (23%), 7-14 (15%), 14-21 (40%) and (↓) 0-21 (25%). ▪ (↓*) Absolute weight of brain (11.9%), heart (25.6%) and kidneys (25.6%) in females. ▪ (↑*) Relative weight in females of brain (13.4%) and liver (15.8%) and (↑n.s.) of kidneys (3.6%). ▪ (↓*) Whole body in females (16.5%) ***F3*** <u>1000 ppm</u> ▪ (↓*) Live pups/litter on day 4 (pre-culled). ▪ (↓*) Bodyweight on day 7 (12%), 14 (15%) and 21 (19%). ▪ (↓*) Bodyweight gain on days 4-7 (23%), 7-14 (16%), 14-21 (27%) and (↓) 0-21 (21%). ▪ (↓*) Absolute weight of heart (34%) in females and testes (26.3%) and kidneys (23.1%) in males. ▪ (↑*) Relative weight of brain in males (21.3%) and females (21.4%). ▪ (↓*) Whole body in males (23.0%) and females (19.1%) <u>400 ppm</u> ▪ (↓*) Absolute weight of heart (10.6%) in females. ▪ (↓*) Relative weight of heart in females (19.3%). ▪ (↓n.s.) Live pups/litter on day 4 (pre-culled). **CROSS MATING** After 1st mating, P0 animals were mated again crossing dose groups. 1000 ppm males were mated with 0 ppm females and 1000 ppm females with 0 ppm males. 40 and 400 ppm animals were mated again but not crossing groups. Results indicated that 1000 ppm males mated with 0 ppm females showed impairment in reproductive indices with only 2 females that delivered pups.	
Fertility study in male rats Laboratory: Stauffer Enviromental Health Center Guideline: n.a. GLP: Yes Rat strain: CR Crl CD (SD) BR 80 males and 240	Purity: 86.3%. Proportion of isomers not indicated Oral (diet) Test substance was mixed with acetone and added to rodent meal Doses (only males): 0, 100, 600 and 1000 ppm equivalent to 0, 5.7, 35.8 and 60.7 mg/kg bw/day	<u>Mortality</u>: one animal at 100 ppm was sacrificed due to poor condition. <u>Clinical signs</u>: the animal that died at 100 ppm was dehydrated and at necropsy several calculi were found in the urinary bladder and one in the kidney. <u>Bodyweight</u>: (↓*) dose-dependent during dosing period but only losses at 1000 ppm on week 7, 8, 9, 10, 12 and 13 and at 600 ppm on week 8 were ≥ 10%. <u>Bodyweight gain:</u> (↓) weeks 0-4 from 100 ppm (11-18%) and on week 0-10 from 600 ppm (15%-21%). <u>Food consumption</u>*:* (↓) weeks 0-4 (10%) and 0-10 (11%) at 1000 ppm. **After 10 weeks of treatment** <u>1000 ppm:</u> *Reproductive parameters* ▪ Only 2/40 females were pregnant.	**Wilcynski, S.L, Killinger, J.M. (1984)** **(IIA, 5.8.2/1)**

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
females: 20 males/dose 60 females/dose **Study acceptable**	1st mating: 20 males treated daily with the test substance during 10 weeks. After this period they were mated (2 females/male). At mid-gestation 10 males and all females were sacrificed. Recovery period of 13 weeks for the remaining 10 males. 2nd mating: 10 males with 20 females.	▪ (↓*) Fertility index in males (10%) and females (5%) with respect to controls (95% in males and 72.5% in females). ▪ (↓*) Mating index in females (47.5% with respect to 92.5% of controls). ▪ The only two pregnant females showed the following decreases: - (↓*) Corpora lutea/litter (12.0 against 15.6 of controls). - (↓*) Implantations/litter (3.0 against 14.0 of controls). - (↓*) Implantation index (24.5% with respect to 92.3% of controls). - (↓*) Viable fetuses/litter (3.0 with respect to 13.7 of controls). *Pathology and organ weights* ▪ (↓*) Absolute and relative weight of testes (53% and 49% respectively). ▪ Testes: (↑*) Incidence of small and flaccid organ, atrophy, dyspermatogenesis, multifocal interstitial cell hyperplasia and vascular degeneration. ▪ Epididymides: (↑*) Incidence of small size, sperm degeneration and tubular epithelial hyperplasia after 10 weeks. ▪ Prostate: (↑*) Incidence of lymphocytic inflammation *Sperm analysis:* ▪ (↓*) Sperm count after 10 weeks (97%). ▪ No data available for sperm motility. ▪ (↑*) Abnormal sperm after (21.5 times with respect to control) ▪ (↑*) FSH level. 600 ppm: *Reproductive parameters* There was a dose-dependent decrease in all parameters, considerably low for the fertility index (75%), but it was only significant in case of the ↓no. viable fetuses/litter (11.8 *vs.* 13.7 of controls). *Pathology and organ weights* ▪ (↓*) Absolute and relative weight of testes (32% and 23%). ▪ Testes: (↑*) incidence of small and flaccid organs, atrophy and dyspermatogenesis. ▪ Epididymides: (↑*) incidence of small size and sperm degeneration. *Sperm analysis:* ▪ (↓*) Sperm count (64%) and (↓*) sperm motility (17%) with respect to controls. ▪ (↑*) Abnormal sperm after (8.1 times with respect to control). **After 13 weeks of recovery** 1000 ppm: *Reproductive parameters* ▪ (↓*)Implantation index (75% with respect to 89.3% of controls). *Pathology and organ weights* ▪ (↓*) Absolute and relative weight of testes (29% and 24%). ▪ Testes: (↑*) incidence of small and flaccid organs and atrophy. ▪ Epididymides: (↑*) incidence of small size and sperm degeneration. *Sperm analysis:* ▪ (↓*) Sperm count (55%) and (↓*) sperm motility (28%) with respect to controls. ▪ (↑*)Abnormal sperm (4.1 times with respect to control). 600 ppm: *Pathology and organ weights* ▪ (↑*) Incidence of small testes. ▪ (↑*) Incidence of small epidydimides and sperm degeneration. *Sperm analysis* ▪ (↓n.s.) Sperm count (18%) and sperm motility (14%). ▪ (↑n.s.) Abnormal sperm (3.1 times with respect to control)	

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
Mechanism of action study in male rats (1st) Laboratory: Stauffer Enviromental Health Center Guideline: n.a. GLP: Yes Rat strain: Crl CD (SD) BR 40 males and 440 females 10 males/dose **Study acceptable to provide additional information**	Purity: 92.5%. Proportion of isomers not indicated Oral (diet) Vehicle: admixed with Mazola corn oil Doses (males): 0, 20, 100 and 400 mg/kg bw/day Mating on day 0 (pre-treatment). Treatment on day 7 during 5 consecutive days. Afterwards 10 weekly matings (days 14-77).	No mortality and clinical signs were observed at any dose level. Bodyweight: (↓*) on day 14 (6%) at 400 mg/kg bw/day. Bodyweight gain: (↓) on days 0-7 (11%) and days 7-14 (50%) at 400 mg/kg bw/day. Food consumption: (↓*) on days 7-11 (22%) at 400 mg/kg bw/day. **Reproductive parameters** No effect in fertility indices and no biologically reductions in corpora lutea, implantations, viable foetuses or early/late resorptions, implantation index or implantation viability.	**Wilcynski, S.L, Killinger, J.M. (1985a)** **(IIA, 5.8.2/2)**
Mechanism of action study in male rats (2nd) Laboratory: Stauffer Enviromental Health Center Guideline: n.a. GLP: Yes Rat strain: CR Crl CD (SD) BR 84 males/group **Study acceptable**	Purity: 92.5%. Proportion of isomers not indicated Oral (diet) Vehicle: test substance was mixed with acetone and administered in the diet Doses (males): 0 and 1000 ppm equivalent to 0 and 56.1 mg/kg/bw/d Time of exposure: 10 weeks. 7 animals/group were sacrificed after 24, 48 and 96 hours of treatment and 1, 2, 4, 6, 8, 10 weeks after. The remaining groups were sacrificed after 10, 20, 30 weeks of the end of treatment.	All the effects are referred to the only dose of 1000 ppm. ▪ No mortality and clinical signs were observed at any dose level. ▪ (↓*) Bodyweight on days 1 and 2 and weeks 1, 4 and 10 but not greater than 10% in any case. ▪ (↓) Bodyweight gain on week 0-1 (48%), 0-4 (32%) and 0-10 (25%). Within recovery period bodyweight gain recovered to levels comparable to controls. ▪ (↓*) Food consumption on day 1 (61%), 2 (31%), 4 (11%) and 7 (11%). ▪ (↓*) Mean absolute and relative weight of epididymis and testes at week 6, 8 and 10 that did not achieved control values after recovery period in the case of testes. ▪ Spermatid analysis in testes: – (↓*)Spermatid concentration from week 6 onwards that remained depressed until the end of recovery period. – (↑*) Spermatid step 17 and (↓*) spermatid step 18 and 19 from week 2. These effects persisted at the end of recovery period. ▪ (↓*) Sperm count in the cauda epididymides from week 6 and from week 4 in caput epididymides. During the recovery period the sperm concentration recovered markedly. ▪ (↓*) Sperm motility in cauda significant from week 6 of treatment that recovered during recovery. ▪ Increase of abnormal sperm in caput and cauda from week 4 onwards significant from week 6. In caput there was abnormal sperm during the recovery period but it appeared normal at the end of this period. In cauda the percentage of abnormal sperm was marked at the end of recovery period (significant after 20 and 31 weeks of recovery). ▪ Increase on week 6-10 of FSH (significant on week 8) and LH (significant on week 6-10) that returned to normality during recovery. Microscopic findings In the testes, germ cell displacement and failed spermatid release were evident since the first 24 hours. Later, at week 4, the germ cell degeneration and depletion occurred along with Sertoli cell vacuolation (week 6 onwards) that were observed until the week 10. The Sertoli cell vacuolation and germ cell depletion were still evident in half of the animals during the recovery period of 31 weeks. In the epididymis, immature germ cells and cellular debris appeared in caput since week 1 and later in cauda (week 2) and predominated for the	**Wilcynski, S.L, Killinger, J.M. (1985b)** **(IIA, 5.8.2/3)**

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no./group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>treatment period. These findings can be considered reversible, since the animals recovered by week 31 after the dosing period. Additionally, Flurochloridone produced a noticeable lack of sperm observable by week 8 in both caput and cauda of epididymis in almost all of the treated animals. For this lesion, the recovery period was insufficient, since some animals did not recover.</td><td></td></tr>
<tr><td>Fertility study in male rabbits
Laboratory: Stauffer Enviromental Health Center
Guideline: n.a.
GLP: Yes
New Zealand White rabbit
96 females and 48 males
12 males and 24 females/group
Study acceptable</td><td>Purity: 86.3%. Proportion of isomers not indicated
Oral (diet)
Vehicle: test substance mixed acetone with diet
Doses (males): 0, 35, 220 and 1400 ppm equivalent to 0, 1.0, 5.9, 33.9 mg/kg/bw/d
Time of exposure: 10 weeks.
12 males/group were mated with 2 females each. On week 10 they were remated with the same females. After 2nd mating 6 males were sacrificed and the six remaining were sacrificed after recovery (5 weeks)</td><td>Parental toxicity
One male was sacrificed at 35 ppm due to nosematosis. No clinical signs were observed during treatment.
(↓*) Food consumption during weeks 3-7 at 1400 ppm.
Histopathology: at 1400 ppm it was observed increased incidence of hepatic biliary hyperplasia sometimes accompanied by increased periportal fibrous connective tissue and a mononuclear cell infiltrate showing haematopoietic differentiation. At terminal sacrifice, 1 male showed minimal and 1 had mild hyperplasia and 4 male showed minimal and 1 had mild extramedullary haematopoiesis. The persistence of these hepatic lesions in this group suggests that regression resulting from treatment was not complete after recovery period. According to the study report these liver findings suggest early cirrhotic changes.
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>35</th><th>220</th><th>1400</th></tr>
<tr><td>Liver, incidence (grading)</td><td colspan="4">After 10 weeks treatment</td></tr>
<tr><td>Biliary hyperplasia with/without fibrosis</td><td>0/6 (-)</td><td>0/6 (-)</td><td>0/6 (-)</td><td>4/6 (1.3)</td></tr>
<tr><td>Extra medullary haematopoiesis</td><td>1/6 (1.0)</td><td>3/6 (1.1)</td><td>1/6 (1.0)</td><td>4/6 (1.3)</td></tr>
<tr><td>Liver, incidence (grading)</td><td colspan="4">After 5 weeks recovery</td></tr>
<tr><td>Biliary hyperplasia with/without fibrosis</td><td>2/6 (1.0)</td><td>1/6 (1.0)</td><td>1/6 (1.0)</td><td>2/6 (1.5)</td></tr>
<tr><td>Extra medullary haematopoiesis</td><td>3/6 (1.0)</td><td>1/6 (1.0)</td><td>3/6 (1.0)</td><td>5/6 (1.2)</td></tr>
</table>
Grade of alteration: 1 = Minimal (very slight), 2 = Mild (slight), 3 = Moderate, 4=Moderately-Severe, 5 = Severe (marked)
Reproductive parameters
No effects in sperm concentration, motility or percentage of abnormal sperm. No effects in fertility indices and in the reproductive organs.</td><td>Wilcynski, S.L, Killinger, J.M. (1985c)
(IIA, 5.8.2/4)</td></tr>
<tr><td>Effect on nonhuman primate sperm production
Laboratory: Stauffer Enviromental Health Center
Guideline: n.a.
GLP: Yes
Cynomolgus monkey
28 male
7 male/group
Study acceptable</td><td>Purity: 86.3%. Proportion of isomers not indicated
Oral (naso-gastric intubation)
Vehicle: Mazola corn oil
Doses (males): 0, 1, 8, 64 mg/kg bw/day
Time of exposure: 5 days/week during 12 weeks.</td><td>No mortality.
Clinical signs: only observed at 64 mg/kg bw/day during the first 6 weeks of study. 2 monkeys vomited after dosing ant on of them repeated three additional times during the study. Another monkey exhibited reduced activity and ataxia one day during treatment may due to aspiration of dose preparation.
Bodyweight: no significant differences.
Bodyweight gain: (↓*) remarkable on week 1 at 64 mg/kg bw/day and dose-dependent along week 1-12.
Food consumption: (↓) on week 1-12 (20%) at 64 mg/kg bw/day. There were significant decreases the first three weeks of study recovering to normal level on week 5.
Clinical biochemistry
64 mg/kg bw/day
▪ (↑*) Triglyceride concentration on week 4 (153%), week 8 (85%) and week 12 (100%) not correlated with any other indications of liver disease or increases in cholesterol.
▪ (↓*) Hemoglobin concentration on week 4 (16.4%), week 8 (11.1%) and week 12 (12.6%) but not significant when compared to pre-treatment values.</td><td>Wilcynski, S.L, Killinger, J.M. (1985d)
(IIA, 5.8.2/5)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
		▪ (↓*) Hematocrit concentration on week 4 (14.8%) and week 8 (11.0%). The reduction on week 12 (10.8%) was not statistically significant. The values were not significant when compared to pre-treatment values. ▪ (↓n.s.) RBC concentration was reduced on week 4 (14.5%), week 8 (10.3%) and week 12 (10.6%). *Sperm analysis* 64 mg/kg bw/day ▪ No relevant changes in sperm concentration, sperm motility and sperm count. ▪ (↑*) Abnormal sperm on week 4 (27%). There was also a non-statistically significant reduction on week 12 (27%). The increase in the incidence of abnormal sperm at 64 mg/kg bw/day at week 4 can be considered incidental since comparable increase was observed at 1 mg/kg bw/day at week 7.	

Table 31: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study	Reference
21-day dietary range-finding in rats Method: OECD 407 GLP: Yes Rat strain: Sprague Dawley 10 rats/sex/dose **Study acceptable** Deviations: no histopathological examinations were performed and only livers and kidneys were weighed	Purity: 85.8%. Proportion of isomers not indicated Oral (diet): test item was dissolved with acetone and mixed with diet and 1% v/w corn oil. Doses: 0, 500, 1200, 3000, 8000 and 20000 ppm equivalent to: Males: 41.2, 91.7, 230.6, 624, 1017 mg/kg bw/day. Females: 44, 106.6, 242.4, 648.1 and 1226 mg/kg bw/day.	It was observed small testes and prostate in males and hypoplasia of the female genital tract at 20000 ppm equivalent to 1017 and 1226 mg/kg bw/day for males-females respectively.	**Ouellette, R.E. (1982) (5.3.1/01)**
3-month dietary toxicity study in rats Method: OECD 408 GLP: Yes Rat strain: Sprague Dawley 20 rats/sex/dose **Study acceptable** Deviations: blood clotting, epididymides and uterus were not determined.	Purity: 85.8%. Proportion of isomers not indicated Oral (diet): test item was dissolved in acetone and mixed with diet and 1% v/w corn oil. Doses: 0, 80, 400 and 2000 ppm equivalent to: Males: 5.4, 26.6 and 137.5 mg/kg bw/day. Females: 6.2, 31.4 and 154.6 mg/kg bw/day.	Absolute and relative testicular weights were significantly lower than controls (aprox. 50%) at 2000 ppm and were accompanied by altered consistence in testes, reduced size in epididymides and altered seminal vesicles. These changes are suggestive of a degenerative or/and atrophic process in the gonads. All animals at 2000 ppm showed bilateral testicular atrophy (moderate to severe degree of atrophy of the primary and secondary spermatocytes with the basal layer or spermatogonia remaining intact) and occasionally the Leyding cells appeared relatively hyperplasic. Moreover microtubular hyperplasia of the epididymides epithelium was seen at this dose level. Significantly increases in absolute and relative ovary weights were observed from 400 ppm but it was not accompanied by any macroscopical or histopathological effect.	**Ouellette, R.E. (1982b) (5.3.2/1)**

Type of study/data	Test substance,	Relevant information about the study	Reference
Chronic toxicity and oncogenicity study in rats Method: B.33 GLP: Yes Rat strain: Sprague Dawley 60 rats/sex/dose except at 400 ppm with 70 rats/sex **Study acceptable** Deviations: none.	Purity: 86.3%. Proportion of isomers not indicated Oral (diet): test item was dissolved in acetone (0.05% w/w) and mixed with diet. Doses: 0, 40, 100 and 400 ppm equivalent to: Males: 1.5, 3.9 and 15.7 mg/kg bw/day. Females: 2.0, 4.8 and 19.3 mg/kg bw/day.	Treatment related effects were observed at 400 ppm: Testes: general discoloration, enlargement, fluid content or reduction in size at necropsy. Microscopic findings were atrophy of the seminiferous tubule with a variable interstitial cell hyperplasia, tubule dyspermatogenesis, interstitial edema and medial hypertrophy/degeneration of small muscular arteries. Epididymes: general discoloration and reduction in the size at necropsy. Microscopic findings were characterized by extensive degeneration of spermatic elements and microtubular hyperplasia. The microtubular hyperplasia may be secondary to alterations in epididymal epithelial "clear cells".	**Sprague, G.L. (1985a) (5.5/02)**

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In the multigeneration reproductive study in rats (Downs and Minor, 1983) tested dose levels were 0, 40, 400 and 1000 ppm equivalent to 0, 2.8, 27.7 and 70.0 mg/kg bw/day. **Parental toxicity** was manifested in all generations at the top dose level of 1000 ppm. At this dose level significant reduction of bodyweight occurred during premating phase. Reduction was observed in P0 males on days 28 and 63 and in both sexes of P1 and P2 on days 0, 28 and 63. Reductions were also observed on day 133 in P1 males and on day 140 in P2 females. In P2 females there was also a significant decrease of bodyweights during gestation and lactation. It has to be taken into consideration that for P1 and P2 parental animals bodyweight was depressed in the beginning of the study (day 0). At this same dose level bodyweight gain decreased on days 0-28 in males of P0 and in both sexes of P1 and P2 and on day 0-63 in all parental animals. There was also a reduction of gain during gestation in P2 females. Food consumption was reduced during premating phase (day 0-63) in P1 males and both sexes of P2, during gestation in P1 and P2 females and during lactation in P2 females. The terminal bodyweights were significantly reduced in P0 males and in both sexes of P1 and P2 parental animals. At 400 ppm it was observed a reduction in the bodyweight gain in P0 females on day 0-63.

At the top dose level of 1000 ppm there were significant variations of absolute and relative organ weights apart from reproductive organs (commented below). In P0 there was a decrease in the absolute weight of kidney (males) and increase in liver (females). There was a decrease of the absolute weight of kidney and liver in P1 and P2 animals (both sexes) and of heart (P1 females and in both sexes of P2). The decrease of the absolute weight of brain in P1 males was significant but only of 3.7%. Relative weights were increased in the case of liver (P0 females), brain (males of P1 and both sexes of P2) and heart (males of P1). At dose levels of 400 and 40 ppm there were some variations in the weight of some organs but they were considered incidental and not relevant. Accordingly, the **NOAEL** for **parental toxicity** was established in 40 ppm equivalent to 2.8 mg/kg bw/day.

There were clear effects affecting **reproductive performance** at 1000 ppm in all parental animals to give pups. Some of these effects were associated with significant depression of the values of mating index in P0 and P1 and delivered after mating, gestation index and fertility index of all parental generations. The number of females with implant sites was significantly reduced in P1 and P2. The pups born/litter, the live birth index and the live pups per litter on day 0 were significantly decreased in P1 to give F2 pups. Necropsy findings confirmed that male reproductive organs (testes and epididymides) were severely affected after treatment at 1000 ppm. Testes absolute and relative weight decreased relevantly in all parental generations and increase incidence of small size and atrophy in this organ was seen in all parental animals.

Dyspermatogenesis and interstitial cell hyperplasia were also significantly observed in testes of P1 and P2 and significant vascular degeneration in P1 but non-significant in P2. In the epididymides it was observed significant increased incidence of small size, sperm cell degeneration and tubular epithelial hyperplasia in all parental animals besides increased prominent clear cells in P2. At 400 ppm there were some cases without statistical significance in P2 of small testes and epidydimides, atrophy in testes and sperm degeneration and tubular cell hyperplasia in epidydimides. It has to be taken into account that the incidence of these effects increased at a higher dose in a dose-dependent manner. The sperm analysis performed in P0 and P1 revealed significant increases of abnormal sperm with respect to controls from 400 ppm and significant reduction in the sperm motility at 1000 ppm in both generations. The cross mating of the 1000 ppm males with 0 ppm females showed an extreme reduction in the fertility index not observed in the cross mating of the 1000 ppm females with the 0 ppm males. This fact pointed out to the specificity of the fertility impairment in males. Therefore it can be concluded that reproductive performance was clearly affected by the active substance at 1000 ppm and there was also abnormal sperm from 400 ppm. **NOAEL** for **fertility** was established in **40 ppm** equivalent to 2.8 mg/kg bw/day.

Pup toxicity manifested by significant reduction of bodyweight and bodyweight gain at 1000 ppm for all generations and significant variations in the absolute and relative weight of several organs. Bodyweight was reduced on day 21 in F1, on days 14 and 21 in F2 and on days 7, 14 and 21 in F3. Bodyweight gain was decreased on days 0-21 for all generations. Besides, it was significantly reduced in F1 on days 14-21 and in F2 and F3 on days 4-7, 7-14 and 14-21. The absolute weight of some organs was reduced in the case of heart (females of F2 and F3), brain (F2 males), kidneys (F1 and F2 females and F3 males) and testes (F3). Relative weight was increased for brain (F2 females and both sexes of F3) and liver (F2 females). The increase in the relative weight of kidneys in F2 females was significant but only of 3.6%. Whole body was significantly decreased in F2 females and both sexes of F3 at 1000 ppm. At this dose level of 1000 ppm it was observed a significant decrease in the survival on day 0-4 in F2 pups and in the number of live pups/litter on day 0 in F2 pups and on day 4 (pre-culled) in F2 and F3 pups. Besides, the pups born/litter, the live birth index and the viability index of F2 pups were significantly decreased. At 400 ppm in F3 the decrease in live pups per litter on day 4 (pre-culled) was not statistical significant and though the relative heart weight decrease in females was statistically significant there was no indication of dose-related trend and the values were in the same range as the values in the other generations. **NOAEL** for **pup toxicity** was set at 400 ppm equivalent to 27.7 mg/kg bw/day.

Another fertility study in rats was performed in order to determine the effects of fluorochloridone on male fertility (Wilcynski and Killinger, 1984) with doses of 0, 100, 600 and 1000 ppm equivalent to 0, 5.7, 35.8 and 60.7 mg/kg bw/day. Male rats were treated daily during 10 weeks and then each male was mated with 2 females. After a recovery period of 13 weeks males were mated again with one female each. Male parental animals showed significant bodyweight losses at 1000 ppm during treatment and further 3 weeks after the end of dosing. Reduction of bodyweight at 600 ppm on week 8 was considered incidental. There were decreases in bodyweight gain from 100 ppm on week 0-4 and from 600 ppm on week 0-10. The decrease in food consumption was observed at 1000 ppm on week 0-4 and week 0-10. **NOAEL** for **general toxicity** was not established considering the effects at 100 ppm equivalent to 35.8 mg/kg bw/day.

Reproductive indices at 1000 ppm were severely affected by treatment. Only 2 females of a total of 20 became pregnant at this dose level. Fertility and mating indexes decreased significantly and the pregnant females showed significant reduction in the corpora lutea/litter, implantations/litter, implantation index and viable foetuses/litter. The viable foetuses/litter was

also significantly reduced at 600 ppm. The absolute and relative weight of testes decreased markedly and showed significant increased incidence of small size, flaccidity, atrophy and dyspermatogenesis from 600 ppm. Besides, at 1000 ppm significant vascular degeneration and multifocal interstitial cell hyperplasia was observed in this organ. It was also seen effects on the epidydimides from 600 ppm of significant small size and sperm degeneration and tubular epithelial hyperplasia but only at 1000 ppm. Lymphocitic inflammation of prostate at 1000 ppm was observed significantly. The sperm analysis revealed clear significant reduction of sperm motility at 600 ppm (no data at 1000 ppm) and in the sperm count from 600 ppm. The abnormal sperm was significantly increased from 600 ppm and the levels of FSH were significantly high at 1000 ppm.

After recovery period of 13 weeks some indicators of fertility impairment persisted mainly at 1000 ppm but also at 600 ppm. Fertility indices recovered with the exception of the implantation index that remained significantly low at 1000 ppm. Small size of testes and epidydimides and sperm degeneration in this last organ was observed significantly from 600 ppm. It was also seen from 600 ppm but statistically significant only at 1000 ppm reduction in the sperm motility and the sperm count and increase in the abnormal sperm from 600 ppm. The absolute and relative weight of testes was significantly decreased at 1000 ppm. At this same dose level it was also observed significant flaccidity and atrophy in the testes. Accordingly, complete reversibility of the effects affecting fertility of males was not confirmed after the recovery period 13 weeks from 600 ppm. **NOAEL** for **reproductive toxicity** was established at 100 ppm equivalent to 5.7 mg/kg bw/day.

A 1st mechanism of action study in male rats (Wilcynski and Killinger, 1985a) was performed to determine the mechanism and/or site of action of flurochloridone in the impairment of the fertility in male rats. Dose levels were 0, 20, 100 and 400 mg/kg bw/day. In this study, males were mated on day 0 and subsequently treated on day 7 with the active substance during 5 consecutive days. After the end of dosing the animals were mated 10 weekly consecutive times (day 14-77 of study). Top dose level of 400 mg/kg bw/day caused significant reduction of food consumption during the week of dosing (day 7-11). There were also significant reductions of bodyweight on day 14 and bodyweight gain decreases on day 0-7 and 7-14. **NOAEL** for **general toxicity** was 100 mg/kg bw/day. No effect on fertility parameters were observed so that **NOAEL** for **fertility** was established 400 mg/kg bw/day. The absence of effects on fertility is probably due to the short dosing period (5 consecutive days of dosing).

A 2nd mechanism of action study was performed in male rats (Wilcynski and Killinger, 1985b) during a period of exposure of 10 weeks with 0 and 1000 ppm equivalent to doses of 0 and 56.1 mg/kg bw/day. The dose level of 1000 ppm caused significant reduction of the bodyweight on days 1 and 2 and weeks 1, 4 and 10 but not greater of 10% in any case. The bodyweight gain decreased during the periods of weeks 0-1, 0-4 and 0-10. Food consumption decreased significantly during first week. There was a significant decrease in the mean absolute and relative weight of epididymides and testes at week 6, 8 and 10 that did not achieved control values after recovery period in the case of testes.

Sperm parameters revealed a significant reduction in the sperm count in cauda and caput epididymides from week 6 and week 4 respectively. The cauda sperm motility was also significantly decreased from week 6. The levels of FSH and LH on week 6-10 were increased. Besides, an increase of the abnormal sperm from week 4 onwards was observed in caput and cauda significant from week 6. All these effects on sperm disappeared during recovery period with the exception of the abnormal sperm especially in cauda. The spermatid concentration analysis in testes showed significant decrease from week 6 that remained depressed after recovery period. Changes were observed after two weeks onwards at which a significant increase in the concentration of the step 17 spermatids and significant decrease in concentrations in step

18 and 19 happened. All these effects on spermatids did not reach normality after recovery period.

Microscopic findings on testes showed spermatogenic disruption from a near normal state at 24 hours to severe effects on week 8 and 10 that did not disappear completely at the end of recovery period. Minimal to mild germ cell displacement and mild failed spermatid release were observed form the first 24 hours and persisted during the 10 weeks of treatment. The more severe effects were germ cell depletion and germ cell degeneration from week 4 along with Sertoli cell vacuolization from week 6 that also persisted until week 10. Germ cell depletion and Sertoli cell vacuolization remained during the recovery period until week 31.

In the epididymides, immature germ cells and cellular debris appeared in caput since week 1 and later in cauda on week 2 and predominated for the treatment period. These findings can be considered reversible, since the animals recovered by week 31 after the dosing period. Additionally, it was observed a noticeable lack of sperm observable by week 8 in both caput and cauda of epididymis in almost all of the treated animals. For this lesion, the recovery period was insufficient, since some animals did not recover.

Taking into account the whole available data of this study it can be said that findings in testes are indicative of spermatogenic cycle disturbance. It has to be noted that Sertoli cell damage is recognized with the formation of vacuoles and this disturbance disrupts the structural or metabolic support of the germ cells, resulting in germ cell degeneration or exfoliation into the tubular lumen. Spermatid retention is also associated with damage in Sertoli cells regarding its role in the release of spemartids. In the study, initially the primary effects appear to be the mild germ displacement and the failure release of the spermatids observed in the ratio increase of step 17: step 18 and 19 of the spermatid development cycle. As the length of the treatment increases, germ cell depletion and germ cell degeneration along with Sertoli cell vacuolization occurred. These effects resulted in a depletion of sperm in cauda and caput epididymides and immature germ cells and cellular debris appeared in caput and cauda. The increases of LH and FSH from week 6 can be regarded as secondary to testicular toxicity. Germ cell depletion along with Sertoli cell vacuolization remained after 31 week of recovery period. It also remained after treatment abnormal sperm, increase of step 17: step 18 and 19 spermatids and decrease spermatid concentration. Accordingly, spermatogenic cycle is disturbed by flurochloridone.

There is also available a fertility study in male rabbits (Wilcynski and Killinger, 1985c) with doses of 0, 35, 220 and 1400 ppm equivalent to 0, 1, 5.9 and 33.9 mg/kg bw/day. Parental toxicity was manifested by a significant reduction in the food consumption during weeks 3-7 at the top dose level of 1400 ppm. At this dose level it was observed increased incidence of hepatic biliary hyperplasia sometimes accompanied by increased periportal fibrous connective tissue and a mononuclear cell infiltrate showing haematopoietic differentiation. The importance of these histopathological findings is commented in chapter 10.12 for STOT RE. **NOAEL** for **general toxicity** was 220 ppm equivalent to 5.9 mg/kg bw/day. No effects on fertility parameters or reproductive organs were observed. **NOAEL** for **reproductive toxicity** was established at 1400 ppm equivalent to 33.9 mg/kg bw/day.

The effect of fluorochloridone in sperm production was analysed in nonhuman primates (Wilcynski and Killinger, 1985d) with an exposition to active substance of 5 days/week during 12 weeks of doses of 0, 1, 8 and 64 mg/kg bw/day. There were some clinical signs at 64 mg/kg bw/day affecting two monkeys with vomiting after dosing and to another monkey with ataxia and reduced activity after aspiration of dose preparation. These clinical signs seem to be associated with the nasogastric administration and not related to treatment. At this dose level food consumption decreased significantly during the first three weeks recovering to normal level on week 5. Food consumption was reduced during weeks 1-12. With respect to bodyweight at 64

mg/kg bw/day there was a remarkable bodyweight gain loss on week 1 and dose-dependent decreases along week 1-12. Clinical biochemistry revealed at 64 mg/kg bw/day significant increase in the concentration of triglycerides but not correlated with any other indications of liver disease or increases in cholesterol. Decreases in the levels of haemoglobin and haematocrit were observed on weeks 4, 8 and 12 but not significant when compared to pre-treatment values. Reductions in RBC were seen without statistical significance. **NOAEL** for **general toxicity** was set at 8 mg/kg bw/day.

The only effect relevant for fertility was a significant increase in the incidence of abnormal sperm at 64 mg/kg bw/day at week 4 that can be considered incidental since comparable increase was observed at 1 mg/kg bw/day at week 7 and no correlation between doses is possible. Therefore, **NOAEL** for **reproductive toxicity** was established at 64 mg/kg bw/day.

In a 21-day dietary range finding study in rats (Ouellette, 1982) tested dose levels were 0, 500, 1200, 3000, 8000 and 20000 ppm equivalent to 0, 41.2, 91.7, 230.6, 624, 1017 mg/kg bw/day for males and 0, 44, 106.6, 242.4, 648.1 and 1226 mg/kg bw/day for females. No histopathological examinations were performed in this study and only livers and kidneys were weighed. It was observed small testes and prostate in males and hypoplasia of the female genital tract at 20000 ppm equivalent to 1017 and 1226 mg/kg bw/day for males-females respectively.

In a 3-month dietary toxicity study in rats (Ouellette, 1982b) tested dose levels were 0, 80, 400 and 2000 ppm equivalent to 5.4, 26.6 and 137.5 mg/kg bw/day for males and 6.2, 31.4 and 154.6 mg/kg bw/day for females. It was observed absolute and relative testicular weights significantly lower than controls (aprox. 50%) at 2000 ppm accompanied by altered consistence in testes, reduced size in epididymides and altered seminal vesicles. These changes are suggestive of a degenerative or/and atrophic process in the gonads. All animals at 2000 ppm showed bilateral testicular atrophy (moderate to severe degree of atrophy of the primary and secondary spermatocytes with the basal layer or spermatogonia remaining intact) and occasionally the Leyding cells appeared relatively hyperplasic. Moreover microtubular hyperplasia of the epididymides epithelium was seen at this dose level.

In a chronic toxicity and oncogenicity study in rats (Sprague, 1985a) tested dose levels were 0, 40, 100 and 400 ppm equivalent to 0, 1.5, 3.9 and 15.7 mg/kg bw/day for males and 0, 2.0, 4.8 and 19.3 mg/kg bw/day for females. Effects observed at the top dose level of 400 ppm identified male testes and epididymes as target organs. In testes, general discoloration, enlargement, fluid content or reduction in size was observed at necropsy. Microscopic findings were atrophy of the seminiferous tubule with a variable interstitial cell hyperplasia, tubule-dyspermatogenesis, interstitial edema and medial hypertrophy/degeneration of small muscular arteries. In epididymis it was observed at necropsy general discoloration and reduction in the size. Microscopic findings in this organ were characterized by extensive degeneration of spermatic elements and microtubular hyperplasia. The microtubular hyperplasia may be secondary to alterations in epididymal epithelial “clear cells”.

10.10.3 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A, known human reproductive toxicant) or from animal data (Category 1B, presumed human reproductive toxicant). No human data available are available for flurochloridone and then classification in Category 1A is not appropriate.

According to the CLP criteria a 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 reproductive toxicity in the absence of other toxic 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 effects.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic 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 the other toxic effects.

The MSCA is of the opinion that the impairment in fertility in male rats is clear and univocal with testes and epididymides as main targets. The toxicity of flurochloridone on fertility was confirmed in the performed mechanistic study (Wilcynski and Killinger, 1985b) in which clear disturbance of the spermatogenic cycle was observed at the tested dose level of 56.1 mg/kg bw/day. This is supported by the microscopic findings in testes and epididymides. Germ cell degeneration and depletion along with Sertoli cell vacuolization was seen as a marker of injury in the testes that also remained after the recovery period. Spermatid failed release and increased ratio of step 17: step 18 and 19 of the spermatid development cycle were also observed, probably related with Sertoli cell damage. This effect in the spermatogenic cycle caused lack of sperm in epididymides and the immature germ cells and cellular debris appeared in caput since week 1 and later in cauda on week 2 predominating for the treatment period. These effects on the reproductive organs can justify the alterations in the sperm analysis and the reduction in the absolute and relative weight of testes and epidydimides during treatment. Even if parental toxicity was manifested by bodyweight, bodyweight gain and food consumption reductions during treatment, in the MSCA opinion it cannot explain the alterations observed at microscopic level in the gonads. Besides, some of the effects, including germ cell depletion along with Sertoli cell vacuolization, remained during recovery period with absence of parental toxicity since bodyweight gain recovered in treated groups and bodyweight and food consumption were comparable to controls. Accordingly, flurochloridone can be regarded as fertility toxicant for male rats.

The effects affecting testes and epididymides, sperm and fertility parameters observed in a multigeneration reproductive study in rats (Downs and Minor, 1983) and in a fertility study in male rats (Wilcynski and Killinger, 1984) from 28 and 35.8 mg/kg bw/day respectively, can be regarded as a direct consequence of the disturbance of the spermatogenic cycle observed in the mechanistic study. The same applies for the effects in the oncogenicity study (Sprague, 1985a) at 15.7 mg/kg bw/day and in the short-term toxicity studies in rats (Oulette, 1982; Oulette, 1982b).

However, it has to be taken into consideration that similar effects to those seen in rats were not observed in reproductive organs in other species in fertility studies in rabbits (Wilcynski and Killinger, 1985c) and non-human primates (Wilcynski and Killinger, 1985d) and in a sixth-month dietary study in dogs (Blair, 1983), in a 28-day dietary range finding study in mice (Oulette, 1982a) and in a 24-month dietary oncogenicity study in mice (Sprague, 1985b). Accordingly, effects on fertility occurred only in rats and reasonably can be considered specific to this species.

In conclusion, the MSCA considers that the clear and consistent evidence of reproductive toxicity in male rats with absence of similar effects in other species suggests the specific sensitivity of the active substance in rats. No information is available on the mechanism toxicity of flurochloridone in the reproductive system and the extent of the effects for humans was not challenged. Hence, relevancy in humans cannot be excluded. On balance and having weighed all the available evidence, the MSCA is

of the opinion that flurocloridone requires classification for adverse fertility effects. Taking into account the specific sensitivity in rats with effects considered not secondary to parental toxicity and the absence of observations in other species, it is considered more appropriate to place the substance in category 2 rather than in category 1B. It is proposed to classify flurochloridone for reproductive toxicity in category 2 (**H361f: Suspected of damaging fertility**) according to the CLP criteria.

10.10.4 Adverse effects on development

Table 32: Summary table of animal studies on adverse effects on development

† *Increase/decrease relative to controls denoted by ↑/↓; *p<0.05; **p<0.01; n.s.: non-significant*

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
Teratology study in rats Laboratory: WIL Research Laboratories Inc. Method: B.31 GLP: Yes Rat strain: Charles River SD COBS CD 25 females/dose **Study acceptable** Deviations: vehicle was administered at 10 ml/kg instead maximum of 4 ml/kg	Purity: 86.3%. Proportion of isomers not indicated Oral (gavage) Vehicle: Mazola corn oil Doses: 0, 25, 100 and 400 mg/kg bw/day Exposure from day 6 to day 15 of gestation Sacrifice on day 20	**MATERNAL TOXICITY** The survival was 100% in all groups. 400 mg/kg bw/day ▪ Dried red material around the nose, mouth or eyes, brown urogenital staining or red urogenital matting during the treatment period. ▪ (↓*) Bodyweight on day 9 (7%), 12 (9%), 16 (14%) and 20 (16%). ▪ (↓**) Bodyweight gain on day 6-9 (>100%), 12-16 (70%), 16-20 (27%), 6-16 (113%) and 0-20 (55%), (↓*) on day 9-12 (75%) and (↓n.s.) on day 0-6 (21%). ▪ (↓**) Food consumption on day 6-9 (46%), 9-12 (29%), 12-16 (31%) and also on day 6-16 (40%) and 0-20 (17%). ▪ (↓) Net bodyweight gain on day 0-20 (67%) and (↓) uterus weight (47%). 100 mg/kg bw/day ▪ (↓*) Bodyweight gain on day 6-9 (>100%) and (↓n.s.) on day 6-16 (20%) and 0-20 (13%). The relevancy of some of these decreases can be questioned considering that the reduction in grams was exiguous and that the absolute weights remained in levels comparable to controls. ▪ (↓)Net bodyweight gain (13%). **REPRODUCTIVE INDICES** 400 mg/kg bw/day ▪ (*↓) Implantation sites (12.2 *vs.* 14.3 of controls). **FOETAL TOXICITY** *(incidence in foetuses/litters; statistical analysis provided for litter incidence; malformations or variations in italics were not registered in historical control data provided)* 400 mg/kg bw/day ▪ 3 total resorptions out of 25 pregnant females (12%). ▪ (↓*) Mean fetuses/litter (8.2 *vs.* 13.5 of controls). ▪ (↑*) Post-implantation losses (4.0 *vs.* 0.8 of controls) corresponding to significant early resorptions (3.7 *vs.* 0.8 of controls). Late resorptions (0.3 *vs.* 0 of controls) were non-significant. ▪ (↓*) Mean foetal weight (25.7%). ▪ ↑External malformations: non-significant omphalocele (1%/9.5%) out of the range of historical controls (0-0.3% for fetuses and 0-4.5% for litters). **Foetal anasarca* (3.6%/23.8%) and *total no. of external malformations (5.6%/42.9%). *Open eye lid* (0.5%/4.8%) and *adactyly* (0.5%/4.8%) was also observed though in a low incidence and without statistical significance. ▪ ↑Visceral malformations: **diaphragmatic hernia* (95%/95.2%), **heart/great vessel anomaly* (86%/100%), **retinal folded* (6%/23.8%), **malpositioned incisors* (14%/33.3%), **undescended testes* (34%/71.4%) and *total no. of visceral malformations (100%/100%). *Malpositioned ovaries* (2%/4.8%), *gonad(s) absent* (1%/4.8%) and *thymus absent* (1%/4.8%) occurred but in a lower incidence and without statistical significance. ▪ ↑Visceral variations: *renal papilla/ureter effects (32%/66.7%) out of the historical control range (0-7.5% for fetuses and 0-31.8% for litters), *major blood vessel variation (6%/19%) out of the historical control range (0-1.1% for fetuses and 0-7.7% for litters) and **enlargement trachea* (35%/61.9%). ▪ ↑Skeletal malformations: **exoccipital-cervical vertebrae defect* (83.3%/100%), **malformed/fused sternebrae* (86.5%/100%), **thickened ribs* (54.2%/84.2%), *bent limb bones (35.4%/68.4%) out of the historical control range (0-0.6% for fetuses and 0-4.5% for litters) and *total no. of skeletal malformations (100%/100%). *Severe malaligned sternebrae* (1%/5.3%) and *malformed clavicle* (1%/5.3%) occurred but in a lower	**Nemec M.D. (1983a)** **(5.6.2/03)**

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
		incidence and without statistical significance. ▪ ↑Skeletal variations: *bent ribs (14.6%/42.1%) out of the historical control range (0-2.1% for fetuses and 0-8% for litters), *13th rib reduced ossification (13.5% /57.9%) out of the historical control range (0-6.4% for fetuses and 0-21.7% for litters) and *hyoid unossified (40.6%/73.7%) out of the historical control range (0.7-15.7% for fetuses and 9.1-45% for litters). 100 mg/kg bw/day ▪ (↓*) Mean foetal weight (8.6%). ▪ ↑Visceral malformations: **diaphragmatic hernia* (40.2%/87.5%), **heart/great vessel anomaly* (4.7%/51.2%) and *total no. of visceral malformations (44.4%/95.8%). Non-significant *malpositioned incisors* (1.2%/4.2%) and *undescended testes* (1.2%/4.2%) were important since they were relevant at a higher dose level. ▪ ↑Visceral variations: *renal papilla/ureter effects (11.8%/58.3%) out of the historical control range (0-7.5% for fetuses and 0-31.8% for litters) and non-significant major blood vessel variation (1.2%/8.3%) relevant since observed at a higher dose level and out of the historical control range (0-1.1% for fetuses and 0-7.7% for litters). ▪ ↑Skeletal malformations: **exoccipital-cervical vertebrae defects* (7.1%/51.2%) and *total no. of malformations (44.4%/95.8%). There was also non-statistically significant *malformed/fused sternebrae* (4.2%/16.2%), *thickened ribs* (1.2%/8.3%) and *severe malaligned sternebrae* (0.6%/4.2%) considered relevant since they occurred at the top dose level. ▪ ↑Skeletal variations: *13th rib reduced ossification (7.7%/51.2%) out of the historical control range (0-6.4% for fetuses and 0-21.7% for litters). The incidence of *hyoid unossified (6.5%/33.3%) was within the range of historical controls (0.7-15.7% for fetuses/9.1-45% for litters) while the non-significant incidence of bent ribs (1.8%/4.2%) was within the historical controls only for litters (0.2-1%-0-9.5%). 25 mg/kg bw/day ▪ ↑External malformations: non-significant omphalocele (0.3%/4.2%) that fell into the range of historical controls (0-0.3% for fetuses and 0-4.5% for litters) and non-significant *fetal anasarca* (0.6%/4.2%) not observed at 100 mg/kg bw/day. ▪ ↑Visceral malformations: *diaphragmatic hernia* (1.2%/8.3%) and *heart/great vessel anomaly* (0.6%/4.2%) not significant but considered relevant since these effects followed a dose-related trend at higher doses. ▪ ↑Visceral variations: non-significant renal papilla/ureter effects (3.7%/25%) within the historical control range (0-7.5% for fetuses/0.31.8% for litters).	
Teratology study in rats Laboratory: WIL Research Laboratories Inc. B.31 GLP: Yes Rat strain: Charles River SD COBS CD 25 females/dose **Study acceptable** Deviations:	Purity: 86.3%. Proportion of isomers not indicated Oral (gavage) Vehicle: Mazola corn oil Doses: 0, 0.2, 2, 10, 20 and 100 mg/kg bw/day Exposure from day 6 to day 15 of gestation Sacrifice on day 20	**MATERNAL TOXICITY** The survival was 100% in all groups. Hair loss was observed but at all dose levels, including controls. 100 mg/kg bw/day ▪ (↓*) Bodyweight gain on day 6-9 (67%), day 12-16 (12%), day 16-20 (14%) and also (↓**) on day 6-16 (19%) and (↓*) days 0-20 (13%). The relevancy of some of these decreases can be questioned considering that the reductions in grams were exiguous and that the absolute weights remained in levels comparable to controls. ▪ (↓) Uterus weight (16.4%). **REPRODUCTIVE INDICES** No relevant effects. **FOETAL TOXICITY** *(incidence in foetuses/litters; statistical analysis provided for litter incidence; malformations or variations in italics were not registered in historical control data provided)* 100 mg/kg bw ▪ (↓*) Fetal weight (10.8%).	**Nemec M.D. (1984a)** **(IIA/5.6.2/02)**

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
vehicle was administered at 10 ml/kg instead maximum of 4 ml/kg		▪ ↑External malformation: non-significant *micrognathia (0.5%/4.8%)*. It has to be noted that agnathia (more severe lesion) was observed in historical controls (0-0.4% for fetuses and 0-5% in litters). ▪ ↑Visceral malformation: **diaphragmatic hernia* (44.6%/86%), *heart/great vessel anomaly (16.5%/57%) out of the historical control range (0-1.8% for fetuses and 0-5% for litters) and no. of *total visceral malformations (52.5%/90%). ▪ ↑Visceral variations: non-significant renal papilla/distended ureter (15.8%/47.6%) above the historical control range (0-7.5%/0-31.8%) and non-significant major blood vessels (2.9%/14.3%) also above the historical control range (0-1.1%/0-7.7%). ▪ ↑Skeletal malformations: **exoccipital-cervical vertebrae defect* (4.3%/27%) and *total malformations (9.2%/41%). There were non-significant bent limb bones (1.4%/9.1%) out of the historical control range (0-0.6%/0-4.5%), *malformed/fused sternebrae* (2.8%/9.1%) and *thickened ribs* (0.7%/4.5%). ▪ ↑Skeletal variations: *unossified 5th and 6th sternebrae (50.4%/91%) above the historical control range for fetuses (15.6-36.6%) but not for litters (52.2-100%) and *slight/moderate sternebrae malaligned (9.9%/41%) above the historical control range (0-4.3%/0-28.6%). There was also non-significant 13th rib reduced ossification (2.1%/13.6%) within historical control range (0-6.4%/0-22.7%). 20 mg/kg bw/day ▪ ↑External malformation: non-significant *micrognathia* (0.5%/4.8%). It has to be noted that agnathia (more severe lesion) was observed in historical controls (0-0.4% for fetuses and 0-5% in litters). ▪ ↑Skeletal variations: *unossified 5th and 6th sternebrae (22.6%/62%) but within the range of the historical controls for fetuses (15.6-36.6%) and litters (52.2-100%). 10 mg/kg bw/day No effects observed. 2 mg/kg bw/day ▪ ↑Skeletal variations: *unossified 5th and 6th sternebrae (14.1%/63%) but within the range of the historical controls for fetuses (15.6-36.6%) and litters (52.2-100%). 0.2 mg/kg bw/day ▪ ↑External malformation: non-significant *micrognathia* (0.3%/4.2%). It has to be noted that agnathia (more severe lesion) was observed in historical controls (0-0.4% for fetuses and 0-5% in litters). ▪ ↑Skeletal variations: *unossified 5th and 6th sternebrae (15.7%/63%) but within the range of the historical controls for fetuses (15.6-36.6%) and litters (52.2-100%).	
Teratology study in rabbits Laboratory: WIL Research Laboratories Inc. B.31 GLP: Yes New Zealand white rabbit 18 females/dose **Study acceptable** Deviations: it is	Purity: 86.3%. Proportion of isomers not indicated Oral (gavage) Vehicle: Mazola corn oil Doses: 0, 5, 20 and 60 mg/kg bw/day Exposure from day 6 to day 18 inclusive of gestation Sacrifice on day 29	**MATERNAL TOXICITY** The survival was 100% in all groups. One dam of each group aborted between days of gestation 20-28 but were considered unrelated to treatment. 60 mg/kg bw/day ▪ (↓) Urination and defecation. ▪ (↓**) Bodyweight gain on days 6-12 (>100%) and the entire treatment period between days 6-18 (97%). However, the relevancy of these decreases can be questioned considering that the reductions in grams were exiguous and that the absolute weights remained in levels comparable to controls. There were also significant increases in other day intervals since the bodyweight gain for the whole period (day 0-29) was greater at this dose level than controls. ▪ (↓n.s.) Food consumption on days 6-12 (17%) and (↓**) days 12-18 (32%). However there was also a significant increase of 75% on days 24-29. ▪ (↓)Uterus weight (12%).	**Nemec M.D. (1983b)** **(IIA/5.6.2/03)**

Method, guideline, deviations if any, species, strain, sex, no./group	Test substance, dose levels duration of exposure	Results	Reference
not detailed the incidence/onset of clinical signs		20 mg/kg bw/day ▪ (↓n.s.) Bodyweight gain on days 6-12 (74%), 12-18 (21%) and 6-18 (57%). The relevancy of these reductions can be questioned considering that the reductions in grams were exiguous and that the absolute weights remained in levels comparable to controls. **FOETAL TOXICITY** *(incidence in foetuses/litters; statistical analysis provided for litter incidence; malformations or variations in italics were not registered in historical control data provided)* 60 mg/kg bw/day ▪ (↓n.s.) Post-implantation losses (19.5% *vs.* 7.4% of controls). ▪ 2 total resorptions attributed to maternal toxicity. ▪ ↑External malformations: *microph-/anophtalmia* (1.1%/7.7%) non-significant and considered spontaneous due to single incidence. ▪ ↑Visceral variations: major blood vessel variation (2.3%/15.4%) non-significant and within the range of historical controls for foetuses (0-10.3%) and litters (0-36.4%). ▪ ↑Skeletal malformations: vertebral anomalies (2.3%/7.7%) non-significant and within the historical control range for litters (0-9.1%) but not for fetuses (0-1.5%). ▪ ↑Skeletal variations: **sternebrae thread-like attached* (17%/38.5%). Hyoids arches bent (6.8%/23.1%) non-significant and within the historical control range for litters (0-27.3%) but not for fetuses (0-4.4%). Sternebrae 5th and 6th unossified (36.4%/76.9%) was non-significant but out of the range of historical controls for fetuses (12.5-24.2%) and litters (27.3-72.7%). Non-significant 13th rudimentary rib(s) (18.2%/61.5%) was within the historical control range for fetuses (0-23%) and litters (0-82.4%) and with no clear dose relationship. 20 mg/kg bw/day ▪ ↑Visceral variations: major blood vessel variation (2.1%/14.3%) non-significant and within the range of historical controls for foetuses (0-10.3%) and litters (0-36.4%). 5 mg/kg bw/day ▪ ↑Visceral variations: major blood vessel variation (5.8%/13.3%) non-significant and within the range of historical controls for foetuses (0-10.3%) and litters (0-36.4%).	

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a teratogenicity study in rats (Nemec, 1983a) tested dose levels were 25, 100 and 400 mg/kg bw/day. At 100 mg/kg bw/day decreased net bodyweight corresponding to the bodyweight minus the uterus weight was observed. At this same dose level there was a significant reduction in the bodyweight gain on day 6-9 and not significant reductions on day 6-16 and 0-20. However, the relevancy of some of these decreases can be questioned considering that the reductions in grams were exiguous and that the absolute weight remained in levels comparable to controls. Maternal toxicity was clear at 400 mg/kg bw/day with relevant decreases in bodyweight (day 16 and 20) and reductions of bodyweight gain and food consumption along the treatment period (day 6-16) and during gestation (day 0-20). Net bodyweight, implantation sites and uterus weight were also reduced at this dose level. **NOAEL** for **maternal toxicity** was set at 25 mg/kg bw/day.

Slight but significant reduction of the mean fetal weight was observed at 100 mg/kg bw/day and evident at 400 mg/kg bw/day. At this last dose level it was observed also 3 total resorptions and significant increase in the incidence of post-implantation losses, early resorptions and significant

reduction of the mean foetuses/litter. External, visceral and skeletal malformations/variations are the following.

From 25 mg/kg bw/day it was observed an increase in the incidence of visceral malformations such as diaphragmatic hernia and heart/great vessel anomaly in a low incidence and without statistical significance at 25 mg/kg bw/day but important since the increased incidence of these effects appeared to be in the initial dose-effects level and followed a dose-related trend at higher dose levels. It has to be pointed out that there was no incidence in historical control data for both malformations. Besides, it has to be considered that no apparent association was observed between the finding of hernia and cardiovascular malformation (many foetuses had one or the other, and many others both) suggesting that they occurred by two different mechanisms.

From 100 mg/kg bw/day there was a relevant increase in the total number of visceral and skeletal malformations. Exoccipital-cervical vertebrae defect was observed as the main significant skeletal malformation from this dose level. Malformed/fused sternebrae, thickened ribs and severe malaligned sternebrae were seen without statistical significance though they were considered relevant since were dose-dependent at higher doses. Relevant skeletal variation of 13th rib reduced ossification was observed from this dose level out of the historical control range. Non-significant increase incidence of bent ribs was out of the historical control range for foetuses but not for litters. Visceral malformations such as non-significant malpositioned incisors and undescended testes were observed from this dose level in a low incidence. However, they were considered relevant since the incidence was increased at a higher dose level. With respect to visceral variations it was significant observed renal papilla/ureter effects out of the historical control range. Besides, non-significant major blood vessel variation was considered important since it occurred at higher incidence increasing the administered dose and it was out of the historical control range at this dose level.

At the top dose level of 400 mg/kg bw/day it was increased significantly the total number of external malformations. Omphalocele was found to be not statically significant but out of the range of historical controls. Significant fetal anasarca with no incidence in historical controls was also observed. Non-significant low incidence of open eye lid and adactyly occurred. It was observed visceral malformations (significant retinal folded and non-significant low incidence of malpositioned ovaries, gonad(s) absent and thymus absent), visceral variations (significant enlargement trachea), skeletal malformations (significant bent limb bones out of the historical control range and low incidence of non-significant malformed clavicle) and skeletal variations (significant hyoid unossified and bent ribs out of the historical control range).

Taking into account the relevant developmental effects that happened from the lowest tested dose level, **NOAEL** for **development** was **not established**.

In other teratogenicity study in rats (Nemec, 1984a) dose levels tested were 0.2, 2, 10, 20 and 100 mg/kg bw/day. Maternal toxicity was observed at 100 mg/kg bw/day with significant bodyweight gain reductions during treatment on day 6-16 and on day 0-20. However, the relevancy of these decreases can be questioned considering that the reductions in grams were exiguous and that the absolute weights remained in levels comparable to controls. **NOAEL** for **maternal toxicity** was established at 20 mg/kg bw/day.

Fetal bodyweight was significantly reduced at 100 mg/kg bw/day and clear signs affecting development were seen with significant increase in the number of total visceral and skeletal malformations at this dose level. Main observed visceral malformations were significant diaphragmatic hernia and heart/great vessel anomaly out of the historical control range. External malformation of micrognathia was observed with no clear dose-response relationship and without statistical significance. Incidence of this lesion compared to the historical controls of agnathia, a more severe lesion, was slightly above for foetuses and within the range for litters. Main visceral variations of renal papilla/distended ureter and major blood vessel variations were found to be not

statistically significant but both above the historical control range. Skeletal malformations at this dose level were significant exoccipital-cervical vertebrae defect and non-significant malformed/fused sternebrae, thickened ribs and bent limb bones (out of the historical control range only for bent limb bones). Significant skeletal variations were unossified 5th and 6th sternebrae, above the historical control range for fetuses but not for litters, and slight/moderate sternebrae malaligned above the historical control range for both foetuses and litters. The effects observed on development at lower doses than 100 mg/kg bw/day were not considered relevant. **NOAEL** for **development** was established at 20 mg/kg bw/day.

In a teratogenicity study in rabbits (Nemec, 1983b) tested dose levels were 5, 20 and 60 mg/kg bw/day. Doses were selected based on results from a range-finding study. The maximum dose of 60 mg/kg bw/day was expected to produce some degree of maternal and embriotoxicity and the lowest dose to be no-effect level. There were signs of maternal toxicity at 60 mg/kg bw/day with clinical signs of urination and defecation and significant bodyweight gain and food consumption decreases. Bodyweight gain reductions during treatment (day 6-12 and 6-18) were compensated with increases in other intervals of the study since the gain for the whole period (day 0-29) was greater at this dose level than controls. At 20 mg/kg bw/day there were also reductions on bodyweight gain on day 6-12, 12-18 and 6-18 without statistical significance. The relevancy of these reductions in bodyweight gain can be questioned since they were exiguous and that the absolute weights remained in levels comparable to controls. Food consumption was reduced at 60 mg/kg bw/day on days 6-12 and 12-18 but increased on day 24-29. Effects on bodyweight gain and food consumption indicated losses during treatment and later recovery. **NOAEL** for **maternal toxicity** was set during EFSA evaluation at 5 mg/kg bw/day due to the bodyweight gain loss at 20 mg/kg bw/day.

Foetotoxicity was manifested at 60 mg/kg bw/day by an increase in the post-implantation losses though non-statistically significant and 2 total resorptions attributed to maternal toxicity. Major blood vessel variation was observed from 5 mg/kg bw/day but without statistical significance and within the historical control range. Besides, there was no clear dose-response for foetuses values. With respect to skeletal malformations and variations the main clear effect was the significant increased incidence of sternebrae thread-like attached (variation) at 60 mg/kg bw/day. At this dose level it was also observed non-significant vertebral anomalies (malformation), non-significant sternebrae 5^{th} and 6^{th} unossified out of the historical control range for foetuses and litters and non-significant hyoids arch bent (variation) out of the historical control range for foetuses but not for litters. Effects at lower doses were not considered relevant. **NOAEL** for **development** was established at 20 mg/kg bw/day.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for developmental toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with development in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A, known human reproductive toxicant) or from animal data (Category 1B, presumed human reproductive toxicant). There is no human data available on flurochloridone therefore classification in Category 1A is not appropriate.

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 development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect

on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of the other toxic effects.

Flurochloridone produced reproducible malformations in a clearly dose-related manner in the two rat teratology studies. This happened at the top dose level of 400 mg/kg bw/day of Nemec (1983a) study with presence of maternal toxicity but also at 100 mg kg/bw/day in both rat studies with slight maternal toxicity manifested by bodyweight gain and net bodyweight decreases. In the MSCA opinion malformations and variations that occurred at this dose level cannot be explained as a consequence of these bodyweight reductions. Accordingly, the effects are not considered secondary of maternal toxicity. Immediately lower tested dose levels below 100 mg/kg bw/day were 25 mg/kg bw/day (Nemec, 1983a) and 20 mg/kg bw/day (Nemec, 1984a). No developmental effects were observed at 20 mg/kg bw/day while visceral malformations of diaphragmatic hernia and heart/great vessel anomaly were observed at 25 mg/kg bw/day without statistical significance but relevantly since they occurred at higher doses in a dose-dependent manner representing initial phases of these malformations. Maternal toxicity was not seen at 25 mg/kg bw/day. It has to be noted that visceral malformations such as diaphragmatic hernia or heart/great vessel anomaly besides fetal anasarca and omphalocele rarely occur spontaneously in the control population. Therefore teratogenicity of the active substance was observed at dose levels equal or greater than 25 mg/kg bw/day.

Results obtained in the rabbit study (Nemec 1983b) at 60 mg/kg bw/day are not as clear as those observed in rat since it is less sensitive and they occurred in presence of maternal toxicity during treatment (urination and defecation, bodyweight gain and food consumption decreases and 2 total resorptions). Non-significant vertebral anomalies (malformation) out of the historical control range only for fetuses and some skeletal variations such as significant sternebrae thread-like attached and hyoids arches bent (out of historical controls for fetuses and non-significant) were observed at this dose level. Major blood vessel variation non-significant and within the range of historical controls for both foetuses and litters and with no clear dose-response along all doses was observed. A higher incidence in this variation and also in the malformation heart/great vessel anomaly was observed in rat at higher doses and consequently the possibility of the development of these lesions in rabbit at doses above 60 mg/kg bw/day could occur. This top dose level selected after a range finding study was expected to produce some maternal toxicity and embriotoxicity, but regarding the toxicity observed, the MSCA is of the opinion that higher dose levels could have been tested in order to rule out an increase in the incidence of malformations/variations that actually occurred in rats at higher dose levels. Consequently, the effects observed are not as important as those observed in rat but even in presence of maternal toxicity they can be regarded as indicative of effect on development.

The MSCA considers that there is clear evidence that flurochloridone is teratogenic in rats. Rat seems to be more sensitive but tested dose levels in rabbit are considered not sufficiently high to rule out severe effects on development in this species and thus the teratogenicity potential of flurochloridone in rabbit cannot be discarded. Accordingly, taking into account the whole available data, the severity of the malformations observed and the incidence in both tested species, the relevancy in humans cannot be ruled out. It is proposed to classify flurochloridone for reproductive toxicity in category 1B (**H360D: May damage the unborn child**) according to the CLP criteria.

10.10.7 Adverse effects on or via lactation

This classification is intended to indicate when a substance may cause harm due to its effects on or via lactation. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

Reproductive studies available in section 10.10.1 do not provide evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk. Toxicokinetics studies do not indicate the likelihood that the substance can be potentially present in breast milk (see section 9).

In conclusion, data available for flurochloridone does not indicate effects on or via lactation.

10.11 Specific target organ toxicity-single exposure

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations, and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. Acute toxicity studies are included in section 10.1.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

According to the results of the acute inhalation study (Decker, U., Knuppe, C., Ullrich, A., 2004; see Table 14) respiratory tract irritation was not observed after administration of flurochloridone.

Narcotic effects were not observed in acute toxicity studies.

STOT SE 1 and 2

STOT-SE Category 1 and 2 is assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity. In this context ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

Table 33: Summary table of relevant effects for STOT SE below cut-off values for classification

Species, route, dose levels and author	Results
Acute oral toxicity study in rats **Howell, A.M. (1979)** Doses: 0, 2000, 2500, 3200, 3600 (only males), 4000, 4500 and 5000 mg/kg bw *Guideline value for classification: ≤ 2000 mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw (STOT SE 1)*	**Clinical signs** 2000 mg/kg bw: mild to severe depression, salivation, diarrhoea, blood-like stains around eyes, nose and mouth, yellow stains around the ano-genital area, ruffled fur and alopecia. Severe diarrhoea also occurred in controls during the first 24 hours. They were reversible in six days. **Necropsy** 2000 mg/kg bw: no relevant findings.
Acute oral toxicity study in mice **Ullmann, L. (1985)** Doses: 0, 1000, 3000 and 5000 mg/kg bw *Guideline value for classification: ≤ 2000 mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw (STOT SE 1)*	**Clinical signs** 1000 mg/kg bw: sedation, dyspnoea, ataxia, curved body position and ruffled that disappeared on day 3 after administration. It has to be noted that the same clinical signs were observed in controls with the same reversibility. **Necropsy** 1000 mg/kg bw: no pathologic changes in survivors.

Acute oral toxicity study in rats **Sieber, M. (2011)** Doses: 300 and 2000 mg/kg bw *Guideline value for classification: ≤ 2000 mg/kg bw (STOT SE 2); ≤ 300 mg/kg bw (STOT SE 1)*	**Clinical signs** 2000 mg/kg bw: swaying gait, dragging of fore and rear limbs, decreased activity, prostration, hunched posture and ruffled fur were observed after treatment in all animals until test day 2 (day of sacrifice of 2/3 animals). The surviving still showed decreased activity and ruffled fur on test day 3, but no clinical signs thereafter until the end of the study. 300 mg/kg bw: 3/6 animals treated showed swaying gait, dragging of fore and rear limbs and decreased activity after treatment on test day 1. No clinical signs from test day 2 until the end of the observation period were observed. **Necropsy** One animal treated with 300 mg/kg bw showed haemorrhagic lungs upon scheduled necropsy. It has to be noted that mortality during the study did not occur at this dose level.

10.11.2 Comparison with the CLP criteria

No signs were observed to be regarded for classification for STOT SE 3 according to CLP Regulation (respiratory tract irritation and narcotic effects)

The only effects observed in the range for STOT SE 1 (guidance value for classification: ≤ 300 mg/kg bw) in Sieber study (swaying gait, dragging of fore and rear limbs and decreased activity) at 300 mg/kg bw were reversible on test day 2 and probably associated to test gavage administration. Consequently they are not indicative of 'significant' or 'severe' changes and therefore not regarded for STOT SE 1.

STOT 2 (guidance value for classification: ≤ 2000 mg/kg bw and <300 mg/kg bw) is not regarded considering the following:

- Effects in mice study (Ullmann, 1985) at 1000 mg/kg bw are not relevant since they occurred also in controls with the same reversibility (day 3).
- Effects observed in rat studies in the range for STOT SE 2 (guidance value for classification: ≤ 2000 mg/kg bw and <300 mg/kg bw) are covered by acute toxicity as Acute Tox.4; H302 regarding lethality observed in an acute oral toxicity study in rats (Sieber, 2011) in the range of concentration (300-2000) mg/kg bw (see section 10.1).

10.11.3 Conclusion on classification and labelling for STOT SE

Flurochloridone does not require classification for STOT SE according to CLP Regulation.

10.12 Specific target organ toxicity-repeated exposure

Table 34: Summary table of animal studies on STOT RE

NOAELS have been copied from the DAR for information only

† *Increase/decrease relative to controls denoted by ↑/↓; *p<0.05; **p<0.01; n.s.: non-significant*

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Dietary 21-day range finding study in rats Laboratory: Stauffer Enviromental Health Center OECD 407 GLP: Yes Rat strain: Charles River Sprague Dawley 10 rats/sex/dose **Study acceptable** Deviations: no histopathological examinations were performed and only livers and kidneys were weighed. *Guideline value for classification: ≤ 428.6 mg/kg bw/day (21 day study)*	Purity: 85.8% Proportion of isomers not indicated Oral (diet): test item was dissolved with acetone and mixed with diet and 1% v/w corn oil. Doses: 0, 500, 1200, 3000, 8000 and 20000 ppm equivalent to: Males: 41.2, 91.7, 230.6, 624, 1017 mg/kg bw/day. Females: 44, 106.6, 242.4, 648.1 and 1226 mg/kg bw/day.	**20000 ppm** ▪ One female died on day 6. At necropsy was found hemorrhagic enteritis and hematuria indicating renal disease. ▪ Dehydration and dark yellow stains on the pelvic region. This last effect was considered a sign of exposition rather than a toxicological sign. Bodyweight and food consumption: ▪ (↓*) Bodyweight in males and females on day 7 (36%/33%), 14 (45%/40%) and 21 (54%/49%). ▪ (↓) Bodyweight gain (remarked) on day 0-21 (>100% for both sexes). ▪ (↓*) Food consumption in males and females on week 1 (74%/71%), week 2 (52%/53%) and week 3 (62%/50%). Haematology: ▪ (↓*) White blood cells (WBC) in males (60%) and females (52%) but not dose-related. ▪ (↓*) Red blood cells (RBC) in males (22%) and in females (8%). ▪ (↓*) Hemoglobin (Hb) in males (25%) and (↓n.s.) females (19%). ▪ (↓*) Hematocrit (Hct) in males (24%) and females (13%). Blood chemistry: ▪ (↑*) Blood urea nitrogen (BUN) in males (25%) and females (90%). ▪ (↑*) γ-glutamyl transpeptidase (γ-GT) (>100% in both sexes). ▪ (↑*) Total and direct bilirubin (>100% in both sexes). ▪ (↑*) Cholesterol (>100% in both sexes). ▪ (↑*) Serum glutamic pyruvic transaminase (SGPT) in males (79%) and (↑n.s.) in females (>100%). ▪ (↓*) Serum glutamic oxoloacetic transaminase (SGOT) in both sexes (30%/24%) but not dose-related. ▪ (↓*) Proteins in both sexes (12%/7%) but no dose-related. ▪ (↑*) Alkaline phosphatase (AP) in females (67%) not dose-related ▪ (↓*) Plasma cholinesterase (plasma ChE) in females (52%) with no clear dose-relationship. Organ weights: ▪ (↓*) Terminal bodyweights in males (54%) and females (50%). ▪ Liver: (↑*) relative weight in males (96%) and females (120%). Variations in absolute weight were not dose-dependent. ▪ Kidney: (↓*) absolute weight in males (40%) and females (29%) and (↑*) relative weight in males (30%) and females (42%). Macropathology: ▪ Discoloration of liver in 4/10 males and 4/9 females ▪ Discoloration of spleen in 10/10 males and 9/9 females. ▪ Small thymus in 8/10 males and 8/9 females, small testes (10/10) and prostrate (10/10) and small uterus (9/9), vagina (6/9), cervix (4/9) and ovaries (2/9). ▪ Body fat reduced in 10/10 males and 8/9 females. **8000 ppm** Bodyweight and food consumption: ▪ (↓*) Bodyweight in males and females on day 7 (18%/16%), 14 (14%/16%) and 21 (18%/18%). ▪ (↓) Bodyweight gain in males and females (remarked) on day 0-21 (59% / >100%). ▪ (↓*) Food consumption in males and females on week 1 (39%/41%). (↓n.s.) and not clearly dose-related in females on week 2 (18%) and also on week 3 (12%). Haematology: ▪ (↓*) RBC (8%) in females. ▪ (↓*) Hct (10%) in females. ▪ (↓n.s.) Hb in males (11%) and females (14%).	**Oulette, R.E. (1982)** **(IIA/5.3.1/01)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Blood chemistry: ▪ (↑*) Total bilirubin in males (50%) and females (>100%). ▪ (↑*) Direct bilirubin in females (100%). ▪ (↑*) Cholesterol in males (95%) and females (>100%). Organ weights: ▪ (↓*) Terminal bodyweights in males (18%) and females (9%). ▪ Liver: (↑*) Relative weight in males (49%) and females (50%). ▪ Kidney: (↓*) Absolute weight in males (15%) and females (11%). (↑n.s.) of relative weight in females (10%). Macropathology: ▪ Discoloration of liver in 5/10 females ▪ Discoloration of spleen in 7/10 males and 3/10 females. **3000 ppm** Bodyweight and food consumption: ▪ (↓*) Bodyweight in females on day 14 (13%) and 21 (12%). ▪ (↓) Bodyweight gain in males and females on day 0-21 (32%/77%). It has to be noted that initial male bodyweight in controls was low compared to the treated group (221 g vs. 240 g). ▪ (↓*) Food consumption in males and females on week 1 (17%/24%). Blood chemistry: ▪ (↑*) Cholesterol in males (29%) and females (66%). ▪ (↑*) Total (100%) and direct bilirubin (>100%) in females. Organ weights: ▪ (↓*) Terminal bodyweights in females (13%). ▪ Liver: (↑*) Relative weight in males (17%) and females (17%). ▪ Kidney: (↑*) Relative weight in females (15%). Macropathology: ▪ Discoloration of liver in 1/10 males and 1/10 females and spleen in 1/10 males and 3/10 females. **1200 ppm** Bodyweight and food consumption: ▪ (↓) Bodyweight gain in males and females on day 0-21 (29%/41%). It has to be noted that initial male bodyweight in controls was low compared to the treated group (221 g vs. 239 g). ▪ (↓*) Food consumption in females on week 1 (12%). Blood chemistry: ▪ (↑*) Total bilirubin (100%) in females. **500 ppm** Bodyweight and food consumption: ▪ (↓) Bodyweight gain in females on day 0-21 (23%). Blood chemistry: ▪ (↑*) Total bilirubin (100%) in females. NOAEL: **1200 ppm** corresponding to **91.7** and **106.6 mg/kg bw/day** for males and females respectively.	
A 28-day dietary range-finding study in mice Laboratory: Stauffer Enviromental Health Center OECD 407 GLP: Yes Mice strain: Charles River $B_6C_3F_1$ 10 mice/sex/dose **Study acceptable as additional information**	Purity: 85.8% Proportion of isomers not indicated Oral (diet): test item was dissolved with acetone and mixed with diet and 1% v/w corn oil. Doses: 0, 50, 200, 700, 3000 and 10000 ppm equivalent to: Males: 8.3,	**10000 ppm** ▪ One female died on day 2 spontaneously and was replaced. Another female was found dead on day 21. Deaths were considered unrelated to treatment. Bodyweight and food consumption: ▪ (↓*) Bodyweight was reduced in males and females on day 8 (13%/16%), day 14 (17%/15%) and on day 28 (19%/23%). ▪ (↓) Bodyweight gain was reduced at this dose level on day 0-28 compared to controls (1 gram of gain *vs*. 4 grams of control in males and 1 gram of gain *vs*. 5 grams of controls in females). ▪ (↓*) Food consumption was significantly reduced on week 1 in males (50%) and on week 2 in females (25%). Blood chemistry: ▪ (↑*) Cholesterol in males (52%). Levels in females were high but control values for this sex were not available due to low collected samples (0). ▪ (↑n.s.) SGPT in both sexes (61%/40%). ▪ (↑n.s.) BUN in females (26%) and not dose-related	**Ouelette, R.E., Sauerhoff M. V. (1982a)** **(IIA/5.3.1/02)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Deviations: no haematology was performed. Only livers and kidneys were weighted and microscopically examined. No individual data are contained in the report. *Guideline value for classification: ≤ 300 mg/kg bw/day (28 day study)*	33.6, 122.5, 528.8, 1586.0 mg/kg bw/day. Females: 9.2, 38.2, 137.2, 535.1, 1841.3 mg/kg bw/day.	Organ weights: ▪ (↓*)Terminal bodyweights in males (19%) and (↓n.s.) in females (15%). ▪ Liver: (↑*) Absolute weight in males (46%) and females (51%) and (↑*) relative weight in males (79%) and females (78%). ▪ Kidney: (↓*) Absolute weight in males (22%) and females (11%). Histopathology*:* ▪ Hepatocyte hypertrophy in 10/10 males and 8/8 females and necrotic hepatitis in 3/10 males. **3000 ppm** Bodyweight and food consumption: ▪ (↓*) Bodyweight in males and females on day 14 (8%/5%) and on day 28 (12%/14%). ▪ (↓) Bodyweight gain was reduced on day 0-28 at this dose level compared to controls (2 grams of gain *vs.* 4 grams of controls in males and 3 grams of gain *vs.* 5 grams of controls in females). ▪ (↓*) Food consumption significantly reduced on week 1 (25%) in males. Blood chemistry: ▪ (↑*) BUN in males (46%) but not dose-related. Organ weights: ▪ Liver: (↑*) Absolute weight in males (31%) and females (36%) and (↑*) relative weight in males (37%) and females (37%). ▪ Kidney: (↓*) Absolute weight in males (12%). Histopathology: ▪ Hepatocyte hypertrophy in 8/10 males and 6/10 females. NOAEL: **700 ppm** corresponding to **122.5** and **137.2 mg/kg bw/day** for males and females respectively.	
3-month subchronic dietary toxicity study in rats Laboratory: Stauffer Enviromental Health Center OECD 408 GLP: Yes Rat strain: Charles River Sprague-Dawley 20 rats/sex/dose **Study acceptable** Deviations: blood clotting time, epididymides and uterus weights were not determined. *Guideline value for classification: ≤ 100 mg/kg bw/day (90 day study)*	Purity: 85.8% Proportion of isomers not indicated Oral (diet): test item was dissolved with acetone and mixed with diet and 1% v/w corn oil. Doses: 0, 80, 400 and 2000 ppm equivalent to: Males: 5.4, 26.6, 137.5 mg/kg bw/day. Females: 6.2, 31.4, 154.6 mg/kg bw/day.	**2000 ppm** ▪ 2 females died due to an anesthetic overdose during blood collection. These deaths were unrelated to treatment. Bodyweight: ▪ (↓*) Bodyweight was significantly reduced in males on day 7 (7%), day 28 (8%), day 42 (11%) and in males and females on day 84 (13%/10%). ▪ (↓) Bodyweight gain was reduced in males and females on day 0-7 (26%/41%), 0-28 (17%/26%) and 0-84 (20%/23%). Organ weights: ▪ (↓*) Terminal bodyweights of males (14%) and females (10%). ▪ Liver: (↑*) relative weight in males (7%) and females (14%) not clearly dose-related in females. ▪ Kidney: (↓*) Absolute weight in males (8%) and (↑*) relative weight in females (10%). ▪ Testes: (↓*) Absolute and relative weight (53% and 46% respectively). ▪ Ovaries: (↑*) Absolute (23%) weight and ↑relative weight (37%) Gross pathology and histopathology*:* ▪ Testes: small/soft in 10/20 and tubular atrophy in 20/20 (moderate to severe degree of atrophy of the primary and secondary spermatocytes with the basal layer or spermatogonia remaining intact). Occasionally the Leyding cells appeared relatively hyperplasic. ▪ Epididymides: small/soft in 15/20, sperm degeneration in 20/20 and tubular hyperplasia in 19/20. ▪ Seminal vesicles: small/soft/unequal size in 9/20. **400 ppm** Bodyweight: ▪ (↓*) Bodyweight was significantly reduced in males on day 84 (6%). ▪ (↓) Bodyweight gain was reduced in males and females on day 0-7 (11%/18%), 0-28 (9%/18%) and 0-84 (10%/11%). Organ weights: ▪ Ovaries: (↑*) Absolute weight (26%) and (↑*) relative weight (32%). NOAEL: **80 ppm** corresponding to **5.4** and **6.2 mg/kg bw/day** for males and females respectively.	**Ouelette, R.E., Sauerhoff M. V. (1982b)** **(IIA/5.3.2/ 1)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
6-month dietary study in Beagle dogs Laboratory: Stauffer Enviromental Health Center OECD 409 GLP: Yes Dog strain: Beagle 6 dogs/sex/dose **Study acceptable** Deviations: The study duration was with 6 months longer than the 13 weeks requested by the guidelines. *Guideline value for classification: ≤ 50 mg/kg bw/day (180 day study)*	Purity: 85.8% Proportion of isomers not indicated Oral (diet): test item was dissolved in acetone and mixed with diet. Doses: 0, 50, 225 and 1000 ppm equivalent to: Males: 1.7, 7.1, 30.0 mg/kg bw/day. Females: 1.6, 7.3, 32.0 mg/kg bw/day.	No mortality occurred. Soft stool and mucoid diarrhoea with red spots slight to moderate was observed during all six months in the majority of treated animals and in all dogs of control groups. Slight to moderate lacrimation and emesis were seen in similar incidence in control and treated dogs and were considered not to be-related to treatment. **1000 ppm** ▪ A slightly increased incidence of irregular heart rate in both sexes. The incidence within the top dose animals decreased towards the end of treatment. Bodyweight gain: ▪ (↓) Bodyweight gain was reduced in females on week 0-4 (22%), week 0-13 (27%) and week 0-26 (30%). The cumulative gain on week 0-26 in females considering initial values of bodyweight was 23.3% for controls and 15.8% for this dose level. Haematology: ▪ (↓*) Hct on month 3 (9%) and (↓**) 6 (9%) in males. ▪ (↓*) Hb on month 3 (10%) and (↓**) on month 6 (11%) in males and (↓*) on month 5 (9%) in females. **225 ppm** Organ weights: ▪ (↓*) Relative weight of heart (12%) in females but not dose-related. NOAEL: **225 ppm** corresponding to **7.1** and **7.3 mg/kg bw/day** for males and females respectively.	**Blair, M. (1983)** **(IIA/5.3.2/ 2)**

Other studies relevant for STOT RE

Other long-term exposure studies, such as on carcinogenicity and reproductive toxicity, can also provide evidence of specific target organ toxicity that could be used in the assessment of classification.

Chapter 10.9: 2-years long term toxicity study in rats (Sprague, G.L., 1985a) and mice (Sprague, G.L., 1985b).

Chapter 10.10.1: multigeneration study in rats (Downs and Minor, 1983), fertility study in male rats (Wilcynski and Killinger, 1984), mechanism of action study in male rats (1st) (Wilcynski and Killinger, 1985a), mechanism of action study in male rats (2nd) (Wilcynski and Killinger, 1985b) fertility study in male rabbits (Wilcynski and Killinger, 1985c) and effect on non-human sperm production (Wilcynski and Killinger, 1985d)

Chapter 10.10.4: two teratogenicity study in rats (Nemec, 1983a; Nemec, 1984a) and a teratogenicity study in rabbits (Nemec, 1983b).

These studies are properly summarized in the corresponding chapters. It has to be noted that most of the effects observed in these studies are not considered for STOT RE. Firstly, effects on reproductive organs are covered by reproductive toxicity. Besides, findings below cut-off values are clinical signs, decreases of bodyweight, bodyweight gain, food consumption or net bodyweight that are effects of toxicological importance but by themselves are not considered to support classification for STOT RE. Variations in organ weights were not accompanied of other findings indicating organ dysfunction. The only effect found relevant for STOT RE 2 in these studies is summarized in Table 35.

Table 35: Summary table of relevant effects of other studies for STOT RE below cut-off values for classification

<table>
<tr><th>Species, route, duration and author</th><th>Dose levels (mg/kg bw/day)</th><th>Effective dose (mg/kg bw/day): effects for repeated exposure toxicity found below cut-off values</th></tr>
<tr><td>Fertility study in male New Zealand White rabbit
Oral (diet)
70 days
Wilcynski, S.L. and Killinger, J.M. (1985c)
Guideline value for classification: ≤ 129 mg/kg bw/day (70 day study)</td><td>Doses (males): 0, 35, 220 and 1400 ppm equivalent to 0, 1.0, 5.9, 33.9 mg/kg/bw/d</td><td>33.9 mg/kg bw/day:
Histopathology: it was observed increased incidence of hepatic biliary hyperplasia sometimes accompanied by increased periportal fibrous connective tissue and a mononuclear cell infiltrate showing haematopoietic differentiation. At terminal sacrifice, 1 male showed minimal and 1 had mild hyperplasia and 4 male showed minimal and 1 had mild extramedullary haematopoiesis. The persistence of these hepatic lesions in this group suggests that regression resulting from treatment was not complete after recovery period. According to the study report these liver findings suggest early cirrhotic changes.
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>35</th><th>220</th><th>1400</th></tr>
<tr><td>Liver, incidence (grading)</td><td colspan="4">After 10 weeks treatment</td></tr>
<tr><td>Biliary hyperplasia with/without fibrosis</td><td>0/6 (-)</td><td>0/6 (-)</td><td>0/6 (-)</td><td>4/6 (1.3)</td></tr>
<tr><td>Extra medullary haematopoiesis</td><td>1/6 (1.0)</td><td>3/6 (1.1)</td><td>1/6 (1.0)</td><td>4/6 (1.3)</td></tr>
<tr><td>Liver, incidence (grading)</td><td colspan="4">After 5 weeks recovery</td></tr>
<tr><td>Biliary hyperplasia with/without fibrosis</td><td>2/6 (1.0)</td><td>1/6 (1.0)</td><td>1/6 (1.0)</td><td>2/6 (1.5)</td></tr>
<tr><td>Extra medullary haematopoiesis</td><td>3/6 (1.0)</td><td>1/6 (1.0)</td><td>3/6 (1.0)</td><td>5/6 (1.2)</td></tr>
</table>
Grade of alteration: 1 = Minimal (very slight), 2 = Mild (slight), 3 = Moderate, 4=Moderately-Severe, 5 = Severe (marked)</td></tr>
</table>

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a 21-day dietary range finding study in rats (Oulette, 1982) tested dose levels were 0, 500, 1200, 3000, 8000 and 20000 ppm equivalent to 0, 41.2, 91.7, 230.6, 624 and 1017 mg/kg bw/day for males and 0, 44, 106.6, 242.4, 648.1 and 1226 mg/kg bw/day for females.

Mortality and clinical signs were only observed at the top dose level of 20000 ppm. One female died on day 6 with signs of renal disease after necropsy (haemorrhagic enteritis and haematuria). The only clinical sign at this dose level related to treatment was dehydration.

Bodyweight was significantly reduced at doses ≥ 8000 ppm in both sexes. At 3000 ppm there were significant reductions of bodyweight in females on days 14 and 21 that did not happen in males. There were reductions of bodyweight gain from 500 ppm in females and from 1200 ppm in males. Food consumption was markedly decreased at 20000 ppm on weeks 1, 2 and 3. There were also significant reductions on week 1 from 1200 ppm in females and from 3000 ppm in males. Reductions on week 2 and 3 below 20000 ppm were not significant in most cases and not dose-related.

Haematology was affected by treatment at doses ≥ 8000 ppm. Reduction of haematocrit (Hct) and red blood cells (RBC) was observed significantly from 8000 ppm in females and at 20000 ppm in males. Haemaglobin (Hb) was reduced from 8000 ppm in both sexes with decreases greater than 10% (biologically relevant) significant only in males at 20000 ppm. At this top dose level white blood cells (WBC) was reduced in both sexes but not dose-related along tested dose levels.

Blood chemistry was affected by treatment at doses ≥ 3000 ppm. Significant increase in the cholesterol level was observed from 3000 ppm in both sexes and also significant elevated direct bilirubin from 3000 ppm in females and total bilirubin from 500 ppm in females. In males, total bilirubin was significantly increased from 8000 ppm and direct bilirubin at 20000 ppm. At this top dose level, there was a significant increase of γ-glutamyl transpeptidase (γ-GT) and blood urea

nitrogen (BUN) in both sexes and serum glutamic pyruvic transaminase (SGPT) only significant in males. Other observations at this dose level (alkaline phosphatase (AP), serum glutamic oxoloacetic transaminase (SGOT), protein levels and plasma cholinesterase) had no dose-dependency and are of doubtful toxicological relevance.

The absolute weight of kidney was reduced in both sexes from 8000 ppm and the relative weight of kidney was increased in both sexes at 20000 ppm. In the case of liver, the observed variations in the absolute weight, some of them significant, did not show a clear dose-dependent tendency across different dose levels. However the relative weight of this organ in both sexes was increased from 3000 ppm. Terminal weights were significantly reduced from 8000 ppm in males and 3000 ppm in females.

Macropathology revealed findings in liver and spleen with discoloration of both organs. In liver this effect was observed at 20000 ppm in males and with unclear dose-relationship from 8000 ppm in females. In spleen the effect was marked from 8000 ppm in both sexes and it was also observed in lower incidence at 3000 ppm. These effects on spleen can be related to alterations in haematopoietic system regarding the haematological changes observed from 8000 ppm. At 20000 ppm it was observed small thymus (both sexes), cervix, uterus, vagina, ovaries, prostrate and testes.

Considering the effects at 3000 ppm **NOAEL** was established at **1200 ppm** equivalent to **91.7** and **106.6 mg/kg bw/day** for males and females respectively.

In a 28-day dietary range finding study in mice (Oulette, 1982a) tested dose levels were 0, 50, 200, 700, 3000 and 10000 ppm equivalent to 0, 8.3, 33.6, 122.5, 528.8 and 1586 mg/kg bw/day for males and 0, 9.2, 38.2, 137.2, 535.1 and 1841.3 mg/kg bw/day for females.

No mortality or clinical signs were associated to treatment. Bodyweight was significantly reduced in both sexes from 3000 ppm on day 14 and 28 and on day 8 at 10000 ppm. Bodyweight gain was affected by treatment on day 0-28 from 3000 ppm. Food consumption was significantly decreased in males on week 1 from 3000 ppm and in females on week 2 at 10000 ppm. Cholesterol was high at 10000 ppm in both sexes but for female controls values were not available due to insufficient quantity of samples. Increases in BUN were not dose-related and increases of SGPT at 10000 ppm in both sexes not significant.

There was a significant increase in the absolute and relative weight of liver from 3000 ppm in both sexes and significant reduction in the absolute weight of kidney from 3000 ppm in males and at 10000 ppm in females. Histopathology revealed hepatocyte hypertrophy in both sexes from 3000 ppm and necrotic hepatitis in males at 10000 ppm. At 10000 ppm terminal weight in males was significantly decreased and non-significantly in females but greater than 10%.

Considering the effects at 3000 ppm, **NOAEL** was established at **700 ppm** equivalent to **122.5** and **137.2 mg/kg bw/day** for males and females respectively.

In a 3-month dietary study in rats (Oulette, 1982b) tested dose levels were 0, 80, 400 and 2000 ppm equivalent to 0, 5.4, 26.6 and 137.5 mg/kg bw/day for males and 0, 6.2, 31.4 and 154.6 mg/kg bw/day for females.

No mortality or clinical signs were associated to treatment. Bodyweight was significantly decreased from 400 ppm on day 84 in males but reduction was greater than 10% only at 2000 ppm. At this top dose level there was also reductions in males on day 42 and in females on day 84. Reductions on day 7 and 28 in males at 2000 ppm were significant but exiguous (<10%). Bodyweight gain was reduced in both sexes from 400 ppm on day 0-7, 0-28 and 0-84. It has to be noted that reduction in males on day 0-28 at 400 ppm was lower than 10%. Food consumption was not affected by treatment.

At dose levels ≥ 400 ppm it was observed a significant increase in the absolute and relative weight of ovaries. At 2000 ppm there was significant increase in the relative weight of liver in both sexes. In

kidney the absolute weight decreased significantly in males and the relative weight increased significantly in females at this same dose level. The terminal weights in both sexes were significantly decreased at 2000 ppm.

Absolute and relative testicular weights were significantly lower than controls at 2000 ppm and were accompanied by reduced size and altered consistence in testes, epididymides and seminal vesicles. These changes are suggestive of a degenerative or/and atrophic process in the gonads.

Histopathology revealed in all animals at 2000 ppm bilateral testicular atrophy (moderate to severe degree of atrophy of the primary and secondary spermatocytes with the basal layer or spermatogonia remaining intact). Occasionally the Leyding cells appeared relatively hyperplasic. Moreover, in the epididymides, microtubular hyperplasia of the epididymides epithelium and spermatic degeneration were seen at this dose level.

Taking into account the effects observed at 400 ppm, **NOAEL** was established at **80 ppm** equivalent to **5.4** and **6.2 mg/kg bw/day** for males and females respectively.

In a 6-month dietary study in dogs (Blair, 1983) tested dose levels were 0, 50, 225 and 1000 ppm equivalent to 0, 1.7, 7.1 and 30.0 mg/kg bw/day for males and 0, 1.6, 7.3 and 32 mg/kg bw/day for females.

No mortality occurred. The only significant clinical sign potentially due to treatment was irregular heart rate in both sexes at the top dose level of 1000 ppm. It happened in absence of histopathological evidences in heart and it was seen decrease in the incidence within the top dose animals towards the end of treatment.

Bodyweight gain was reduced in females at 1000 ppm on week 0-4, 0-13 and 0-26. The cumulative gain during weeks 0-26 in females at this dose level considering the initial bodyweights was 23.3% in controls and 15.8% in treated animals.

Haematology revealed at 1000 ppm significant decreased in the level of haematocrit on month 3 and 6 in males and haemoglobin on month 3 and 6 in males (> 10%) and on month 5 in females (< 10%).

Considering the effects observed at 1000 ppm, **NOAEL** was established at **225 ppm** equivalent to **7.1** and **7.3 mg/kg bw/day** for males and females respectively.

Other relevant studies for STOT RE

In the fertility study in male rabbits (Wilcynski and Killinger, 1985c) at 33.9 mg/kg bw/day it was observed after exposure period of 10 weeks increased incidence of hepatic biliary hyperplasia sometimes accompanied by increased periportal fibrous connective tissue (4/6 males) and mononuclear cell infiltrate showing haematopoietic differentiation after exposure period (4/6 males). After recovery period of 5 weeks, 2/6 males showed biliary hyperplasia and 5/6 males had extramedullary haematopoiesis indicating the persistence of these hepatic lesions what suggests that regression resulting from treatment was not complete. Grading for both lesions was from minimal to mild. Dose-dependency was only clear for biliary hyperplasia after exposure period but not for recovery period. For extramedullary haematopoiesis dose-relationship was not clear. According to the information included in the study these observations in the rabbit suggest that mild early cirrhotic changes were associated with flurochloridone treatment.

10.12.2 Comparison with the CLP criteria

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The CLP criteria state that STOT-RE is assigned on the basis of findings of ‘significant’ or ‘severe’ toxicity. In this context ‘significant’ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. ‘Severe’ effects

are generally more profound or serious than ‘significant’ effects and are of a considerably adverse nature which significantly impact on health.

Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated oral dose toxicity study (rat) are seen at or below 10 mg/kg bw/day.

Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated-dose oral toxicity study in rat are seen to occur in case the limit value is greater than 10 and lower or equal to 100 mg/kg bw/day.

The only effects below cut-off values for STOT RE classification that could be considered indicative of impairment in organs were the following:

In a 21-day dietary study in rats (Oulette, 1982) at dose level of 230.6-242.4 mg/kg bw/day in males and females respectively (below cut-off value of 428.6 mg/kg bw/day for STOT RE 2), effects in liver were observed in both sexes with increased levels of cholesterol and increase in the relative weight of this organ and also elevated total and direct bilirubin in females. Discoloration of liver in 1/10 males and 1/10 females was observed but with no dose-dependency across different dose levels. These effects could be considered indicative of initial stages of liver damage but not sufficiently important at the observed dose level to be considered for a STOT RE.

At this same dose level discoloration of spleen was observed in 1/10 males and 3/10 females. This probably indicates initial stages of effect on the haematopoietic system considering the increase in the incidence at higher dose levels with alterations in haematological parameters. However, haematological parameters remained comparable to controls at this dose level. The effect is not sufficiently relevant for STOT RE.

In a fertility study in male rabbits (Wilcynski and Killinger, 1985c) liver damage was also observed. At 33.9 mg/kg bw/day after a time of exposure of 10 weeks it was observed in liver increased incidence in biliary hyperplasia sometimes accompanied by increased periportal fibrous connective tissue and mononuclear cell infiltrate showing haematopoietic differentiation. Both lesions were observed below extrapolated cut-off value for STOT RE 2 (129 mg/kg bw/day) and did not show complete regression after recovery period of 5 weeks.

Bile duct hyperplasia accompanied by increased periportal fibrous connective tissue is considered an adverse and non-reversible effect for liver and thus potentially relevant for STOT RE. According to the information included in the study these observations are indicative of early cirrhotic type liver changes associated with treatment. It has to be noted that this lesion was graded from minimal to mild and dose-dependency was not clear for recovery period.

Extramedullary haematopoiesis is a well-recognized process in which the body attempts to maintain erythrogenesis in response to an alteration in the normal production of red blood cells. It can be related to disturbance of the haematopoietic system (anaemia) but it can also have other causes such as active immune responses to pathogens and also with bone marrow myelofibrosis. In the study no changes in the haematological parameters (haematocrit, haemoglobin, red and white blood cells and platelets) were observed in the treated group compared to controls. Bone marrow histopathological observation was not recorded. This lesion was graded from minimal to mild and dose-dependency was not clear. The MSCA is of the opinion that relevancy of extramedullary haematopoiesis is doubtful.

In a 6-month dietary study in dogs (Blair, 1983) effects on blood below cut-off value of 50 mg/kg bw/day for STOT RE 2 were seen. At 30-32 mg/kg bw/day for male and females respectively, blood appeared to be the main target of flurochloridone considering the decrease in the level of haematocrit on month 3 and 6 in males and haemoglobin on month 3 and 6 in in males (>10%) and month 5 in females (< 10%). These reductions in blood parameters occurred occasionally

during the 6-month dosing period and they are not regarded as a strong evidence for STOT RE. Macropatholgy and histopahology did not reveal changes associated to haematological effects. Higher tested dose levels are not available to observe the evolution of the incidence of haematological parameters. Taking into account the whole available data and also the absence of effects in blood in other species below cut-off values (mice and rat) classification for STOT RE is not required.

10.12.3 Conclusion on classification and labelling for STOT RE

Effects in blood and spleen are not considered a strong evidence to consider classification after repeated exposure as it has been commented in the previous chapter. The only findings that could support a classification for STOT RE are those found in the fertility study in male rabbits in liver (below cut-off values for STOT RE 2).

As it has been commented before, the toxicological relevance of extramedullary haematopoiesis in liver is doubtful. There was not dose-dependency after exposure and recovery period and the grading of the lesion was from minimal to mild.

Biliary hyperplasia is considered an adverse and non-reversible effect for liver but some uncertainties have been observed in the fertility study in male rabbits regarding this effect. Firstly, dose-dependency is not clear, particularly after recovery period (the incidence was similar in treated group and controls). Besides, severity of this lesion was also graded from minimal to mild and was not found in other species such as mice, rat and dog. In MSCA opinion, there is not sufficient evidence, taking the whole available data, to support a classification for STOT RE 2.

Data available indicates that flurochloridone does not require classification for STOT RE.

10.13 Aspiration hazard

Regarding the available data for the toxicity of flurochloridone included in this dossier besides the physicochemical properties of the active substance it does not seem to pose an aspiration toxicity hazard to humans. There are no data in humans indicating evidence of this toxicity and flurochloridone is a solid organic substance but not a hydrocarbon.

The MSCA is of the opinion, with the current data available on flurochloridone, that classification due to aspiration hazard is not required.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

A brief summary of relevant studies on degradation, listed in the Draft Assessment Report (DAR) and Addendum, is reported below. From all available data on Flurochloridone only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 36: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability: OECD 301 E	No evidence of biodegradation in 28 days. The reference compound was degraded to 98% in the same period.	Finally accepted after the comments received during the PRAPeR review. Addendum I to Vol. III, B8. 2007. The substance is not readily biodegradable.	Douglas, M. T. & Pell, 1985
Hydrolysis: - OECD 111 - OPPTS 835.2110 - SETAC (Europe), Part 9 and JMAFF Guideline 2-6-1	DT_{50} values obtained at 50 ºC: pH 5 >1 year pH 7 >1 year pH 9 >1 year	Hydrolytically stable at pH 5, 7 and 9. Both isomers are also stable to hydrolysis.	Adam, D. 2006
Water-sediment study: Germany BBA Guideline Part IV, 5-1	Metabolic pathway of Flurochloridone		Shaw, D. 1996a
Water-sediment study: Germany BBA Guideline Part IV, 5-1	Metabolic pathway of Flurochloridone		Shaw, D. 1996b
Recommendations of FOCUS Group: FOCUS Degradation Kinetics, 2006.	First order half-lives: Substance: DT_{50} whole system (days) Flurochloridone: 14.3 R406639: 52.3 R42819: 261	Substance degradation was recalculated.	Van der Gauuw, A. 2004c
Photolysis: - SETAC 1995 - US EPPA OPPTS 835.2210	First order half-lives: DT_{50} = 15.9 – 16.5 days (^{14}C-carbonyl-label) DT_{50} = 17.4 – 18.1 days (^{14}C-phenyl-label)	A rapid photodegradation of the substance was observed, while it was stable in the dark controls.	Van der Gaauw, A. 2004a

11.1.1 Ready biodegradability

Douglas et al., 1985.

The ready biodegradability was tested according to OECD 301 E guideline. The test was performed with Flurochloridone technical in a culture medium inoculated with activated sludge over 28 days.

Flurochloridone showed no evidence of biodegradation over the course of the study. No time-dependent change in DOC was found and the mean measured concentration of 8.12 mg DOC/L for

the samples taking after 7, 14, 21, 27 and 28 days was in close agreement with the concentration at the start of the test.

Table 37: Dissolved organic carbon DOC in mg/L and biodegradation results

Test substance	Day						Biodegradation
	0	7	14	21	27	28	
Flurochloridone	8.1	6.3	11.0	6.9	6.2	10.2	-
Sodium benzoate	38.8	5.7	8.8	1.2	0.7	0.7	98 %

The suitability of the test system was demonstrated by the findings for the reference substance sodium benzoate, which was degraded to 98% in the same period.

At first, the study was considered not relevant but supplementary and a new ready biodegradability study of Flurochloridone was required. After the period of comments during the PRAPeR review of the substance, no further information was required and Flurochloridone was considered not readily biodegradable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Ketague, D. B., 1983.

The hydrolytic degradation of Flurochloridone was investigated with the technical product (mixture (3:1) of *trans/cis* isomers) in buffer solutions of pH 4.2, 7.0 and 9.2, over 30 days.

At 40ºC Flurochloridone was stable at pH levels 4.2 and 7.0. At pH 9.2, about 25% hydrolysis was observed after 30 days.

AT 60ºC, approximately 12% hydrolysis was observed at pH 4.2 after 30 days. At pH 7.0 and 9.2, significantly higher amounts were hydrolysed and half-lives of 17.6 and 6.0 days were calculated, respectively (linear regression, firs-order kinetics).

This study was not acceptable since:

- The purity of 1:3 mixture *cis/trans* Flurochloridone was not reported
- DT_{50} at 20ºC was not provided; variation in temperature was higher than recommended (± 2)
- The analytical method employed was not proved to be specific and sensitive for the determination of Flurochloridone (no information on the validation of the method was provided).

Lee, K. S. et al., 1985.

The hydrolytic degradation of Flurochloridone was investigated at 25ºC and 40ºC in buffer solutions of pH 5, 7 and 9, over 30 days.

Flurochloridone was found to be stable at 25°C and all pH levels. At 40°C, it was also stable at pH 5, but slight hydrolysis could be observed at pH 7 and pH 9. Under these conditions, DT_{50} values of 190 and 140 days were calculated, respectively (pseudo first order).

Table 38: Hydrolysis of Flurochloridone (*trans/cis* (3:1) mixture) in buffer solutions over 30 days

Temperature	**25°C**			**40°C**		
pH	**5**	**7**	**9**	**5**	**7**	**9**
Hydrolysis	0%	0%	0%	0%	10.4%*	17.9%*
DT_{50}	> 400 days	> 400 days	> 400 days	> 400 days	190 days	140 days
	stable	stable	stable	stable	Slight hydrolysis	Slight hydrolysis

* Decrease in actual concentration compared to Day 0

At 40°C and pH 9, five hydrolysis products were isolated and identified, but none exceeded 1.8% of the initial radioactivity. Flurochloridone accounted for 85.1% and the recovery was 95.7%. The structure of the most polar fraction, amounting to about 5.7%, could not be identified.

The study was considered not acceptable since:

- It was not performed with pure Flurochloridone
- Information on the analytical method for monitoring Flurochloridone at different times by GC was not provided and the method was not proved to be specific and sensitive for the determination of Flurochloridone.
- For the identification and quantification of the hydrolysis products, some combined chromatographic and spectrometric techniques were used but the spectra are not provided and the quantification after isolation by TLC was not considered adequate.

Taking into account the lack of details on the methods above and their conclusions, the next study was performed and it was considered acceptable.

Adam, D., 2006.

The hydrolytic degradation of Flurochloridone was investigated at 50ºC in buffer solutions of pH 5, 7 and 9, over 5 days. This study demonstrated that Flurochloridone is hydrolytically stable at pH values of 5, 7 and 9 at 50ºC under sterile conditions in the dark. Both isomers (*cis* and *trans*) are stable to hydrolysis.

This study is a preliminary test in which less than 10% hydrolysis of the test substance occurred after 5 days. Therefore, no further testing was required according to the guideline.

Table 39: Hydrolysis of [^{14}C]-Flurochloridone in buffer solution at 50°C after 5 days.

pH	Total Flurochloridone	DT_{50}
5	< 10%	> 1 year
7	< 10%	> 1 year
9	< 10%	> 1 year

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

11.1.4.2 Inherent and enhanced ready biodegradability tests

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Water-Sediment studies

Shaw, D., 1996a.

Aerobic degradation of Flurochloridone in two natural water/sediment systems, both derived from a stream (i.e. Old Basing (Hampshire, UK) and Virginia Water (Berkshire, UK)), has been investigated for 100 days at 20ºC.

Findings:

Physical-chemical properties:

- For Old Basing test systems, the pH ranged between 7.0 and 7.9 (mean 7.6) and the oxygen concentration between 16% and 70% (mean 29%) in the water phase. The redox potential of the sediment ranged from -91 mV to -197 mV (mean -138 mV) indicating that the sediment remained anaerobic during the test period.
- For Virginia Water test systems, the pH ranged between 6.7 and 8.1 (mean 7.6) and the oxygen concentration between 17% and 72% (mean 36%) in the water phase. The redox potential of the sediment ranged from -15 mV to -236 mV (mean -114 mV). The redox potential of the water from both systems remained relatively constant throughout the test period.

Mass balance:

- For Old Basing, total recovery of radioactivity was in the range 97.2-101.3%. The distribution of the radioactivity between the water and the sediment phase was approximately even by Day 7. By the end of the study after 100 days, only 6.9% was measured in the water phase and, a total of 88% (extractable and non-extractable fractions) in the sediment phase.
- For Virginia Water, total recovery of radioactivity was in the range 93.4-99.5%. The distribution of the radioactivity between the water and the sediment phase was approximately even by Day 14. By the end of the study, 21.4% was measured in the water phase and, a total of 68% (extractable and non-extractable fractions) in the sediment phase.

Flurochloridone

In the water phase, Flurochloridone accounted for about 80% in the water phase of both systems on Day 0 and decreased in concentration to less than 1% by the end of the study.

In the sediment phase, the concentration of Flurochloridone increased to 42.2% by Day 14 (Old Basing) and to 26.7% by Day 2 (Virginia Water). Thereafter, its concentration fell to 9.2% and 2.5% by the end of the study in Old Basing and Virginia Water, respectively.

R42819

In the water systems, it was identified as relevant metabolite exceeding 10%, but only in Virginia Water. Its maximum concentration was 23.2% and was measured on Day 30. The maximum concentration of this metabolite in the Old Basing system was only 6.4%.

In the sediment phase, this metabolite was relevant in both systems. The maximum concentrations were 38.7% (Old Basing) and 41.4% (Virginia Water).

R406639

This metabolite slightly exceeded 10% in the Old Basing system (10.5% on Day 30).

Other radioactive components

The thin-layer chromatographic system also resolved a number of other radioactive components, designated T1 to T7. Components T3 to T6 were each present in very low proportions and were quantified together in all cases. Component T7 was apparently present in sediment only.

Component T1 (immobile in the TLC system) amounted to about 10% in combined (water and sediment) samples of Virginia Water system and to about 7% in Old Basing system, with most in the water phase. HPLC chromatography of the water samples from Day 30 and Day 60 revealed that T1 was comprised of a number of separate components, none of which represented more than about 5%.

Components T3 to T6 were each present in very low proportions and were quantified together in all cases.

Non-extractable residues increased throughout the test period to 25.7% and 17.9% on Day 100 in the sediment of Old Basing and Virginia Water, respectively.

Small amounts of volatile compounds were collected until the end of the study and most (4.1% Old Basing, 6.9% Virginia Water) was 14CO2.

Table 40: Distribution of radioactivity in water/sediment systems treated with [2-14C-pyrrolidone] Flurochloridone (values are means of duplicate samples and give percentage of applied radioactivity)

Day	0	0.25	1	2	7	14	30	60/61	100
Old Basing									
Water	83.8	78.2	76.5	63.0	43.8	26.9	16.6	10.1	6.9
T 1	2.3	2.1	4.4	2.8	3.3	4.3	2.9	2.5	2.7
T 2	\	\	\	\	\ 1.1	\ 1.9	0.4	0.7	0.9
T 3, 4, 5, 6	} 1.5	} 1.3	} 3.3	} 3.7	)	)	0.6	0.4	0.2
R 406639	|	|	|	|	1.5	2.5	3.4	1.3	0.9
R 42819	)	)	)	)	2.1	3.6	6.4	3.9	1.8
Flurochloridone	79.7	74.4	68.5	56.1	35.6	14.3	2.9	0.7	0.2
Others	0.3	0.3	0.4	0.6	0.3	0.5	0.2	0.7	0.4
Sediment	17.5	21.4	23.4	36.7	53.1	71.1	82.7	88.0	87.2
Extractable	*17.1*	*20.7*	*20.6*	*35.3*	*49.4*	*63.1*	*70.4*	*68.3*	*61.5*
T 1	\	0.2	\	0.3	0.7	1.7	3.0	2.9	3.0
T 2	|	\	} 0.3	\ 0.2	\ 0.5	0.2	0.6	0.8	1.1
T 3, 4, 5, 6	} 0.7	} 0.3	)	)	)	0.7	1.1	1.3	1.4
T 7	|	|	\ 0.4	0.1	0.3	0.6	0.8	2.1	1.2
R 406639	|	)	)	0.7	2.7	5.3	10.5	9.2	9.3
R 42819	)	0.2	2.5	0.3	5.0	12.6	29.3	38.7	36.4
Flurochloridone	16.5	20.1	17.5	33.6	40.3	42.2	25.3	13.4	9.2
Others	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1	< 0.1	< 0.1
Not extractable	*0.5*	*0.8*	*2.8*	*1.4*	*3.7*	*8.0*	*12.4*	*19.7*	*25.7*
$^{14}CO_2$	-	< 0.1	<0.1	0.1	0.2	0.4	0.6	1.7	4.1
Other volatiles	-	< 0.1	0.2	0.1	0.2	0.3	0.4	0.4	0.4
RECOVERY	101.3	99.6	100.0	99.8	97.2	98.6	100.3	100.1	98.5
Virginia Water									
Water	91.5	76.3	75.0	61.8	50.8	53.2	35.5	29.3	21.4
T 1	2.3	2.5	3.1	3.2	3.1	4.1	4.7	6.5	4.1
T 2	\	\ 1.0	\ 1.3	\	\	\ 2.7	\ 2.7	2.0	1.2
T 3, 4, 5, 6	} 2.6	)	)	} 1.8	} 3.2	)	)	0.6	0.9
R 406639	|	0.5	0.8	)	)	1.8	1.5	0.9	1.1
R 42819	)	0.6	2.8	1.5	8.6	14.9	23.2	18.4	13.2
Flurochloridone	86.3	71.5	66.6	54.9	35.5	29.1	3.3	0.8	0.5
Others	0.4	0.3	0.5	0.6	0.5	0.5	0.3	0.4	0.4
Sediment	5.5	17.0	23.0	35.6	45.8	45.6	61.2	64.1	68.1
Extractable	*5.4*	*16.2*	*20.6*	*32.1*	*41.3*	*39.8*	*49.4*	*48.3*	*50.2*
T 1	\	\	\	0.3	1.1	1.3	2.2	2.3	2.9
T 2	|	} 0.2	} 0.4	\ 0.5	0.1	0.2	0.3	0.5	0.6
T 3, 4, 5, 6	} 0.3	)	)	)	0.6	0.9	1.0	1.1	1.4
T 7	|	\ 0.2	\ 0.2	\ 0.5	\ 0.9	\ 1.2	\ 1.8	0.4	\ 1.6
R 406639	|	)	)	)	)	)	)	1.3	)
R 42819	)	0.9	4.9	4.2	18.7	27.3	40.7	40.5	41.4
Flurochloridone	5.0	14.9	15.0	26.7	19.9	9.0	3.5	2.3	2.5
Others	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.1	0.2	0.1
Not extractable	*0.2*	*0.8*	*2.4*	*3.5*	*4.5*	*5.8*	*11.8*	*15.8*	*17.9*
$^{14}CO_2$	-	<0.1	<0.1	0.1	0.4	0.5	0.9	5.0	6.9
Other volatiles	-	0.1	0.1	0.2	0.5	0.4	1.0	0.5	0.4
RECOVERY	97.0	93.4	98.0	97.6	97.4	99.5	98.5	98.8	96.7

The study is considered valid.

Shaw, D. 1996b.

Aerobic degradation of Flurochloridone in two natural water/sediment systems, both derived from a stream (i.e. Old Basing (Hampshire, UK) and Virginia Water (Berkshire, UK)), has been investigated for 100 days at 20ºC.

Findings:

Physical-chemical properties:

- For Old Basing test systems, the pH ranged between 7.2 and 78.0 (mean 7.7) and the oxygen concentration between 17% and 66% (mean 34%) in the water phase. The redox potential of the sediment ranged from -36 mV to -194 mV (mean -125 mV) indicating that the sediment remained anaerobic during the test period. . The initial biomass values of the sediments were 268 µg C/g sediment and at the end of the test period, the value had change to 92 µg C/g sediment.
- For Virginia Water test systems, the pH ranged between 6.6 and 8.1 (mean 7.6) and the oxygen concentration between 16% and 63% (mean 36%) in the water phase. The redox potential of the sediment ranged from -16 mV to -229 mV (mean -120 mV). The redox potential of the water from both systems remained relatively constant throughout the test period. The initial biomass values pf the sediments were 12 g C/g sediment and at the end of the test period the value had change to 33 g C/g sediment

Mass balance:

- For Old Basing, total recovery of radioactivity was in the range 96.8-101.9%. The distribution of the radioactivity between the water and the sediment phase was approximately even by Day 7. By the end of the study after 100 days, only 6.1% was measured in the water phase and 88% (extractable and non-extractable fractions) in the sediment phase.
- For Virginia Water, total recovery of radioactivity was in the range 96.5-100.8%. The distribution of the radioactivity between the water and the sediment phase was approximately even by Day 14. By the end of the study, 20.3% was measured in the water phase and 73.7% (extractable and non-extractable fractions) in the sediment phase.

Flurochloridone

In the water phase, Flurochloridone accounted for about 86% in the water phase of both systems on Day 0 and decreased in concentration to less than 1% by the end of the study.

In the sediment phase, the concentration of Flurochloridone increased to 37.2% by Day 7 (Old Basing) and to 25.6% by Day 2 (Virginia Water). Thereafter, its concentration fell to 9.3% and 2.4% by the end of the study in Old Basing and Virginia Water, respectively.

R42819

In the water systems, it was identified as relevant metabolite exceeding 10%, but only in Virginia Water. Its maximum concentration was 21.6% and was measured on Day 30. The maximum concentration of this metabolite in the Old Basing system was only 5.3%.

In the sediment phase, this metabolite was relevant in both systems. The maximum concentrations were 36.5% (Old Basing) and 46.9% (Virginia Water) found on Day 100.

R406639

This metabolite slightly exceeded 10% in the Old Basing system (10.6% on Day 30, 10.2% on Day 61).

Other radioactive components

The thin-layer chromatographic system also resolved a number of other radioactive components, designated T1 to T6. Components T3 to T6 were each present in very low proportions and were quantified together in all cases, but did not exceed 2% in any test system. Component T7 was apparently present in sediment only.

Component T1 (immobile in the TLC system) amounted to about 10% in combined (water and sediment) samples of Virginia Water system and to about 6% in Old Basing system, with most in the water phase. HPLC chromatography of the water samples from Day 30 and Day 60 revealed that T1 was comprised of a number of separate components, none of which represented more than about 4%.

Component T2 was present in both water and sediment and, where it was quantified separately, represented together maximum about 2% (water plus sediment).

Non-extractable residues increased throughout the test period to 30.1% and 18.6% on Day 100 in the sediment of Old Basing and Virginia Water, respectively.

Small amounts of volatile compounds were collected until the end of the study. About 1-2% were trapped as $^{14}CO_2$ and about 2-3% as organic volatiles.

Table 41: Distribution of radioactivity in water/sediment systems treated with [U-14C-phenyl] Flurochloridone (values are means of duplicate samples and give percentage of applied radioactivity)

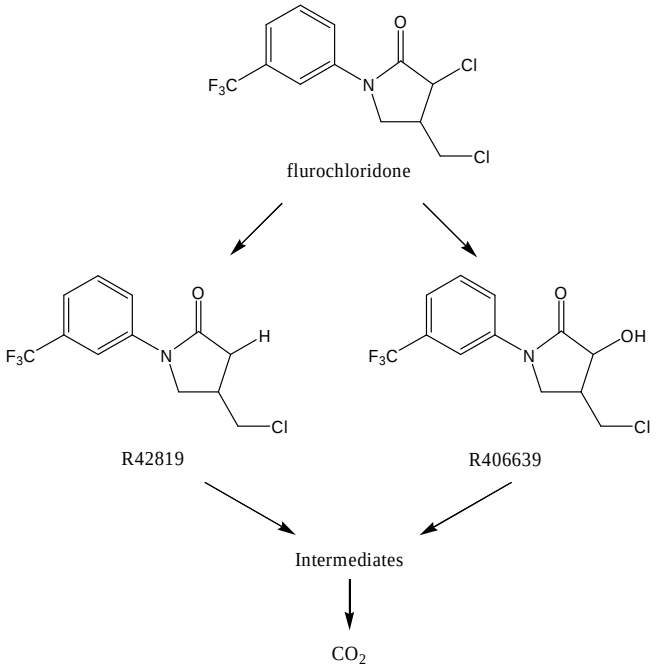
Day	0	0.25	1	2	7	14	30	60/61	100
	Old Basing								
Water	90.0	79.6	73.9	64.3	50.4	42.7	14.6	10.1	6.1
T 1	2.1	2.0	2.7	2.4	2.3	4.2	3.1	3.5	3.3
T 2	⎫	⎫	⎫	⎫	⎫ 1.2	⎫ 1.2	0.5	0.6	0.5
T 3, 4, 5, 6	⎬ 1.7	⎬ 2.0	⎬ 1.8	⎬ 1.1	⎭	⎭	0.3	0.7	0.2
R 406639	⎪	⎪	⎪	⎪	1.4	2.8	2.2	1.0	0.4
R 42819	⎭	⎭	⎭	⎭	0.6	5.3	4.6	3.5	1.5
Flurochloridone	86.0	75.4	68.8	60.7	44.4	28.8	3.8	0.7	<0.1
Others§	0.3	0.3	0.6	0.3	0.5	0.5	0.2	0.2	0.2
Sediment	11.9	21.7	24.2	32.2	45.6	57.3	83.6	87.3	88.4
Extractable	*11.6*	*19.3*	*22.9*	*31.0*	*43.3*	*51.5*	*69.0*	*65.7*	*58.3*
T 1	⎫	⎫	⎫	0.3	0.2	1.0	2.0	2.8	3.1
T 2	⎪	⎬ 0.2	⎬ 0.2	⎫ 0.2	⎫ 0.4	⎫ 0.7	0.5	0.7	0.8
T 3, 4, 5, 6	⎬ 0.4	⎭	⎭	⎭	⎭	⎭	1.0	1.4	1.4
R 406639	⎪	0.2	0.4	0.6	3.0	5.6	10.6	10.2	7.3
R 42819	⎭	0.2	0.2	0.4	2.6	10.7	26.4	33.1	36.5
Flurochloridone	11.2	18.7	22.1	29.5	37.2	33.5	28.7	17.5	9.3
Others	<0.1	0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
Not extractable	*0.3*	*2.4*	*1.3*	*1.3*	*2.3*	*5.8*	*14.6*	*21.7*	*30.1*
$^{14}CO_2$	-	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.3	1.1
Other volatiles	-	0.1	0.1	0.3	0.9	0.9	1.1	1.6	2.3
RECOVERY	101.9	101.3	98.1	96.8	96.8	100.8	99.4	99.3	97.8
	Virginia Water								
Water	92.6	86.1	79.0	60.3	49.1	48.8	18.0	27.9	20.3
T 1	2.0	2.3	2.4	2.5	3.0	4.5	6.6	5.7	4.1
T 2	⎫	⎫	⎫	⎫	⎫ 1.0	⎫ 1.7	⎫ 3.1	1.3	1.3
T 3, 4, 5, 6	⎬ 2.2	⎬ 2.4	⎬ 2.0	⎬ 1.8	⎭	⎭	⎭	0.6	0.4
R 406639	⎪	⎪	⎪	⎭	1.1	1.7	1.2	0.8	0.8
R 42819	⎭	⎭	⎭	3.6	7.3	17.1	21.6	19.0	13.3
Flurochloridone	88.0	80.9	74.1	52.1	36.6	23.6	5.4	<0.3	<0.3
Others	0.4	0.5	0.5	0.4	0.1	0.4	0.4	0.4	0.2
Sediment	5.0	14.6	20.7	37.0	46.7	48.8	59.7	66.8	73.7
Extractable	*4.9*	*14.0*	*19.1*	*33.8*	*41.6*	*42.2*	*45.8*	*51.5*	*55.1*

T 1	)	)	)	0.3	0.9	0.9	2.1	2.0	2.7
T 2	|	} 0.2	} 0.3	) 0.4	) 0.6	0.2	0.4	0.5	0.6
T 3, 4, 5, 6	} 0.2	)	)	)	)	0.6	1.0	1.0	1.1
R 406639	|	0.2	0.3	0.5	1.1	1.4	1.5	1.4	1.5
R 42819	)	0.5	1.4	7.1	21.3	28.6	37.1	44.3	46.9
Flurochloridone	4.6	13.1	17.1	25.6	17.7	10.7	3.8	2.5	2.4
Others	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	0.1
Not extractable	*0.1*	*0.7*	*1.6*	*3.2*	*5.1*	*6.6*	*13.9*	*15.3*	*18.6*
total system									
$^{14}CO_2$	-	<0.1	<0.1	<0.1	<0.1	0.1	0.7	2.4	2.2
Other volatiles	-	0.1	0.1	0.5	0.7	0.6	1.3	2.6	3.1
RECOVERY	97.5	100.8	99.8	97.8	96.5	98.3	99.6	99.6	99.2

The study is considered valid.

Flurochloridone rapidly disappeared from water/sediment systems. A rapid movement of the applied radiolabel was observed from the water into the sediment. The metabolite R42819 was found to be relevant in the sediments of both systems and in the water phase of one system. A second metabolite (R406639) slightly exceeded 10% of the applied radioactivity, but only in the sediment of one system.

Figure 1: Proposed metabolic pathway of Flurochloridone in water/sediment systems



Van der Gauuw, A. (2004c).

In this study, DT_{50} values were calculated according to Timme, Frehse and Laska (1986), using laboratory data from Shaw, D. 1996a/b. These data were re-evaluated according to FOCUS Kinetics guidance.

a) Whole system. Degradation rates for the total system were calculated using various types of kinetics (including root 1st and 1.5 order). In order to derive single first-order (SFO) total-system half-lives suitable for use in FOCUS surface water modelling, degradation rates for Flurochloridone and its metabolites R406639 and R42819 were re-calculated using Model Maker. First-order kinetics was used for each transformation pathway.

   These values of DT_{50} were considered relevant for risk assessment.

Table 42: Summary of first-order total system half-lives for Flurochloridone, R406639 and R42819 in the whole system

Study	System	^{14}C.Iabelling position of Flurochioridone	DT_{50} (days)		
			Flurochioridone	**R406639**	**R42819**
Shaw, 1996a	Old Basing	Pyrrolidone	19.6	58.0	210
	Virginia Water	Pyrrolidone	10.3	np.	184
Shaw, 1996b	Old Basing	Phenyl	22.8	36.9	274
	Virginia Water	Phenyl	9.19	66.7	436
Geometric mean			**14.3**	**52.3**	**261**

np: Not performed since it was not possible to fit the experimental data due to values near to the limit of detection

b) Sediment. A different model was used to derive the water and sediment half-lives for Flurochloridone. Since it was not possible to derive a degradation rate for Flurochloridone in the water phase a conservative default value of 1000 days was used according to the recommendations of the FOCUS kinetics group. The rate constant was therefore set to 0.000693 days-1 (corresponding to a half-life of 1000 days) and the data fitted to obtain half-life in sediment. First-order kinetics was used for each transformation pathway.

The geometric mean sediment half-life was 6.3 days for Flurochloridone. For the water phase, a conservative default value of 1000 days was used.

Table 43: Summary of first-order sediment half-lives for Flurochloridone.

Study	**System**	**^{14}C.labelling position of Flurochioridone**	**Sediment half-life for Flurochioridone (d)**
Shaw, 1996a	Old Basing	Pyrrolidone	13.5
	Virginia Water	Pyrrolidone	3.21
Shaw, 1996b	Old Basing	Phenyl	12.8
	Virginia Water	Phenyl	2.93
Geometric mean			**6.3**

According to evaluation, these DT_{50} are not considered valid for risk assessment. Taking into account the Error level $\chi 2$ test obtained (see Table below), the DT_{50} values in sediment are not considered relevant.

Table 44: Statistical evaluation of DT_{50}sed (days) proposed by notifier

System	**Label**	**modification**	**DT_{50}sed**	**M_0**	**Error level $\chi 2$ test**
Old Basin	pyrrolidone	DT_{50}w 1000 days	13.5	87.99	31.4
Virginia system	pyrrolidone	DT_{50}w 1000 days	3.21	87.25	30.4
Old Basin	phenyl	DT_{50}w 1000 days	12.8	91.08	28.8
Virginia system	phenyl	DT_{50}w 1000 days	2.93	90.98	23.4

11.1.4.4 Photochemical degradation

A. van der Gaauw, 2004a.

The rate of photochemical degradation of ^{14}C-carbonyl and ^{14}C-phenyl labelled Flurochloridone was investigated in sterile buffer solution at pH 7.

An application solution for each test substance was prepared and the amount present in each application solution were determined by LSC. Aliquots of these treated buffer solutions were continuously illuminated for up to 15 days. A second application solution was prepared for the ^{14}C-phenyl-labelled Flurchloridone dark control samples.

Findings:

- A rapid photodegradation of Flurochloridone in sterile buffer solution at pH 7 was observed, while it was stable in the dark controls. The amount of ^{14}C-carbonyl and ^{14}C-phenyl-labelled Flurochloridone decreased from 96.9 % and 100 % on Day 0 to 18.9 % and 19.9 % after 15 days of irradiation, respectively.
- Besides the parent compound, the most significant fractions were characterised and identified as R406639 (M5), which increased to account for 41.5 % (^{14}C-carbonyl-label) and 37.7 % (^{14}C-phenyl-label) of the applied radioactivity after 15 days, and hydroxymethyl-pyrrol-2-one (M8), which reached maximum levels of 12.1 % and 11.1 % on day 15 for ^{14}C-carbonyl-label and ^{14}C-phenyl-label, respectively.

Table 45: Photolysis of [carbonyl-^{14}C] and [phenyl-^{14}C]-Flurochloridone in sterile buffer solution of pH 7 (duplicate samples, values given in % of applied radioactivity)

Sampling day	0	0.08	0.73	2	3	5	7	10	13	15
Carbonyl label										
Buffer (balance)	100.0	95.5	99.7	98.8	101.1	94.8	91.1	91.4	94.7	97.4
Flurochloridone	96.9	92.8	86.9	78.2	73.7	55.4	41.9	29.6	22.1	18.9
M1	n.d.	n.d.	n.d.	1.0	n.d.	2.1	2.0	2.5	2.8	3.2
M5 (R406639)	n.d.	n.d.	5.6	10.0	15.0	21.4	26.3	32.2	38.7	41.5
M6	n.d.	n.d.	n.d.	n.d.	1.3	1.5	1.2	1.6	1.5	1.5
M7	n.d.	n.d.	0.3	1.9	2.3	3.5	3.7	4.2	5.9	5.8
M8 (hydroxymethyl)	n.d.	n.d.	1.8	3.1	4.2	6.1	7.8	9.5	11.3	12.1
M9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.0	2.3	3.8	4.0
M10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.5	3.0	4.2	4.3
$^{14}CO_2$	-	<0.1	<0.1	0.2	0.3	0.6	0.8	1.0	1.5	1.8
Other volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	100.0	95.5	99.8	99.0	101.4	95.4	91.9	92.4	96.2	99.2
Phenyl label										
Buffer (balance)	100.0	94.3	93.2	92.9	95.4	94.0	93.9	93.1	92.5	91.1
Flurochloridone	100.0	94.3	85.6	78.4	72.3	59.8	47.8	34.0	24.5	19.9
M1	n.d.	n.d.	n.d.	n.d.	n.d.	1.8	1.4	2.2	2.4	2.9
M5 (R406639)	n.d.	n.d.	5.4	9.3	15.0	20.0	26.5	32.2	36.0	37.7
M6	n.d.	n.d.	n.d.	0.8	1.0	1.3	1.4	1.7	2.2	1.2
M7	n.d.	n.d.	0.4	1.4	2.2	4.0	3.9	5.3	5.7	7.5
M8 (hydroxymethyl)	n.d.	n.d.	1.8	3.1	4.4	5.8	8.1	9.9	11.0	11.1
M9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.5	3.7	4.6	3.2
M10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.8	2.1	3.0	2.9
$^{14}CO_2$	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.2
Other volatiles	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery	100.0	94.3	93.2	92.9	95.4	94.1	94.0	93.2	92.6	91.3

- not performed; n.d. not detected

- The concentrations were submitted to pseudo first-order kinetics in a two-compartment model. DT_{50} values of 6.2 days (^{14}C-carbonyl-label) and 6.8 days (^{14}C-phenyl-label) were

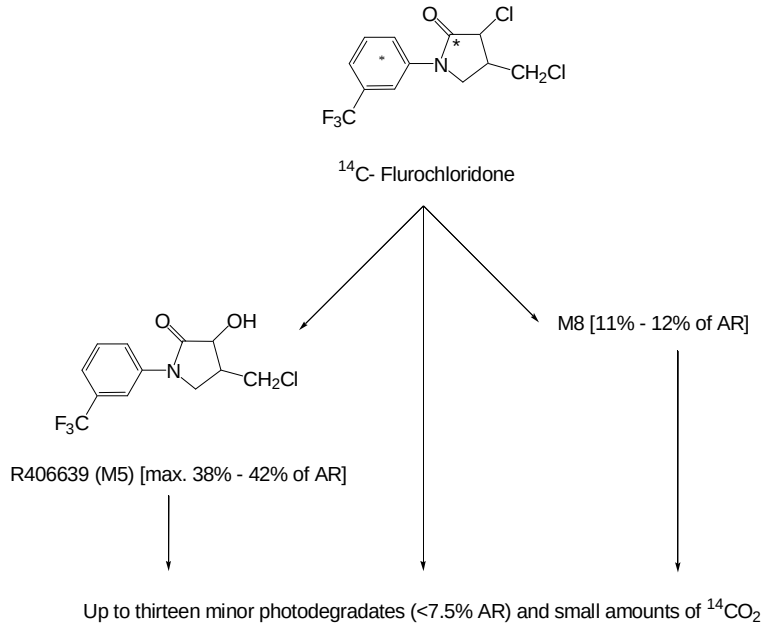
calculated. From the experimental data, environmental photolytic DT_{50} were calculated at different latitudes.

Table 46: Estimated environmental photolytic half-lives of Flurochloridone at different latitudes

	DT_{50} [days]		
Latitude :	50° N	40° N	30° N
[carbonyl-^{14}C]Flurochloridone	16.5	15.9	15.9
[phenyl-^{14}C]Flurochloridone	18.1	17.4	17.4

The proposed metabolic pathway of Flurochloridone in sterile buffer solution at pH 7 when exposed to light is shown below:

Figure 2: Proposed photolytic pathway of Flurochloridone in water (sterile buffer pH 7)



This study was considered valid.

After the period of comments during the PRAPeR review of the substance, in EFSA's view it is unlikely that there would be significant exposure by aquatic organisms to M8 metabolite under more realistic natural conditions. The maximum levels at the end of the study were reached after 38-40 days once equated to natural sunlight days and in natural sediment water systems the relatively more rapid partitioning to sediment and biodegradation would be expected to limit the potential for significant photolytic formation of M8.

G. D. Christian and W. C. Purdy, 1962.

The photodegradation of a mixture of *trans*- and *cis*-Flurochloridone was investigated in deionized water. The DT_{50} of Flurochloridone was calculated to be 6.9 days (first-order kinetics, linear regression). R406639 and R42819 were identified as principal products.

This study was considered supplementary as no Xenon lamp was used, the analytical method for monitoring Flurochloridone at different times was not proved specific and sensitive, volatile products were not trapped and spectra for the identification of the degradation products were not reported and the compounds were not quantified.

K. S. Lee; L. L. Chang; B. N. Giang; D. Kukla, 1985.

The photolytic degradation of Flurochloridone was investigated at 25ºC and pH 7 during 12 days. Significant degradation of Flurochloridone occurred when exposed to light, while there was only negligible reduction in concentration in the dark control. A DT_{50} of 4.3 days was calculated assuming pseudo first-order kinetics.

Calculation assuming pseudo first-order kinetics were also performed for *cis* and *trans* isomer. The data suggest that *cis* isomer is more susceptible toward photolytic decomposition (DT_{50} = 2.4 days) than trans isomer (DT_{50} = 4.3 days). R406639 and R42819 were accounted as principal products but R42819 could not be identified.

This study was considered supplementary as the mass balance is only available for the last day of sampling, the applied radioactivity is below 90% and traps were not used for trapping volatile compounds, the light source is not specified, neither the position of radiolabel carbon.

11.2 Environmental transformation of metals or inorganic metals compounds

11.2.1 Summary of data/information on environmental transformation

11.3 Environmental fate and other relevant information

Adsorption

Rowe, D. and Lane, M.C.G., 1994

The adsorption and desorption properties of Flurochloridone were investigated, according to OECD Guideline 106 (1981), in four soils of three textural classes: sand, dandy clay and sandy loam. The experiment was carried out on all four soils at 16-hour adsorption step followed by a single desorption step over the same period.

Findings:

The recoveries ranged between 90% and 97% of the applied radioactivity for all soils and concentrations. From the mass balance, it could be concluded that within the adsorption period no significant decomposition of the test compound occurred.

Average adsorption partition coefficients (Kd values) ranged from 7.6 in the sand (pH 5.1, 0.6% Corg) to 19 in the sandy loam (pH 8.5, 3.0% Corg). Freundlich adsorption coefficients (K') demonstrated a similar pattern, ranging from 6.5 to 14.

The adsorption data were fitted well by the Freundlich equation with a coefficient of determination R^2 being 1.0. The mean Freundlich exponent over all soils tested was 0.9, demonstrating that the sorption process was slightly non-linear with increasing equilibrium concentrations in the water phase. Adsorption, represented by the Kd values, was shown to follow approximately linearly the organic matter content of the soils ($R^2 > 0.988$).

Average Kd values adjusted for the organic C content of soil (Koc) ranged from 680 in the high pH sandy loam to 1300 in the low pH sand soil. Corresponding coefficients derived from the Freundlich adsorption coefficient (K'oc values) followed a similar pattern and ranged from 490 to 1100 (average 700).

The Koc values for the desorption step were somewhat higher than the adsorption values ranging from 780 to 1500. This indicates that adsorption was not fully reversible, resulting in further reduction in the potential mobility of the compound. Koc values increased with decreasing concentrations.

Table 47: Adsorption and desorption constants of Flurochloridone in four soils

Soil	Adsorption					Desorption	
	K_d	K_{oc}	K'	K'_{oc}	1/n	K_d	K_{oc}
Sandy loam (pH 8.5, 3.0% org. C)	19	680	14	490	0.91	23	780
Sand (pH 5.1, 0.6% org. C)	7.6	1300	6.5	1100	0.92	9.9	1700
Sandy loam (pH 6.7, 1.2% org. C)	10	870	7.8	670	0.89	13	1100
Sandy clay loam (pH 7.5, 1.7% org. C) Hyde	12	720	9.4	540	0.88	16	920
Average		893		700	0.9		1125

This study is considered valid.

11.4 Bioaccumulation

Table 48: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
EEC A.8. Shake flask method with GC/NPD	Log Pow = 3.36 at 20 ºC and pH=7	Finally accepted at the additional report to the DAR 2009	Goodmann 1994a
US EPA Guideline (1979), American Society for Testing and Materials E-35.21 draft 8 (1978) and E-47.01 draft 5 (1982), and Current bioconcentration test methods and theory (Hamelink J.L., 1977)	BCF(at steady-state) 220; BCF(at end of exposure): 292 in whole fish, 76 in edible tissues, 348 in non-edible tissues		Mc Allister, W.A., Franklin, L. and Cohle, P. 1984

11.4.1 Estimated bioaccumulation

11.4.2 Measured partition coefficient and bioaccumulation test data

With regard to the partition coefficient, one EEC A.8 study accomplishing GLP was carried out. The experimental value for the partition coefficient n-octanol/water (log Kow) is 3.36, at 20 ºC and pH=7. RMS in Addendum I pointed out that the effect of pH was not investigated and that a deviation from EEC A.8 was found. Later, at the additional report to the DAR, RMS commented about this study that as no effect of pH was observed for water solubility in the new water solubility study submitted (Weissenfeld, 2009), the effect of pH on the partition coefficient was not expected. The partition coefficient study was finally accepted at the Addendum II.

With regard to the bioaccumulation potential of Flurochloridone, a 28 days dynamic study on uptake, depuration and bioconcentration by bluegill sunfish (*Lepomis macrochirus*) was carried out. The test was separated in two phases, first the uptake phase (28 d) where fish were continuously exposed in a flow-through test system to a constant concentration of 0.089 mg a.s/L (mean value). Then, after the exposure period, fish were held in clean flowing water for a 14 days depuration period. The uptake rate constant (K_1) was 175 ppm in fish/ppm in water/day, the depuration rate constant (K_2) was 0.80 day-1 and the steady-state bioconcentration factor (BCF) for whole fish was determined using steady-state approach and were BCF at steady-state=220 in whole fish, BCF at the end of exposure=292 in whole fish, 76 in edible tissues and 348 in non-edible tissues.

Another study aimed to characterize metabolites in edible tissue of fish, which was taken from fish used at the bioconcentration study described above. RMS concluded from this study that the parent

represented about one third of the total radioactivity, and that a significant fraction corresponded to unknown metabolites, each below 1% of the total residue.

To conclude, on the basis of the available information, whether the substance has a high potential for bioaccumulation in aquatic organisms or not, the BCF value of 292 (in whole fish) from the dynamic study should be compared to the CLP criteria. Thus, Flurochloridone does not meet the criterion established by CLP (the experimentally determined BCF value is <500), so low potential for bioaccumulation is expected. The experimental log Kow value of 3.36 also means that Flurochloridone does not meet the cut-off value established by CLP (log Kow is <4). Nevertheless, as experimental derived BCF values are more preferred than log Kow values for classification purposes, the above mentioned BCF <500 would already determine that low potential for bioaccumulation is expected for this active substance.

11.5 Acute aquatic hazard

A brief summary of the aquatic toxicity studies listed in the Draft Assessment Report (DAR) is reported below. From all available ecotoxicity tests on this substance only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 49: Summary of relevant information on acute aquatic toxicity.

Method	Species	Test material	Results[1]	Remarks	Reference
Fish					
Short-term toxicity to fish: OECD Guideline 203 (1984)	*O. mykiss*	Flurochloridone technical	LC_{50} = 3.0 mg/L (nom)		Douglas, M.T., Handley, J.W., and MacDonald, I.A. 1987
Short-term toxicity to fish: U.S. EPA Ecological Research Series EPA-660/3-75-009 (1975) and American Public Health Association (1975): Standard Methods for the Examination of Water and Wastewater.	*L. macrochirus*	Flurochloridone technical	LC_{50} = 6.7 mg/L (nom)		Cohle, P.R. and McAllister, W.A. 1983
Aquatic invertebrates					
Short-term toxicity to aquatic invertebrates: ASTM guideline "Proposed Standard Practice for Conducting Static Acute Toxicity Tests on Wastewaters with Daphnia" (1981) and recommendations of the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975)	*D. magna*	Flurochloridone technical	EC_{50} = 5.1 mg/L (nom)		Spare, W.C. 1983
Algae					
Algistatic activity on the growth of blue green algae: US EPA Guideline 123-2 (1982)	*A. flos-aquae*	Flurochloridone technical	ErC_{50} (72h) =13.4 mg/L (nom)		Wallace, S.J. and Swarbrick, R.H. 2001
Algistatic activity on the growth of blue green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	Flurochloridone technical	ErC_{50} (72h) = 0.0047 mg/L (mm)		Bätscher, R. 2004a

Toxicity of metabolite R406639 to green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	R406639	ErC_{50}(72h) = 3.3 mg/L (nom)		Bätscher, R. 2004b
Toxicity of metabolite R42819 to green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	R42819	ErC_{50}(72h) = 2.3 mg/L (nom)		Bätscher, R. 2004c
Aquatic plants					
Toxic effects on the duckweed *Lemna gibba*: US EPA FIFRA Subdivision J Guideline 123-2 and OECD Guideline 221	*Lemna gibba*	Flurochloridone technical	$ErC_{50(frond\ number)}$ = 0.06 mg/L (mm)		Woodyer, J.E., Swarbrick, R.H., and Shillaber, N. 2001
Toxicity of metabolite R42819 to *Lemna gibba*: OECD Guideline 221	*Lemna gibba*	R42819	ErC_{50}= 8.2 mg/L (nom)		Bätscher, R. 2003

[1] (mm) for measured concentration, (nom) for nominal concentration.

11.5.1 Acute (short-term) toxicity to fish

Douglas, M.T., Handley, J.W., and MacDonald, I.A. (1987)

Oncorhynchus mykiss were exposed to Flurochloridone technical in an acute toxicity study for a 96 hours period, under dynamic conditions. After a 7 day acclimatisation period, groups of 10 fish (mean length of 4.1 cm and mean weight of 0.99 g at study start) were each exposed to nominal concentrations of 0.56, 1.0, 1.8, 3.2 and 5.6 mg a.s./L and a solvent control (100 µL/L auxiliary solvent). Test vessels (glass aquarium) contained 20 L of test media (loading 0.5 g/L, static volume). The test media was renewed continuously at a rate 6 L per hour (7.2 tank volumes per day). The fish were not fed during the test.

Temperature in water was 14±1°C, the pH values ranged from 7.5 to 7.7 and the dissolved oxygen concentrations from 8.5 to 10.2 mg O_2/L during the 96 hour exposure period. A 16 hour light and 8 hour dark photoperiod occurred during the test.

The test fish were observed daily for mortalities and abnormalities in the behaviour or physical appearance. The mortality data were analysed by the method of Thompson and Weil (1952).

The exposure concentrations of Flurochloridone technical in the test water were verified at 0, 24 and 96 hours in all test concentrations and at 48 and 72 hours in 0.56 and 1.0 mg a.s./L.

Findings: The mean measured concentrations of flurochloridone technical in the test medium ranged from 60% to 125% of nominal after the 96 hour exposure. The exception was that after 96 hours at nominal 5.6 mg/L only 46% of this concentration was measured (2.55 mg/L).

RMS commented the following: the concentration of a.s. in one test concentration dropped below 80% of nominal (46% after 96 hours at nominal 5.6 mg/L). However, this deviation is not considered to have an impact on the overall validity of the study.

The 96-hour LC50 was calculated to be 3.0 mg a.s./L (95% confidence limits: 2.4-3.7 mg a.s./L). The NOEC was determined to be 1.0 mg a.s./L. and the LC50 was determined to be 3.0 mg a.s./L (95% confidence limits: 2.4-3.7 mg/L).

Cohle, P.R. and McAllister, W.A. (1983)

Short-term toxicity of Flurochloridone technical was determinate to bluegill sunfish (*Lepomis macrochirus)* under static conditions over 96 hours. After 48 hour acclimatisation, 10 fish (mean length of 2.1 cm and mean body weight of 0.21 g at study start) were each exposed to nominal concentrations of 1.0, 1.8, 3.2, 5.6 and 10 mg a.s./L and a solvent control (10 mL acetone, equivalent to the quantity used in the highest test concentration). Test concentrations were prepared after correcting for purity. Each test vessel (18.9 L glass vessel) contained 15 L of soft reconstituted water. The fish were not fed during the test.

The test vessels were kept in a water bath at 22±1°C. The pH value ranged from 7.0 to 7.6 and the dissolved oxygen concentrations (DOC) from 5.6 to 8.9 mg O_2/L (62-99% saturation).

Observations: The test fish were observed after 24, 48, 72 and 96 hours for mortality and symptoms of intoxication. Statistical analysis of the mortality data was obtained by employing a computer program developed by Stephan using binomial test. Three different methods were used (binomial, moving average and probit) and the method selected was the one which gives the narrowest confidence limits for the LC_{50}. The LC_{50} values were obtained by non-linear interpolation.

Findings: No mortalities were observed in the control and at test concentrations up to and including 3.2 mg a.s./L during the test period of 96 hours. At 5.6 mg/L, 10% mortality had occurred after 48 hours and 20% after 96 hours. At 10 mg a.s./L, the highest concentration tested, all fish were dead already after 24 hours of exposure. Mortality was accompanied by such effects as surfacing, loss of equilibrium, quiescence and fish resting on the bottom of the test chambers, even at the 3.2 mg a.s./L concentration.

RMS commented that in the study no measurement of samples are mentioned. RMS concluded about this study: No analytical verification for Flurochloridone was done. This shortcoming does not make invalid this study, because test preparations were described with many details. Taking into account the measured test concentrations from the above study, the stability of Flurochloridone in water is demonstrated since measure values after 24 hours are very close to the corresponding values after 96 hours in test medium.

For bluegill sunfish, the 96 hour LC_{50} value was calculated to be 6.7 mg a.s./L. The 96 hour NOEC was determined as 1.8 mg a.s./L, based on the lack of mortality and abnormal effects.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Spare, W.C. (1983)

The acute toxicity of Flurochloridone technical to the water flea *Daphnia magna* Straus was determined under static conditions over 48 hours. Four replicates of 5 daphnids (1st instars, <20 hours old) per treatment were exposed to nominal test concentrations of 0.6, 1.1, 1.9, 3.4 and 6.0 mg a.s./L, a water and a solvent control. Each test chamber (250 mL beaker) contained 200 mL of test solution. The water fleas were not fed during the test.

A photoperiod of 16 hours light and 8 hour dark occurred during the test. Temperature in water ranged from 21 to 22°C and the pH values from 7.0 to 7.6. The dissolved oxygen concentration was between 8.0 and 8.4 mg O_2/L at initiation of the test in the treatment groups and in the controls. After 48 hours, the concentrations have decreased to 7.6 mg O_2/L in the water control, 5.5-6.4 mg O_2/L in the three lower test concentrations, 4.2 mg O_2/L in the solvent control and 3.6 mg O_2/L in the two highest test concentrations.

A reference toxicant ($K_2Cr_2O_7$) was tested concurrently. Its LC_{50} value was calculated to be 0.41 mg/L for the 48h period.

Table 50:

Nominal Concentration (mg/l)	Mortality (%)	
	24 hours	48 hours
0	0	0
0.6	0	0
1.1	0	0
1.9	0	0
3.4	0	10
6.0	25	70
LC_{50} (mg/L)		5.1 (4.4 - 6.1)
NOEC		1.9

Observations: Mortalities were recorded at 24 and 48 hours. The LC_{50} values were calculated using the probit method (Stephan, 1979). All calculations were based on nominal concentrations. No mortality was observed in the two controls and in the nominal concentration up to and including 1.9 mg a.s./L, during the test period of 48 hours. In the 3.4 mg a.s./L treatment group no mortality was found after 24 hours of exposure and 10% of dead daphnids was recorded after 48 hours. At 6.0 mg a.s./L (the highest test concentration) 25% and 70% mortalities were observed after 24 and 48 hours, respectively. The 24 hour LC_{50} value was greater than 6.0 mg/L, which was the highest concentration tested.

The LC_{50} values were calculated using the Probit method. All calculations were based on nominal concentrations. The 48-hour LC_{50} was calculated to be 5.1 mg as./L, and the 48 hour NOEC was determined to be 1.9 mg as./L.

RMS considered this study valid, and added about this report the following: there is no evidence regarding to the maintenance of test concentrations over the 48 hours of exposure. However, from other ecotoxicological tests conducted with Daphnia along 21 days under semi-static conditions it is demonstrated that test substance concentration is kept above 80% nominal after 48 hours without renewal. In addition, from the chemical properties of the test substance, no significant degradation is expected to occur during 48 hours, since hydrolytic DT_{50} value is higher than 100 days. Considering both assumptions, maintenance of test concentration along the test is expected.

The toxicity of Flurochloridone to water flea under 48 hour static condition exposure was LC_{50}= 5.1 mg a.s./L.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

As stated under point 11.5, only relevant tests considered as valid by RMS in the DAR have been included.

Wallace, S.J. and Swarbrick, R.H. (2001)

A test was carried out to determinate the algistatic activity of this substance on the growth of the blue green alga *Anabaena flos-aquae* (CCAP 1403/13A) under static conditions over three days. It was conducted under GLP. Three replicates each were exposed to nominal concentrations of 0.56, 1.0, 1.8, 3.2, 5.6, 10 and 18 mg a.s./L and six replicates to a control. The pH values ranged from 7.30 to 7.53 at the start of the test and from 7.44 to 7.68 at the end of the test. The daily temperature ranged from 24.0°C to 24.3°C.

The concentrations of Flurochloridone in the test solutions were measured at 0 and 72 hours from all concentrations. The overall mean measured concentrations of Flurochloridone ranged from 72% to

99% of nominal. All results are based on mean measured concentrations. The algal cell densities were determined after 24, 48 and 72 hours of exposure by spectrophotometric absorbance using an UV/visible spectrophotometer.

The area under the growth curve and the growth rate were calculated for the 0 to 72 hours interval. The EC values for biomass (EbC_{50}) and growth rate (ErC_{50}) were calculated for the 0-72 hours interval from the area under the growth curve using the Weibull program. These data were examined by one-way analysis of variance and Dunnett's test was used to identify significant differences between the treatment groups and the control (p=0.05) and to determine the LOEC and NOEC values.

Table 51: Analytical determination of flurochloridone and impact on mean cell concentration in algal growth inhibition test with *Anabaena flos-aquae* in a static test system.

Nominal conc. Flurochloridone	**Geometric Mean Measured conc. flurochloridone**	**Mean cell concentration (* 10^4 cell/ml)**			**Mean growth rates (day^{-1})**	**Mean area under growth curve**
(mg a.s./L)	**(mg a.s./L)**	**24 hours**	**48 hours**	**72 hours**	**0-72 hours**	**0-72 hours**
Control	< 0.0089	0.0327	0.1290	0.6277	1.545	0.46
0.56	0.48	0.0355	0.1154	0.5741	1.515	0.42
1.0	0.84	0.0306	0.1254	0.6188	1.540	0.45
1.8	1.5	0.0386	0.1150	0.6211	1.541	0.45
3.2	2.9	0.0417	0.1355	0.5148	1.478	0.42
5.6	5.1	0.0363	0.1062	0.4213	1.412*	0.34*
10	9.9	0.0337	0.0799	0.2389	1.223*	0.22*
18	13	0.0277	0.0395	0.0707	0.817*	0.09*

ErC_{50} (mg a.s/L)	13.4	(10.2-16.6, 95% confidence limits)
EbC_{50} (mg a.s/L)	8.85	(7.59-10.1, 95% confidence limits)
NOErC (mg a.s/L)	2.9	
NOEbC (mg a.s/L)	2.9	

* Mean value significantly different from the solvent control.

The 0-72 hour EC_{50} values for the biomass and growth rate were calculated to be 8.85 mg a.s/L and **13.4 mg a.s/L**, respectively (95% confidence limits: 7.59-10.1 mg as./L and 10.2-16.6 mg a.s./L). Biomass and growth rates were significantly different from the control at 5.1, 9.9 and 13 mg a.s./L. Accordingly, the NOEC for both the biomass and the growth rate was determined to be **2.9 mg a.s./L**, and the LOEC value 5.1 mg a.s./L.

Bätscher, R. (2004a)

This study seeked the determination of the algistatic activity of Flurochloridone on the growth of the blue green alga *Scenedesmus subspicatus*, in a 72 hours test under static conditions. It was conducted under GLP.

At the beginning of the test, 10000 algal cells/mL were inoculated (3 days old) in 50 mL Erlenmeyer flasks. Three replicates each were exposed to nominal concentrations of 0, 0.32, 1.0, 3.2, 10, 32 and 100 µg a.s./L and six replicates to the solvent control (DMF, 100µg/L test water). The flasks were covered with glass dishes and incubated in a temperature-controlled water bath at 23-24°C and continuously illumination and stirring. The pH values ranged from 8.0 to 8.2 at the start of the test and from 7.9 to 8.8 at the end of the test.

The concentrations of Flurochloridone in the test solutions were measured at 0 and 72 hours from all concentrations by HPLC method. Recoveries of spiked test water samples at relevant concentrations ranged between 96% and 108%. The algal cell densities were determined after 24, 48 and 72 hours of exposure with an electronic particle counter. The area under the growth curve (biomass) and the growth rate were calculated for each test flask. Based on these values, the arithmetic mean area and growth rate were determined. The EbC_{50} and ErC_{50} values were calculated by Probit analysis. For the determination of the LOEC and NOEC, the calculated mean biomass and growth rate at the test concentration were tested for significant differences when compared to the solvent control values by a Dunnett- test.

The overall mean measured concentrations of the test substance at the test concentrations from 3.2 to 100 µg/L were in the range of 70% to 88% of nominal. At the two lowest test concentrations of nominal 0.32 and 1.0 µg/L the test concentrations could not directly verified (contamination of analytical samples. However, in the respective application solutions 88% and 86% of nominal were measured, respectively, demonstrating the correct dosage of the test substance. The biological results are based on the mean measured concentrations either in the test media or in the application solutions.

The EC_{50} values for biomass and growth rate were calculated to be 2.1 µg/L and **4.7 µg/L**, respectively. A statistically significant inhibitory effect on the growth (biomass and growth rate) of *Scenedesmus subspicatus* was observed at 0.86 µg/L (LOEC) and higher concentrations. No effects were seen at **0.28 µg/L** (NOEC). The percentage inhibition at 0.28 µg/L and 0.86 µg/L was 1.3% and 5.2% (0-72 hours), respectively.

Table 52: Analytical determination of flurochloridone and impact on mean cell concentration in algal growth inhibition test with *Scenedesmus subspicatus* in a static test system.

Nominal conc. flurochloridone	**Measured concentration flurochloridone (µg a.s/L)**			**Mean cell density (* 10^4 cell/ml)**			**Mean growth rates (day^{-1}) (% inhibition)**	**Mean area under growth curve (% inhibition)**
(µg a.s/L)	**0 h**	**72 h**	**Mean**	**24 h**	**48 h**	**72 h**	**0-72 h**	**0-72 h**
Control				2.87	10.95	58.68	1.36 (-0.4%)	976 (-2.8%)
Solvent control				2.68	10.52	57.73	1.35 (---)	950 (---)
0.32	0.63	1.1	0.86[a] **0.28**[b]	2.87	10.38	54.73	1.33 (1.3%)	915 (3.7%)
1.0	1.48	1.19	1.34[a] **0.86**[b]	2.55	9.82	47.15	1.28* (5.2%)	803* (15.5%)
3.2	2.68	2.64	2.66	2.58	4.53	8.47	0.71* (47.4%)	212* (77.6%)
10	7.96	7.70	7.83	2.12	2.87	2.80	0.34* (74.7%)	93* (90.2%)
32	29	27	28	2.13	2.15	1.75	0.19* (86.2%)	64* (93.3%)
100	76	64	70	2.10	2.22	1.60	0.15* (88.7%)	63* (93.4%)

ErC_{50} (µg a.s/L)	4.7	(1.9-12, 95% confidence limits)
****EbC_{50} (µg a.s/L)**	2.1	(0.2-11 95% confidence limits)
NOErC (µg a.s/L)	0.28	
NOEbC (µg a.s/L)	0.28	

[a] Values corresponding to these concentrations are far from nominal. Probably it is due to contamination of the glass bottles used for sample storage. Contamination of test vessels is excluded because single samples show reliable results (these are shown below, labelled with [b]). This would not be possible if the contamination had occurred before the sampling procedure.

[b] These values correspond to the measured concentration in the application solutions. They were 88 and 86% from nominal, respectively. These will be considered for endpoints calculations.

* Mean value significantly different from the solvent control.

The toxicity values for *Scenedesmus subspicatus* exposed to Flurochloridone for 72 hour were EC_{50} for the biomass and growth rate were calculated to be 2.1 µg a.s./L and 4.7 µg a.s./L respectively. NOEC for both the biomass and the growth rate was determined to be 0.28 µg a.s./L.

Bätscher, R. (2004b) and Bätscher, R. (2004c)

Toxicity of the Flurochloridone metabolites R406639 and R42819 to green algae *Scenedesmus subspicatus* was tested in these two 72 hours algal growth inhibition tests, under static conditions. Both studies were conducted following the guideline OECD 201 and Directive 92/69/EEC, C.3 with deviations. Both were performed under GLP, and they were considered valid by RMS.

Three replicates each were exposed to a range of concentrations of the test substance and six replicates to the control. In the study with R406639, the following nominal concentrations were tested: 0.46, 1.0, 2.2, 4.6 and 10 mg/L. In the study with R42819, the nominal test concentrations were 0.032, 0.1, 0.32, 1.0, 3.2, and 10 mg/L.

The pH values ranged from 7.8 to 8.2 at the start of the test and from 7.8 to 9.4 at the end of the test. The concentrations of the metabolites in the test solutions were measured at 0 and 72 hours from all concentrations. The algal cell densities were determined after 24, 48 and 72 hours of exposure with an electronic particle counter.

The area under the growth curve (biomass) and the growth rate were calculated for each test flask. Based on these values, the arithmetic mean area and arithmetic mean growth rate were determined. The EbC50 and ErC50 values were calculated by Probit analysis. For the determination of the LOEC and NOEC, the calculated mean biomass and growth rate at the test concentration were tested for significant differences when compared to the control values by a Dunnett-test.

The overall mean measured concentrations of the metabolite R406639 were in the range of 89 to 110% of nominal. The overall mean measured concentrations of the metabolite R42819 ranged from 103% to 110. Therefore, the biological results are based on the nominal concentrations.

Table 53: Analytical determination of R406639 and impact on mean cell concentration in algal growth inhibition test with *Scenedesmus subspicatus* in a 72 h static test system.

Nominal conc. R406639	**Aritmetic[a] Mean Measured conc. R406639**	**Mean cell concentration (x 10^4 cell/ml)**			**Mean growth rates (day^{-1}) | (Ir%)**		**Mean area under growth curve (x 10^4) | (I_{AUC} %)**	
(mg a.s/L)	**(mg a.s/L)**	**24 hours**	**48 hours**	**72 hours**	**0-72 hours**		**0-72 hours**	
Control	-	4.42	9.94	54.34	1.33	0.0	937	0.0
0.46	0.443	4.47	10.38	51.10	1.31	1.5	917	2.1
1.0	1.1	4.93	7.85	48.92	1.29	2.8	834	11.0
2.2	2.14	3.40	4.55	13.12	0.86	35.6	288	69.2
4.6	4.21	2.20	2.32	3.68	0.43	67.1	93	90.1
10	8.90	1.52	1.52	1.48	0.13	90.5	31	96.7

[a.] The R406639 measured values at 72 hours are very close to initial. In this case there is not difference in using arithmetic or geometric mean to express the metabolite concentration.

Table 54: Analytical determination of R42819 and impact on mean cell concentration in algal growth inhibition test with *Scenedesmus subspicatus* in a 72 h static test system.

Nominal conc. R42819	Aritmetic[a] Mean Measured conc. R42819	Mean cell concentration (x 10^4 cell/ml)			Mean growth rates (day^{-1}) | (Ir%)		Mean area under growth curve (x 10^4) | (I_{AUC} %)	
(mg a.s/L)	(mg a.s/L)	24 hours	48 hours	72 hours	0-72 hours		0-72 hours	
Control	-	5.20	18.29	104.67	1.55	0.0	1760	0.0
0.032	n.m	5.25	19.6	108.67	1.56	-0.9	1840	-4.6
0.1	n m.	5.32	18.45	105.58	1.55	-0.2	1777	-1.0
0.32	0.352	5.68	18.88	112.83	1.57	-1.6	1884	-7.0
1.0	1.08	5.45	7.75	23.93	1.06	31.8	544	69.1
3.2	3.38	2.80	3.02	3.75	0.44	71.6	125	92.9
10	10.3	2.42	2.40	2.42	0.29	81.2	85	95.2

n.m (no measured). The samples from the test concentrations of nominal 0.032 and 0.10 mg/L were not analysed since these test concentrations were below the determined 72-h NOEC.

[a] The R42819 measured values at 72 hours are very close to initial. In this case there is not difference in using arithmetic or geometric mean to express the metabolite concentration.

Table 55:

	R406639 [mg/L] and 95% confidence limits	R42819 [mg/L] and 95% confidence limits
EbC_{50} (0-72 hours)	1.9 (1.2 - 3.0)	0.99 (0.13 - 7.6)
ErC_{50} (0-72 hours)	3.3 (2.6 - 4.3)	2.3 (1.3 - 4.4)
NOEC	0.46* / 1.0**	0.32* / 0.32**

*biomass

**growth rate

R406639 had a statistically significant inhibitory effect on the growth (i.e. biomass) of *Scenedesmus subspicatus* at 1.0 mg/L (LOEC) and higher concentrations. No effects on biomass were seen at 0.46 mg/L (NOE_bC). The growth rate was affected at 2.2 mg/L, but no effects were seen at **1.0 mg/l** (NOE_rC). The EC_{50} values for biomass and growth rate were calculated to be 1.9 mg/L and **3.3 mg/L**, respectively.

R42819 had a statistically significant inhibitory effect on the growth (biomass and growth rate) of *Scenedesmus subspicatus* at 1.0 mg/L (LOEC) and higher concentrations. No effects were seen at **0.32 mg/L** (NOEC). The EC_{50} values for biomass and growth rate were calculated to be 0.99 mg/L and **2.3 mg/L**, respectively.

The conclusion is that both metabolites R406639 and R42819 showed low acute toxicity to the green algae. According to the values included in the previous paragraphs, the metabolites are by a factor of at least 470 less toxic to *Scenedesmus subspicatus* compared to parent.

Woodyer, J.E., Swarbrick, R.H., and Shillaber, N. (2001)

A study to determinate the toxic effect of Flurochloridone technical on the duckweed *Lemna gibba* was undergone under semi-static conditions, over 14 days. It was conducted under GLP. Deviations to OPPTS and ASTM: High pH values were observed on Days 5 and 9 in the old test solutions due to a slight contamination by the new solutions. However, this was not considered to have an effect on the study, since the contamination occurred after the solutions had been removed from the exposure part of the study.

The test concentrations were nominal 10, 20, 40, 80, 160, 320 and 640 µg as./L. The test solutions were renewed on Day 5 and 9. Three replicates with 3 plants of 4 fronds each (total 12 fronds per replicate) were exposed to the test concentrations and the culture medium control (M-Hoagland's medium, pH 5.0). Temperature in the incubator ranged from 24.8 to 25.3°C and the pH values ranged from 4.3 to 4.6 in the newly prepared test solutions, and from 4.7 to 5.9 in the old test solutions.

The data for frond and weight increase were examined by one-way analysis of variance. Dunnett's test was used to identify significant inhibitions from the culture medium control (p=0.05, one-sided) and to determine the LOEC and NOEC values. The data for percentage inhibition (frond number and dry weight) were analysed using the moving average angle method and the 14-day EC_{50} was calculated using Stephan's method.

The overall mean measured concentrations of tested substance in the new and old test solutions ranged from 69 to 93% of nominal. Therefore, all results are based on the mean measured concentrations of 9.3, 15, 29, 61, 110, 240 and 500 µg a.s./L.

Frond growth (Exposure phase): During the 14 day exposure period, the mean increase in numbers of fronds (ΔFN) in the control (365) was comparable to that at the two lowest test concentrations of 9.3 and 15 µg a.s./L with 370 and 423, respectively. However, the mean increase in numbers of frond were significantly different from the control at the concentrations of 29 to 500 µg a.s./L, with 33 to 92% inhibition. Therefore, the 14day NOEC was determined to be 15 µg a.s./L. The 14 day EC_{50} for frond number increase was calculated to be 60 µg as./L (95% confidence limits: 54 - 67 µg as./L).

Dry weight (dw): The mean dry weight of the control plants at Day 14 was 45.6 mg. At 9.3 and 15 µg as./L, dry weights were comparable to the control with 54.4 and 61.4 mg, respectively. However, there were significant differences from the control at test concentrations of 29 to 500 µg a.s./L, with 44 to 95% inhibition. Therefore, the 14day NOEC was determined to be 15 µg a.s./L. The 14day EC_50 for weight increase was calculated to be 48 µg a.s./L (95% confidence limits: 43 - 53 µg as./L).

Symptoms of toxicity: There were no symptoms noted in the control and at 9.3 and 15 µg as./L. On Day 2, a slight yellowing of the fronds were observed at 110, 240 and 500 µg a.s./L. On Day 5, a slight discolouration of the fronds was observed at 61 µg a.s./L, and most of the fronds at 110, 240 and 500 µg a.s./L were pale and showed reduced root lengths. On Day 14, the fronds were discoloured in the test concentrations 29 to 500 µg a.s./L.

Table 56:

Flurochloridone concentration (µg a.s./L)		**Day 14 frond number (ΔFN)[2]**		**Day 14 dry weight (dw)**	
Nominal	**Overall Measured[1]**	**Mean**	**Inhibition (%)**	**Mean (mg)**	**Inhibition (%)**
Dilution water control	<1.7				
Culture medium control		377	-	45.6	-
10	9.3	382	-[3]	45.4	-[3]
20	15	435	-	61.4	-[3]
40	29	257	33*	26.1*	44*
80	61	85	80*	5.9*	90*
160	110	73	83*	5.0*	92*
320	240	60	87*	4.7*	93*
640	500	41	92*	3.8*	95*

[1] Geometric mean of each set of new and old results (at day 5 and day 9) was calculated and the overall mean measured concentration was calculated as an arithmetic mean of these results from 0 day to 14 day. The highest limit of detection is 1.7 µg a.s/L.
[2] Inoculum (day 0) was 12 fronds per replicate at test initiation there were 5 plants and a total of 15 fronds in each replicate.
[3]. Increase in from number or dry weight when compared with the culture medium control.
* Significantly different compared to pooled control (p<0.05) from the culture medium control.

Based on mean measured concentrations, the overall 14 day NOEC (frond number and dry weight) is **0.015 mg a.s./L**. The 14 day EC_{50} values for increase of frond number and dry weight were calculated to be **0.060** and 0.048 mg a.s./L, respectively.

Bätscher, R. (2003)

R42819 metabolite's toxicity to the aquatic plant *Lemna gibba* was studied in a 7day static growth inhibition test. The study was performed under GLP. Three replicates per test concentrations of nominal 0.46, 1.0, 2.2, 4.6 and 10 mg/L and three control replicates were prepared. At study start, the test vessels were inoculated with three Lemna colonies consisting of four fronds each (twelve fronds per vessel). The test vessels were covered with glass lids and incubated in a temperature-controlled water bath under continuous illumination. During the test the temperature ranged from 23°C to 24°C, and the pH from 7.5 to 8.9.

Duplicate samples of the test media of all test concentrations and the control were analysed for R42819 at the start and end of the test. Frond and colony numbers and toxic effects (e.g. discoloration, sinking, root length or other abnormalities) were determined on Days 3 and 5, and at the end of the test (Day 7). Additionally, the dry weight of a sample of fronds identical to that used to inoculate the test vessels was determined at the start of the test. At test termination, the dry weight of all colonies per test vessel was determined. From the observed parameters, the growth rate, area under the growth curve and final biomass were calculated. The NOEC and LOEC were determined by testing the growth parameters (growth rate, area under the growth curve and final biomass) at the test concentrations on statistically significant differences to the control values by Dunnett's test ($p=0.05$). EC_{50} values were calculated using Probit analysis.

The measured concentrations of R42819 in the test media ranged from 89 to 98% and from 92 to 99% of nominal at the start and the end of the test, respectively. Therefore, the results were calculated using nominal concentrations.

After 7 days of exposure, the growth of *Lemna gibba* was significantly inhibited by this metabolite. Statistically significant reductions in growth rate, area under the growth curve and final biomass were found at 1.0 mg/L and for all concentrations above. The NOEC and LOEC were determined by testing the growth parameters (growth rate, area under the growth curve and final biomass) at the test concentrations on statistically significant differences to the control values by Dunnett's test ($p=0.05$). EC_{50} values were calculated using Probit analysis. Accordingly, the LOEC and NOEC for all growth parameters were established at 1.0 mg/L and **0.46 mg/L**, respectively.

The 7 day EC_{50} values were calculated to be **8.2 mg/L** (95% confidence limits: 6.7-11 mg/L) for growth rate, 5.9 mg/L (95% confidence limits: 4.7-8.1 mg/L) for area under the growth curve and 3.0 mg/L (95% confidence limits: 1.8-5.4 mg/L) for final biomass (based on dry weight). According to these results, the metabolite R42819 is by a factor of at least 60 less toxic to *Lemna gibba* than the parent Flurochloridone.

Table 57:

R42819 concentration (µg a.s./L)		Day 7 frond number[1]	7-day Growth rate		Day 14 dry weight (biomass)	
Nominal	**Overall Measured**	**Mean**	**(1/day)**	**Inhibition (%)**	**Mean (mg)**	**Inhibition (%)**
Culture medium control		135.0	0.35	0	16.1	-
0.46	0.417	134.3	0.35	0.2	16.4	-[2]
1.0	0.916	118.3	0.33*	5.6	13.1*	20.7
2.2	2.08	94.0	0.29*	15.0	9.6*	44.5*
4.6	4.45	65.0	0.24*	30.2	5.8*	70*
10	9.83	34.0	0.15*	57.0	4.4*	79.4*

[1] Inoculum (day 0) was 12 fronds per replicate at test initiation.
[2] Increase in frond number or dry weight when compared with the culture medium control.
* Significantly different compared to pooled control ($p<0.05$) from the culture medium control.

Regarding the other Flurochloridone's metabolite, R406639, no data addressing its toxicity to aquatic plants has been submitted in the original DAR. This metabolite reached up to 14% in water/sediment study (it is considered relevant). However, no additional information is necessary based on the fact that the toxicity of this metabolite in algae *Scenedesmus subspicatus* was several orders of magnitude less toxic than the parent. Then, parent will cover the potential risk due to this metabolite R406639.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

A brief summary of the long-term aquatic toxicity studies listed in the Draft Assessment Report (DAR) is reported below. From all available ecotoxicity tests on this substance only information considered adequate, reliable and relevant for the classification proposal has been included.

Table 58: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
Fish prolonged toxicity test 14 day study: OECD Guideline 204 (1984)[2]	*O. mykiss*	Flurochloridone technical	NOEC(28d) = 0.36 mg/L (mm)	Outdated guideline, the result won't be taken into consideration	Smith, G.J. 1990
D.magna reproduction test: OECD Guideline 211(1984)	*D. magna*	Flurochloridone technical	NOEC(21d) = 0.83 mg/L (mm)		Stewart, K.M., Tapp, J.F., Sankey, S.A., Williams, T.D. and Stanley, R.D. 1990
Effects on sediment dwelling organisms: BBA Guideline "Long-term toxicity test with Chironomus riparius: Development and validation of a new test system " (1995)	*C. riparius*	Flurochloridone technical	NOEC(25d) >0.25 mg/L (nom)	Only as additional information, this endpoint was not accepted for risk assessment	Gentle, W.E. 1997

Algistatic activity on the growth of blue green algae: US EPA Guideline 123-2 (1982)	*A. flos-aquae*	Flurochloridone technical	NOErC =2.9 mg/L (nom)		Wallace, S.J. and Swarbrick, R.H. 2001
Algistatic activity on the growth of blue green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	Flurochloridone technical	NOErC =0.00028 mg/L (mm)		Bätscher, R. 2004a
Toxicity of metabolite R406639 to green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	R406639	NOErC =1.0 mg/L (nom)		Bätscher, R. 2004b
Toxicity of metabolite R42819 to green algae: OECD Guideline 201 and Directive 92/69/EEC, C.3	*S. subspicatus*	R42819	NOErC = 0.3 mg/L (nom)		Bätscher, R. 2004c
Toxic effects on the duckweed *Lemna gibba*: US EPA FIFRA Subdivision J Guideline 123-2 and OECD Guideline 221	*Lemna gibba*	Flurochloridone technical	NOErC = 0.015 mg/L (mm)		Woodyer, J.E., Swarbrick, R.H., and Shillaber, N. 2001
Toxicity of metabolite R42819 to *Lemna gibba*: OECD Guideline 221	*Lemna gibba*	R42819	NOErC = 0.46 mg/L (nom)		Bätscher, R. 2003

[1] (mm) for measured concentration, (nom) for nominal concentration.

[2] In 2014 OECD removed this guidance.

11.6.1 Chronic toxicity to fish

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to analyse the effects of Flurochloridone technical on the survival and behaviour, under flow-through conditions over 28 days. The study was conducted in 1990 in compliance with an international guideline and under GLP.

Juvenile fish were purchased as eyed eggs and were placed under the specific laboratory conditions (15±2°C water temperature, 16 hour light/8 hour dark photoperiod). The eggs hatched between 9 and 15 days after receipt. About two months later, groups of 10 fish were exposed to nominal concentrations of 0.28, 0.57, 1.14, 2.28 and 4.56 mg a.s./L. A dilution water control and solvent (acetone) control were additionally included in the experiment. The test vessels (15 L glass vessels) contained a volume of 13.1 L (26 cm depth, no aeration) and were located in a temperature controlled water bath. The test solutions were renewed at a rate of 1000 mL every 27.6 minutes (6 tank volume replacements per 24 hour period). The fish were fed trout food daily during the test.

Temperature in test solutions ranged from 14.5 to 16.3°C, the pH-value from 7.1 to 7.5 and the dissolved oxygen concentration from 6.4 to 9.5 mg/L. A 16-hour light and 8-hour dark photoperiod occurred during the test with a light intensity between 400 and 800 lux.

Symptoms of toxicity and mortalities were observed daily. The LC_{50} values were estimated using the computer program TOXDAT version 1.61. In addition, body weight and length were recorded at the end of the test.

The mean measured concentrations of Flurochloridone technical were 0.14, 0.36, 0.66, 2.18 and 4.02 mg a.s./L (50 to 96% of nominal) during the 28 day exposure period. The results have been reported based on the mean measured concentrations.

No mortalities were observed in the controls and up to and including 0.66 mg a.s./L during the 28-day exposure. The general symptoms of toxicity noted were immobilisation, erratic swimming and stressed behaviour. No significant effects were seen at 0.14 and 0.36 mg/L during the entire study. Effects were seen first after 14 days of exposure at 0.66 mg/L, after 7 days of exposure at 2.18 mg/L and already on Day 1 at the highest concentration tested, i.e. 4.02 mg/L. Accordingly, the NOEC was determined to be 0.36 mg as./L, based on symptoms of toxicity and mortality.

Based on mean measured values, this substance did not induce any effects (mortality, sublethal, body weight and length) in rainbow trout, when exposed over 28 days, up to and including 0.36 mg a.s./L (NOEC=0.36 mg a.s./L). The 28 day LC_{50} was calculated to be 1.87 mg a.s./L.

This test was considered valid by RMS at the original DAR. Nevertheless, as this test was performed in 1990 according to the OECD Guideline 204 (1984) which has been deleted in 2014 by the OECD, it is nowadays considered out of date, so the results obtained should not be taken into consideration for classification purposes.

11.6.2 Chronic toxicity to aquatic invertebrates

Stewart, K.M., Tapp, J.F., Sankey, S.A., Williams, T.D. and Stanley, R.D. (1990)

The effects of Flurochloridone on survival, growth (body length) and reproduction of *Daphnia magna* was studied in a test under semi-static conditions along 21 days. It was conducted under CLP.

Ten replicates, each containing one daphnia (<24 hours old), were exposed to the nominal concentrations of 0.125, 0.25, 0.5, 1 and 2 mg a.s./L, a water and a solvent control. The test solutions were renewed three times per week. The surviving adults were transferred to the new solutions and the offspring removed from each vessel and counted. The daphnids were fed daily with cultured algae (*Chlorella vulgaris*) and yeast.

A photoperiod of 16 hours light and 8 hours dark was provided during the test. Temperatures in water ranged from 19.1 to 20.7°C. The pH values ranged from 8.18 to 8.36 in freshly prepared test media and from 7.50 to 8.42 in the old test media. The dissolved oxygen concentrations varied between 8.9 and 9.8 mg/L in freshly prepared test media and between 5.1 and 10.0 mg/L in the old test media. In two samples of Day 21 (solvent control and 2 mg/L treatment group) the concentration of the dissolved oxygen was below 5 mg/L, i.e. 4.5 and 4.9 mg/L, respectively.

Mortality and symptoms of toxicity of parent daphnids were recorded daily. From Day 6 on, daily observations were made for the presence of offspring and the numbers of live and dead offsprings were recorded. At the end of the test, the length of each surviving adult was measured. The lengths of surviving adults and the mean number of offsprings in the treatments were compared (one-sided) with the solvent control using Dunnett's test following an analysis of variance.

The concentrations of Flurochloridone were measured on six renewal occasions in the new test solutions and the following renewal days from the old test solutions.

The overall mean measured concentrations of Flurochloridone in the new and old test solutions were 0.11, 0.22, 0.41, 0.83 and 1.8 mg a.s./L and ranged from 82 to 88% of nominal. All results are given as mean measured concentrations.

Adult mortality: there were no adult mortalities in the controls and in the treatment groups up to and including 0.83 mg a.s./L. There was only one dead Daphnia found at 1.8 mg as./L, the highest concentration tested, after 4 days (10% mortality). This was not considered to be a significant reduction in survival. Accordingly, the 21 day LC_{50} was determined to be greater than 1.8 mg a.s./L. No other symptoms of toxicity were observed.

Body length: the mean body lengths of the adult daphnids at the end of the test in the water control was 4.82 mm and 4.76 mm in the solvent control. At test concentrations up to and including 0.83 mg a.s./L, the mean lengths of the surviving adults were not significantly reduced. However, at the maximum concentration of 1.8 mg a.s./L, the mean length (4.48 mm) was significantly less than that of the solvent control (p=0.05).

Reproduction rate: in the controls and in all treatment groups all surviving adult daphnids released the first offspring by Day 9 and completed 5 broods of offspring. The mean number of offspring per parent was 217 and 201 in the water and solvent control, respectively. There was no effect on reproduction up to and including 0.83 mg a.s./L, with a mean number of offspring ranging from 198 to 221 youngs per adult.. However, the mean number of offspring was significantly reduced at the highest concentration of 1.8 mg a.s./L (158 youngs/adult) compared with the solvent control. No dead offspring were observed in any treatment.

Table 59: Chronic toxicity of Flurochloridone technical to the water flea exposed for 21 days (semistatic conditions)

Nominal concentration (mg a.s/L)	**Measured [ ] 0-21 days (mg a.s./L)**	**Mortality (%)**	**Total number of offspring/parent**	**Mean adult length at day 21 (mm)**
Control	< 0.002	0	217	4.82
Solvent control	< 0.002	0	201	4.76
0.125	0.11	0	198	4.73
0.25	0.22	0	203	4.76
0.5	0.41	0	211	4.75
1	0.83	0	221	4.73
2	1.8	10	158*	4.48*

* statistically different from control (p=0.05)

Taking into account these results, the NOEC for length and reproduction was determined to be **0.83 mg a.s./L** based on mean measured concentrations.

Gentle, W.E. (1997)

The sediment-dwelling *Chironomus riparius* was exposed to a technical compound containing 99.5% w/w trans-isomer and 0.1% *cis*-isomer, to determinate long-term toxicity of Flurochloridone. The test was undertaken according to GLP.

The effect of tested compound on the emergence of *Chironomus riparius* was investigated in a sediment-water system over 25 days. A single test concentration of nominal 0.25 mg trans-isomer/L was tested. Four replicates of 25 animals (first instar larvae) were prepared for the test concentration and a control. 24 hours after adding the larvae, the test substance was applied to the water phase of the system.

The concentrations of tested substance in the overlying water were analysed on Days 0, 7 and 25. The measured concentrations of the substance in the overlying water was 0.26 mg a.s./L (102% of nominal) one hour after application and decreased to 0.13 mg as./L (53% of nominal) on Day 7, and to 0.067 mg as./L (27% of nominal) after 25 days.

In both the control and treated systems, first emergence occurred on Day 12 and the last midges to emerge were on Days 22 and 19, respectively. In the control and treated systems the total numbers of emerged midges were 97 and 98 % of the larvae introduced, respectively. The mean time to emergence in the control and treated systems was 13.1 and 13.8 days, respectively, and this difference was found to be statistically significant. However this was not considered to be biologically significant since the time between assessments was one day and the difference was less than one day.

Table 60: Influence of Flurochloridone technical (99% w/w trans isomer) on the development and the emergence of *C.riparius*

Nominal *trans*-flurochloridone concentration	**Measured over lying water concentration (mg/L)**			**Mean emerged**	**Mean emerged time**
(mg/L)	**Day 0**	**Day 7**	**Day 25**	**(%)**	**(days)**
Control	< 0.01[a]	< 0.01 [a]	< 0.01 [a]	97	13.1
0.25	0.26	0.13	0.067	98	13.8

[a] LOD: Limit of determination under the conditions used

The mean time to emergence was determined in the treatment and control and compared by analysis of variance. Flurochloridone trans-isomer had no effects on the emergence of *Chironomus riparius* when exposed to 0.25 mg a.s/ L. However, results from this assay are not accepted for risk assessment purpose.

RMS considered that this study was valid only as additional information, and commented the following: the batch number corresponds to a technical compound containing 99.5% w/w trans-isomer and 0.1% cis-isomer. This rate is quite different from the intended use of formulation product Racer 25 CS (trans/cis 3:1). Although the study is well performed, the results obtained with this test correspond exclusively to the trans isomer. RMS did not support the comments from the notifier considering that this study covers the technical substance as a whole, and considers that the endpoints from this study should be considered just as an approximation.

11.6.3 Chronic toxicity to algae or other aquatic plants

Please refer to previous point 11.5.3. where the toxicity tests with the parent and metabolites on algae and on Lemna are included.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

There are acute toxicity data available for fish, aquatic invertebrates, algae and aquatic plants, covering the three trophic levels. The lowest acute toxicity endpoint is *Scenedesmus subspicatus* ErC_{50}(72h)= 0.0047 mg/l. This endpoint will establish the M factor needed for the CLP environmental classification.

Based on this endpoint Flurochloridone should be classified according to Regulation (EC) No 1272/2008 as:

Aquatic Acute 1 with M factor of 100.

CLP criteria:

- for EC_{50} acute toxicity values below or equal to 1 mg/l [ErC_{50}(72h) = 0.0047 mg/l ≤ 1 mg/l] and
- for M factor, acute toxicity value in the range 0.001 < $L(E)C_{50}$ ≤ 0.01 mg/L.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Regarding aquatic toxicity on a long-term basis, there are reliable chronic toxicity data for aquatic invertebrates, algae and aquatic plants available for this substance. In the original DAR in 2006 a 28 days duration long-term toxicity study with fish was included and it was considered valid at that moment. Nevertheless, it should be pointed out that this study was undertaken following the OECD guideline 204 from 1984, which was deleted later in 2014 by OECD. In addition, that study focused on the survival and behaviour effects to juvenile fishes during the 28 days period, meaning that the exposure does not cover all fish life stages.

At that moment RMS agreed that a fish early-life stage toxicity test was not necessary for Flurochloridone, based on the rapid degradation observed at the two water/sediment studies and also based on the toxicity results for fish obtained in the short-term as well as in the long-term. Therefore, an early life stage test for fish is not available.

RMS considered that none of the criteria accorded at EU level to trigger a fish full-life cycle test were met by the substance (bioaccumulation factor >1000 with <95% elimination during the depuration phase, persistence in water or sediment DT_{90}>100 days, and the value of acute toxicity LC_{50} <0.1 mg/L). Thus, a full-life cycle test was not deemed necessary at the initial evaluation of Flurochloridone, so it is not available now.

In conclusion, regarding all the above, there is a data gap about long-term toxicity of Flurochloridone to fish, which means that according to the CLP criteria the three trophic levels are not covered.

With regard to bioaccumulation, Flurochloridone does not meet the criterion established by CLP as the experimentally determined BCF value in fish is <500, so low potential for bioaccumulation is expected (BCF at the end of the study = 292 in whole fish).

The experimental log Kow value of 3.36 also means that Flurochloridone does not meet the criterion established by CLP (log Kow <4). Nevertheless, as experimental derived BCF values are more preferred than log Kow values for classification purposes, the above mentioned BCF <500 would already determine that low potential for bioaccumulation is expected for this active substance.

Regarding rapid degradability, Flurochloridone is considered not readily biodegradable according to the result of the biodegradation test presented, following OECD 301 E guideline.

Flurochloridone is considered hydrolytically stable at environmentally relevant pH values (pH 5, 7 and 9). In addition, both isomers showed that they are also stable to hydrolysis.

Photolysis seems to play a role in the degradation of Flurochloridone in water (Van der Gaauw, 2004a) with a photolytic half-live of 16 – 18 days (latitudes 30º - 50ºN) and two major metabolites have been identified.

The water/sediment studies (Shaw, 1996a-b) suggest that Flurochloridone disappears rapidly from water/sediment systems. A rapid movement of the applied radiolabel is observed from the

water into the sediment. The kinetic analysis of the data from these studies were re-evaluated in Van der Gaauw, 2004c.

In water phases, Flurochloridone decreases in concentration to less than 1 % by the end of both studies. R42819 metabolite is identified as relevant metabolite exceeding 10 % (about 13 %) but only in the Virginia Water system of both studies.

In sediment phases, the concentration of Flurochloridone increased by the middle of the study and its concentration fells by the end of the study. R42819 metabolite is identified as relevant metabolite (about 40%) in both systems (Old Basin and Virginia Water).

Flurochloridone can be considered as not rapidly degradable in the aquatic environment from the water/sediment system studies carried out. Although short DT_{50} values were calculated for the whole system (DT_{50} = 14.3 days), Flurochloridone is not ultimately degraded. It disappears by dissipation process, R42819 metabolite being identified as relevant metabolite both in water and sediment phases. The aquatic toxicity of the two metabolites is lower than the parent substance's toxicity, based on the available data on algae and on aquatic plant. So they were not considered further in relation to the hazard classification of the active substance.

Due to the results summarized above, Flurochloridone can be considered as a not rapidly degradable substance in the environment, according to CLP criteria.

Following scheme 4.1.1 to classify long-term hazards to the aquatic environment of the substance, as only chronic toxicity data are available for two trophic levels, the following two approaches need to be applied. Finally, the most stringent outcome from both would be taken into consideration to classify the substance.

a) Long-term hazards' assessment according to Table 4.1.0 (b) of CLP Regulation

The lowest chronic toxicity endpoint is *Scenedesmus subspicatus* NOErC(72h)= 0.00028 mg/l. This endpoint will trigger Aquatic Chronic 1 and will also establish the M factor needed for CLP environmental classification category.

Taking into account this endpoint, Flurochloridone should be classified according to Regulation (EC) No 1272/2008 as:

Aquatic Chronic 1 with M factor of 100.

CLP criteria:

- for NOEC chronic toxicity values below or equal to 0.1 mg/l [NOErC(72h) = 0.00028 mg/l ≤ 0.1 mg/l], and
- for M factor, as Flurochloridone is considered as not rapidly degradable and the chronic toxicity value is in the range $0.0001 < L(E)C_{50} \leq 0.001$ mg/L

b) Long-term hazards' assessment according to Table 4.1.0 (b) iii of CLP Regulation (surrogate method)

The lowest acute toxicity endpoint is *Scenedesmus subspicatus* ErC_{50}(72h)= 0.0047 mg/l. This endpoint and the fact that the substance is not rapidly degradable and the experimental bioconcentration factor BCF is <500 would also trigger Aquatic Chronic 1 result, and the same M factor of 100 derived from that acute endpoint pointed above. This approach assessing acute toxicity in combination with rapid degradability or bioaccumulation potential results in the same CLP hazard category for long-term hazards' classification.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Due to all the information and assessment summarized in the previous sections 11.7.1 and 11.7.2, the following classification categories and M factors can be concluded for this active substance:

Flurochloridone meets the CLP Regulation criteria for being classified as Aquatic Acute 1 with M factor of 100.

Flurochloridone meets the CLP Regulation criteria for being classified as Aquatic Chronic 1 with M factor of 100.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No data available.

12.1.2 Comparison with the CLP criteria

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No data available.

13 ADDITIONAL LABELLING

14 REFERENCES

14.1 Physico-chemical properties

Author(s)	Year	Title Source Company, Report No GLP or GEP status (where relevant), Published or not
Goodmann, M	1994a	Flurochloridone - Physico-chemical properties of pure material Zeneca Western Research Center report no. RR 94-053B Agan Chemical Manufacturers Ltd., report no. 90004460 GLP, unpublished
Goodmann, M	1994b	Flurochloridone - Physico-chemical properties of technical material Zeneca Western Research Center report no. RR 94-048B Agan Chemical Manufacturers Ltd., report no. 90004459, GLP, unpublished
Weissenfeld, M.	2006a	Determination of the Vapour Pressure of Flurochloridone RCC Ltd., Switzerland, Report No. A82923 Agan Report No. 90009437 GLP, Unpublished
Tognucci, A	2003a	Determination of the boiling point/boiling range of flurochloridone RCC Ltd. report no. 848297 Agan Chemical Manufacturers Ltd., Report no. 90006082 GLP, unpublished
Tognucci, A	2003b	Determination of the surface tension of an aqueous solution of flurochloridone technical RCC Ltd. report no. 848298 Agan Chemical Manufacturers Ltd., Report no. 90006027 GLP, unpublished
Weissenfeld, M.	2006	Expert Statement: On the Oxidizing Properties of Flurochloridone RCC Ltd., Switzerland, Report No. A83171 Agan Report No. 90009365 Non-GLP, Unpublished
Weissenfeld, M.	2008a	Flurochloridone (trans isomer): Determination of the vapor pressure. Harlan Laboratories Ltd., report no. C14450, 8 December 2008 Agan report no. 90011555 GLP, unpublished
Weissenfeld, M.	2008c	Flurochloridone (trans isomer): Determination of the water solubility. Harlan Laboratories Ltd., report no. C14448, 3 December 2008 Agan report no. 90011554 GLP, unpublished
Weissenfeld, M.	2008d	Flurochloridone (cis isomer): Determination of the water solubility. Harlan Laboratories Ltd., report no. C14426, 24 November 2008 Agan report no. 90011552 GLP, unpublished

Author(s)	Year	Title Source Company, Report No GLP or GEP status (where relevant), Published or not
Weissenfeld, M.	2007	Determination of the solubility of flurochloridone in organic solvents. RCC Ltd, report no. B10394, 15 May 2007 Agan report no. 9009672 GLP, unpublished
Weissenfeld, M.	2008e	Flurochloridone pure: Calculation of the dissociation constant. RCC Ltd, report no. C14415, 6 November 2008 Agan report no. 90011551 Non-GLP, unpublished
Weissenfeld, M.	2009	Flurochloridone: Determination of the water solubility at pH 4, pH 7 and pH 9. Harlan Laboratories Ltd., report no. C14382 Agan report no. 90011550 GLP, unpublished
O'Connor, B.J.	2012	Purified Flurochloridone: Determination of the melting point/melting range Harlan Laboratories Ltd. Project no. 41104682 GLP, unpublished

14.2 Toxicology and metabolism

Author(s)	Year	Title Source Company, Report No GLP or GEP status (where relevant), Published or not
McBain, J.B.	1985	Metabolism of 1-m-trifluoromethylphenyl-3-chloro-4-choromethyl-2-pyrrolidone (R40244) by rats. MRC-85-02 Not GLP, Unpublished
Silcock, R.C.	2001a	Flurochloridone: excretion and tissue distribution of a single oral dose (4 mg/kg) in the rat. GLP, Unpublished
Silcock, R.C.	2001b	Flurochloridone: excretion and tissue distribution of a single oral dose (200 mg/kg) in the rat. UR0673 GLP, Unpublished
Silcock, R.C.	2002	Flurochloridone: Repeated oral dose (4 mg/kg) study in the rat. UR0658 GLP, Unpublished
Kunz, Ch.	2006a	[^{14}C] Flurochloridone: Blood Level, distribution and Metabolism after Single Oral Administration to Male and Female Rats RCC Ltd., Switzerland, Report No. 857436 Agan Report No. 90009067 GLP, Unpublished

Author(s)	Year	**Title** **Source** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**
Kunz, Ch.	2006b	[^{14}C] Flurochloridone: Blood Level, distribution and Metabolism after Single Oral Administration to Male and Female Rabbits RCC Ltd., Switzerland, Report No. 857437 Agan Report No. 90009068 GLP, Unpublished
Howell, A. M.	1979	Toxicology Laboratory Report T 6292 Not GLP, Unpublished
Ullmann, L.	1985	Acute oral toxicity of Racer Technical in mice. Report No 046530 GLP, Unpublished
Sieber, M.	2011	Flurochloridone technical: Acute Oral Toxicity Study in Rats. Acute Toxic Class Method. Harlan Study Report D44331 GLP, Unpublished
Decker, U., Knuppe, C. Ullrich, A.	2004	4-Hour acute inhalation toxicity study in rats. Report No. 850348 GLP, Unpublished
Mutter, L.C.	1985	Dermal sensitisation test with Racer Technical. Report No T-12106 GLP, Unpublished
Arcelin. G.	2006	Fluorochloridone Technical: Contact Hypersensitivity in Albino Guinea Pigs, Maximization-Test. Report No 90009212 GLP, Unpublished
Ouelette, R.E.,	1982	A dietary range-finding study with R-40244 technical in rats. Report No. T 10725 GLP, Unpublished
Ouelette, R.E., Sauerhoff, M. V.	1982a	A 28-Day Dietery Range-Finding Study with R-40244 Technical in Mice Report No. T-10830 GLP, Unpublished
Ouelette, R.E., Sauerhoff, M. V.	1982b	Three-month subchronic dietary toxicity study with R-40244 in rats. Report No. T 10796 GLP, Unpublished
Blair, M.	1983	Six Month Dietary Study with R-40244 Technical in Beagle Dogs. Report No. T 10815 GLP, Unpublished
Jagannath, D.R.	1978a	Mutagenicity evaluation of R-40244 in the Ames salmonella/microsome plate test. T-6350) Not GLP, Unpublished
Jagannath, D.R.	1978b	Mutagenicity evaluation of R-40244 in the Ames salmonella/microsome plate test. T-6153 Not GLP, Unpublished

Author(s)	Year	**Title** **Source** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**
Matheson, D.W.	1978a	Mutagenicity evaluation of R-40244, 5276-20-1 in the mouse lymphoma forward mutation assay. T-6348 Not GLP, Published
Matheson, D.W.	1978b	Evaluation of R-40244 in the in vitro transformation of BALB/3T3 cells assay T-6424 GLP, Unpublished
Snyder, R.D.	1985	Effects of R-40244 on human fibroblast DNA T-12058
Pirovano, R.	1986	Technical Flurochloridone: unscheduled DNA synthesis in cultured HeLa cells (in vitro) RBM M971/1-2 GLP, Unpublished
Majeska, J.B.	1985	R-40244 technical: mutagenicity evaluation in bone marrow micronucleus T-11916 GLP, Unpublished
Sokolowski, A.	2008	Salmonella typhimurium and Escherichia coli reverse mutation assay with flurochloridone technical. Report no. 1182503 GLP Unpublished
Wollny, H-E.	2008	Cell mutation assay at the thymidine kinase locus (TK $^{+/-}$) in mouse lymphoma L5178Y cells with flurochloridone technical. Report no. 1182502 GLP Unpublished
Hoffmann, H.	2008	*In vitro* chromosome aberration test in Chinese hamster V79 cells with flurochloridone technical. Report no. 1182501 GLP Unpublished
Sokolowski, A.	2011	Salmonella typhimurium and Escherichia coli reverse mutation assay with flurochloridone technical. Report no. 1450002 GLP Unpublished
Bohnenberger, S.	2012	*In vitro* micronucleus test in Chinese Hamster V79 cells with flurochloridone technical. Report no. 1450001 GLP Unpublished
Roth, M.	2012	*Micronucleus assay in bone marrow cells of the rat with flurochloridone technical.* Report no. 1464700 GLP Unpublished
Sprague, G. L.	1985a	Chronic toxicity and oncogenicity study with R-40244 technical in rats T-10831 GLP, Unpublished

Author(s)	Year	Title Source Company, Report No GLP or GEP status (where relevant), Published or not
Sprague, G. L.	1985b	24- GLP, Unpublished oncogenicity study with R-40244 technical in mice T-10846 GLP, Unpublished
Downs, J.R., Minor, J.L.	1983	R-40244 Multigeneration reproduction study in rats T-10900 GLP, Unpublished
Nemec, M.D.	1983a	A teratology study in rats with R40244 T-11731 GLP, Unpublished
Nemec, M.D.	1984a	A teratology study in rats with R40244 T-11883 GLP, Unpublished
Nemec, M.D.	1983b	A teratology study in rabbits with R40244 T-11036 GLP, Unpublished
Wilczynski, S.L., Killinger, J.M.	1984	Fertility study with R-40244 technical in male rats T-11729 GLP, Unpublished
Wilczynski, S.L., Killinger, J.M.	1985a	A R-40244 technical mechanism of action study in male rats (1st) T-12657A GLP, Unpublished
Wilczynski, S.L., Killinger, J.M.	1985b	A R-40244 technical mechanism of action study in male rats (2nd) T-12657B GLP, Unpublished
Wilczynski, S.L., Killinger, J.M.	1985c	R-40244 - Fertility study in male rabbits T-11921 GLP, Unpublished
Wilczynski, S.L., Killinger, J.M.	1985d	The effect of R-40244 on nonhuman primate sperm production T-11951 GLP, Unpublished

14.3 Environment

Authors	Date	Title	Testing Facility	GLP	Published
Douglas, M. T., Pell, I. B.	1985	Assessment of ready biodegradability of Racer (technical) Ro-Neet (technical), Devrinol (technical) and Eptam (technical).	n.a.	Yes	No
Katague, D. B.	1983	Hydrolysis of R-40244	n.a.	No	No
Lee, K. S.; Chang, L. L.; Giang, B. N.; Kukla, D.	1985	Hydrolysis and photolysis of R-40244	n.a.	No	No

Authors	Date	Title	Testing Facility	GLP	Published
Adam, D.	2006	^{14}C Flurochloridone: Hydrolysis at three different pH values	n.a.	Yes	No
Shaw, D.	1996a	[2-^{14}C-pyrrolidone]flurochloridone degradability and fate in water-sediment systems	n.a.	Yes	No
Shaw, D.	1996b	[U-^{14}C-phenyl]flurochloridone degradability and fate in water-sediment systems	n.a.	Yes	No
Van der Gaauw, A.	2004c	Estimation of the environmental concentrations of Flurochloridone and its metabolites in surface water – FOCUS Surface Water Steps 1 – 4 calculations.	n.a.	n.a.	No
Van der Gaauw, A.	2004a	[^{14}C]-Flurochloridone: Aqueous photolysis	n.a.	Yes	No
Christian, G. D. ; Purdy, W. C.	1962	The residual current in orthophosphate medium	n.a.	No	Yes
Lee, K. S.; Chang, L. L.; Giang, B. N.; Kukla, D.	1985	Hydrolysis and photolysis of R-40244	n.a.	No	No
Rowe, D.; Lane, M. C. G.	1994	Flurochloridone: Adsorption and desorption properties in soil.	n.a.	Yes	No
Goodman, M.	1994	Flurochloridone: Physico-Chemical properties of pure material (WRC-94-090).	n.a.	Yes	No
McAllister, W.A.; Franklin, L.	1984	Uptake, depuration and bioconcentration of 14C-R-40244 by Bluegill sunfish (*Lepomis macrochirus*).	n.a.	Yes	No
Douglas, M. T.; Handley, J. W.; MacDonald, I.A.	1987	The acute toxicity of Racer technical to rainbow trout (*Salmo gairdneri*)	n.a.	Yes	No
Cohle, P. R., McAllister, W. A.	1983	Acute Toxicity of R-40244 to Bluegill Sunfish (*Lepomis macrochirus*).	n.a.	Yes	No
Spare WC, Gottfried G, Dillon F	1983	The Acute Toxicity of R-40244 to *Daphnia magna* Straus	n.a.	Yes	No
Wallace, S. J., Swarbrick, R. H	2001	Flurochloridone: Toxicity to the blue green alga *Anabaena flos-aquae*.	n.a.	Yes	No
Bätscher, R.	2004a	Toxicity of flurochloridone tech. to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test.	n.a.	Yes	No

Authors	Date	Title	Testing Facility	GLP	Published
Bätscher, R.	2004b	Toxicity of R406639 to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test.	n.a.	Yes	No
Bätscher, R.	2004c	Toxicity of R42819 to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test.	n.a.	Yes	No
Woodyer, J.E., Swarbrick, R. H., Shillabeer, N.	2001	Flurochloridone: Toxicity to duckweed (*Lemna gibba*)	n.a.	Yes	No
Bätscher, R.	2003	Toxicity of R42819 to the aquatic plant *Lemna gibba* in a 7-day static growth inhibition test.	n.a.	Yes	No
Smith, G. J.	1990	Flow-Through Threshold LC50 Determination of Flurochloridone to Rainbow Trout	n.a.	Yes	No
Stewart, K. M., Tapp, J. F., Sankey, S.A., Williams, T.D., Stanley, R.D.	1990	Flurochloridone : Determination of chronic toxicity to *Daphnia magna*	n.a.	Yes	No
Gentle, W. E.	1997	Flurochloridone: BBA Toxicity Test with Sediment-dwelling *Chironomus riparius*	n.a.	Yes	No

15 ANNEXES

Robust summaries of the studies covered in this CLH report are included in the Draft Assessment Report (DAR) of flurochloridone and subsequent addendums:

- Spain, February 2006. Draft Assessment Report (DAR) on the active substance flurochloridone. Prepared by the rapporteur Member State Spain in the framework of Directive 91/414/EEC. [PA]
- Spain, November 2007. Addendum I and Corrigendum I to the Draft Assessment Report (DAR) on the active substance flurochloridone.
- Spain, October 2009. Addendum II to the Draft Assessment Report (DAR) on the active substance flurochloridone.
- Spain, May 2012/September 2012. Addendum Confirmatory Data (Toxicology data and confidential information). [PA]
- Spain, April 2014. Addendum Confirmatory Data (Environmental fate and behavior). [PA]

Other relevant documents relevant for flurochloridone evaluation:

- Spain 2010. Final Addendum to the Additional Report on flurochloridone, compiled by EFSA, August 2010. [PA]
- EFSA (European Food Safety Authority), 2010. Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance flurochloridone. [PA]
- EFSA (European Food Safety Authority), 2010. Conclusion on the peer review of the pesticide risk assessment of the active substance Flurochloridone. EFSA Journal 2010; 8(12):1869. [PA]
- EFSA (European Food Safety Authority), 2013. Conclusion regarding the peer review of the pesticide risk assessment of confirmatory data submitted for the active substance flurochloridone. EFSA Journal 2013;11(3):3116. [PA]
- EFSA (European Food Safety Authority), 2014. Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment of confirmatory data for the active substance flurochloridone. EFSA supporting publication 2014: EN-642. [PA]

All the documents are included in section 13 of IUCLID 6 file. Public available documents are marked with [PA].