Committee for Risk Assessment
RAC

Annex 1

Background document

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

Quinolin-8-ol; 8-hydroxyquinoline

EC Number: 205-711-1
CAS Number: 148-24-3

CLH-O-0000001412-86-60/F

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

Adopted

5 June 2015

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CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 8-hydroxyquinoline

EC Number: **205-711-1**

CAS Number: **148-24-3**

Index Number: **-**

Contact details for dossier submitter: Spanish Competent Authority

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ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Version number: 3 **Date: September 2014**

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ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	8-hydroxyquinoline
EC number:	205-711-1
CAS number:	148-24-3
Annex VI Index number:	n.a.
Degree of purity:	≥ 990 g/kg
Impurities:	No relevant toxicological impurities

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification: 8-hydroxyquinoline

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not currently in Annex VI, Table 3.1 of the CLP Regulation
Current proposal for consideration by RAC	Acute Tox. 3; H301 Eye Dam. 1; H318 Skin Sens. 1; H317 Repr. Cat 2; H361d Aquatic Acute 1; H400 M-factor 1 Aquatic Chronic 1; H410 M-factor 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3; H301 Eye Dam. 1; H318 Skin Sens. 1; H317 Repr. Cat 2; H361d Aquatic Acute 1; H400 M-factor 1 Aquatic Chronic 1; H410 M-factor 10

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Conclusive but not sufficient for classification
2.2.	Flammable gases				Conclusive but not sufficient for classification
2.3.	Flammable aerosols				Conclusive but not sufficient for classification
2.4.	Oxidising gases				Conclusive but not sufficient for classification
2.5.	Gases under pressure				Conclusive but not sufficient for classification
2.6.	Flammable liquids				Conclusive but not sufficient for classification
2.7.	Flammable solids				Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				Conclusive but not sufficient for classification
2.10.	Pyrophoric solids				Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Conclusive but not sufficient for classification
2.13.	Oxidising liquids				Conclusive but not sufficient for classification
2.14.	Oxidising solids				Conclusive but not sufficient for classification
2.15.	Organic peroxides				Conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 3; H301			
	Acute toxicity - dermal				Conclusive but not sufficient for classification

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CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
	Acute toxicity - inhalation				No data
3.2.	Skin corrosion / irritation				Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Eye Dam 1: H318			
3.4.	Respiratory sensitisation				No data
3.4.	Skin sensitisation	Skin Sens. 1; H317			
3.5.	Germ cell mutagenicity				Conclusive but not sufficient for classification
3.6.	Carcinogenicity				Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Repr. 2: H361d			
3.8.	Specific target organ toxicity –single exposure				Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure				Conclusive but not sufficient for classification
3.10.	Aspiration hazard				Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic acute 1, H400: Very toxic to aquatic life Aquatic chronic 1, H410: Very toxic to aquatic life with long lasting effects	Acute M = 1 Chronic M = 10		
5.1.	Hazardous to the ozone layer				Conclusive but not sufficient for classification

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

Labelling:

GHS Pictograms:




GHS05 GHS06 GHS08 GHS09

Signal word: Danger

Hazard statements:
H301: Toxic if swallowed
H317: May cause allergic to skin reactions
H318: Causes serious eye damage
H361d: Suspecting of damaging the unborn child
H400: Very toxic to aquatic life
H410: Very toxic to aquatic life with long lasting effects

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

8-hydroxyquinoline is a substance covered by the third stage of the work programme for review of existing active substances provided for in Article 8(2) of Directive 91/414/EEC concerning the placing of Plant Protection Products (PPP) on the market, with a view to the possible inclusion of this substance in Annex I to the Directive. Spain was the RMS and Probelte S.A. the sole applicant. Data referred to assessment made under PPP Regulation is attached to the IUCLID 5 dossier (Draft Assessment Report, updated May 2010) (EFSA Scientific Report, 28 January 2011) (Addendum I to DAR, July 2010). The overall conclusion from the evaluation was that plant protection products containing 8-hydroxyquinoline fulfilled the safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC. Therefore, the Commission implementing Regulation (EU) 993/2011 decided the approval of the active substance 8-hydroxyquinoline in accordance with the Regulation (EC) 1107/2009 (repealing previous Directive 91/414/EEC).

The conclusions on the peer review of pesticide risk assessment of 8-hydroxyquinoline were published in the EFSA Journal 2011;9(1):1964. EFSA proposed the following classification with regard to mammalian toxicological data, Xn, R22 “Harmful if swallowed”, R41 “Risk of serious damage to eyes”, R43 “May cause sensitization by skin contact” and Repro Cat 3 R63 “Possible risk of harm to the unborn child” and with regard to ecotoxicological data, N; R50/R53 “Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment”.

8-hydroxyquinoline is not currently listed in Annex VI of the CLP Regulation. One REACH registration dossier is available for this substance up to now. This dossier was submitted to ECHA with 8-hydroxyquinoline as a transported isolated intermediate. No robust study summaries appear to have been submitted with this registration dossier.

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

In accordance with article 36(2) of Regulation (EC) 1272/2008 on classification, labelling and packaging of substances and mixtures, being 8-hydroxyquinoline 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 end points. This Annex VI dossier presents a classification and labelling proposal based on the information provided for the assessment of 8-hydroxyquinoline under 91/414/EEC Directive (currently repealed by Regulation (EC) 1107/2009) and also takes into account the information of the European Agency for the Evaluation of Medicinal Products (EMEA) for 8-hydroxyquinoline (document EMEA/MRL/464/98-Final).

2.2 Short summary of the scientific justification for the CLH proposal

No classification and labelling has been proposed for 8-hydroxyquinoline regarding physical and chemical properties.

Justification for the proposal with respect to human health effects:

Acute Tox 3 H301

This classification is based on the reported acute oral LD_{50} value of 177 mg/kg bw obtained in a mice study (Dickhaus and Heisler, 1981b).

Eye Dam. 1 H318

Eye irritation classification meets CLP criteria due to the persistence of a corneal lesion in one animal in a rabbit study until the end of the study on day 20 (Stelter, 2008b).

Skin Sens. 1 H317

No skin sensitisation studies were provided for the inclusion in the Annex I of the Directive 91/414/EEC (currently repealed by regulation (EC) 1107/2009). It is well known that 8-hydroxyquinoline induces skin sensitisation in humans. Patients showed a contact allergy to 8-hydroxyquinoline and to 8-hydroxyquinoline sulphate during a dermatological therapy with the compound (Pevny, 1971; Rothe, 1978; Metzner, 1987). Not sufficient data for sub-categorisation according to CLP are available and classification as Skin Sens. 1 - H317 (May cause an allergic skin reaction) is required.

Repr. Cat. 2 H361d

This classification is supported by the results of the rabbit developmental study (Fascineli, 2006d). There was strong evidence that 8-hydroxyquinoline can impair foetal development based on several cases of external malformation (omphalocele), soft tissue variations (periorbital haemorrhage and retinal fold), skeletal retardations (not ossified and rudimentary sternebrae) and reduction in the number of ossification centres. Besides, in developmental and two generation rat studies (Fascineli, 2006b and 2006c), findings related to developmental toxicity were also seen. Although in presence of maternal toxicity they can be regarded as a supporting evidence of developmental toxicity.

A classification as Repr. Cat. 2 H361d is proposed.

Justification for the proposal with respect to environmental effects:

The toxicity study for algae shown an E_rC_{50} (72h) of 0.71 mg/L, furthermore a 28 days fish NOEC of 0.0099 mg/L was observed. In addition, 8-hydroquinoline was found to be not ready biodegradable according to the OECD 301 D (EC Method C.4-E. Part VI) Closed Bottle Test and it is unlikely for the substance to bioaccumulate in aquatic organisms (log

KOW < 3). As a consequence and according to the CLP Regulation, 8-hydroquinoline should be classified as Aquatic Acute 1, M=1 – Aquatic Chronic 1, M=10.

2.3 Current harmonised classification and labelling

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

No current entry in Annex VI in CLP Regulation.

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

No current entry in Annex VI in CLP Regulation.

2.4 Current self-classification and labelling

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

The self-classification according to the ECHA inventory of notified classification and labelling on 11 September 2014 was:

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)			
Acute Tox. 4	H302	H302		GHS07 Wng			90
Acute Tox. 4	H302	H302		GHS07 Wng			32
Acute Tox. 4	H332	H332					
Aquatic Chronic 3	H412	H412					
Acute Tox. 4	H302	H302		GHS07 GHS09 Wng			6
Aquatic Chronic 1	H410	H410					
Acute Tox. 4	H302	H302		GHS07 Wng			6
Acute Tox. 4	H332	H332					
Not Classified							3
Acute Tox. 4	H302	H302		GHS07 GHS08 Dgr			2
Muta. 2	H341	H341					

		H341		GHS07 GHS08			2
		H302		Wng			
		H332		GHS07			1
		H302		Wng			
		H319		GHS07			1
		H315		Wng			
		H332					
		H335					
		H302					
Acute Tox. 4	H302	H302		GHS07 GHS08			1
Muta. 2	H341	H341		Wng			
Acute Tox. 4	H302						1
Acute Tox. 4	H332			Wng			
Acute Tox. 4	H302	H302		GHS07			1
Acute Tox. 4	H332	H332		GHS08			
Muta. 2	H341	H341		Wng			
Acute Tox. 4	H302	H302		GHS07			1
Aquatic Acute 1	H400			GHS09			
Aquatic Chronic 1	H410	H410		Wng			
Acute Tox. 4	H302	H302		Wng			1
Skin Irrit. 2	H315	H315					
Eye Irrit. 2	H319	H319					
STOT SE 3	H335	H335					
STOT SE 2	H371	H371					
Acute Tox. 4	H302	H302		GHS07			1
Acute Tox. 4	H312	H312		GHS08			
Skin Irrit. 2	H315	H315		Wng			
Eye Irrit. 2	H319	H319					
Acute Tox. 4	H332	H332					
STOT SE 3	H335	H335					
Muta. 2	H341	H341					

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

8-hydroxyquinoline is an active substance for plant protection products approved under Regulation (EC) No 1107/2009 via Commission Implementing Regulation (EU) No 993/2011. Therefore, 8-hydroxyquinoline is subject to harmonised classification and labelling according to article 36(2) of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

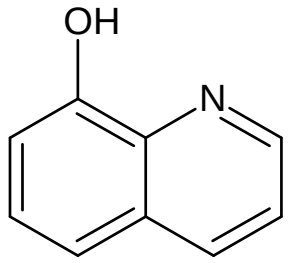
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	205-711-1
EC name:	Quinolin-8-ol
CAS number (EC inventory):	
CAS number:	148-24-3
CAS name:	
IUPAC name:	Quinolin-8-ol
CLP Annex VI Index number:	No entry
Molecular formula:	C_9H_7NO
Molecular weight range:	145.16

Structural formula:



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1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
8-hydroxyquinoline		≥ 99%	

Current Annex VI entry: No entry available.

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
None			

None of the impurities expected from the manufactured process exceeded the maximum specified limit and the content of all impurities was below 1.0 g/kg (0.1% w/w). The impurities are not expected to affect the classification and labelling of the test substance.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

8-hydroxyquinoline is manufactured with a minimum purity of 990 g/kg. 8-hydroxyquinoline contains neither additives nor significant amounts of impurities of toxicological, environmental or ecotoxicological concern.

1.3 Physico-chemical properties

Table 8: Summary of physico-chemical properties

Property	**Value**	**Reference/Comment**
State of the substance	Yellow crystalline solid	Gomez A.G., (2004a)
Melting/freezing point	73.1 to 74.1 ºC (purity: 99.9%)	Gonzalez, M.B. (2004a) EEC A1
Boiling point	265.6 ºC	Gonzalez, M.B. (2004a) EEC A2
Relative density	$D_4^{20} = 1.4309$	Gomez, A.G. (2004a) EEC A3
Vapour pressure	6.7×10^{-3} Pa (20°C) (purity: 99.6%) 1.9×10^{-2} Pa (25°C) (purity: 99.6%) 1.1 Pa (50ºC) (purity 99.6%)	Smeykal, H. (2003) OECD 104
Surface tension	σ = 71.2 mN/m at 20°C (purity: 99.9%) 8-hydroxyquinoline is not a surface active substance	Walter, D. (2004) EEC A5
Water solubility	2.44 g/L (20 ºC) at pH: 4.13 0.663 g/L (20 ºC) at pH: 7.10 0.847 g/L (20 ºC) at pH: 9.15 (purity: 99.82%)	Gil, A.G. (2010) EEC A6

Partition coefficient n-octanol/water	log $P_{O/W}$: 1.264 at 22 ºC (pH 4.13) log $P_{O/W}$: 1.950 at 22 ºC (pH 7.05) log $P_{O/W}$: 1.880 at 22 ºC (pH 9.11) (purity: 99.82%)	Gil, A.G. (2010) EEC A8
Flash point	Not required for the inclusion of the active substance in Annex I of Directive 91/414 because de melting point of 8-hydroxyquinoline is higher than 40 ºC.	
Flammability	8-hydroxyquinoline is not highly flammable. (purity 99.9%)	Gonzalez, M.B. (2004b and 2004c) EEC A10
Explosive properties	8-hydroxyquinoline does not present danger of explosion (purity 99.9%)	Smeykal, H. (2004) EEC A14
Self-ignition temperature	Not auto-inflammable (below the melting point) (purity 99.9%)	Gonzalez, M.B. (2004b and 2004c) EEC A16
Oxidising properties	Structural aspects and estimated thermodynamic properties indicated that 8-hydroxyquinoline has no oxidising properties	Tiemann, J. (2004)
Stability in organic solvents and identity of relevant degradation products	8-hydroxyquinoline at 25ºC (purity 99.9%): n-heptane <10 g/l 1,2-dichloroethane >250 g/l methanol 80-100 g/l acetone >250 g/l ethyl acetate >250 g/l p-xylene 80-100 g/l	Gil, A.G. (2004a) CIPAC MT 181
Dissociation constant	pKa_1 (25 ºC, 99.9%) 4.88±0.01 pKa_2 (25 ºC, 99.9%) 9.45±0.01	Gomez, A.G. (2004b and 2010) OECD 112

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this type of report.

2.2 Identified uses

8-hydroxyquinoline is a preventive and curative fungicide and bactericide used as active substance in plant protection products. Besides, it is also used as a laboratory reagent.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point	Not required for the inclusion of the active substance in Annex I of Directive 91/414 because de melting point of 8-hydroxyquinoline is higher than 40 ºC.	None	
Flammability EEC A10	8-hydroxyquinoline is not highly flammable. (purity 99.9%)	None	Gonzalez, M.B. (2004b and 2004c)
Explosive properties EEC A14	8-hydroxyquinoline does not present danger of explosion (purity 99.9%)	None	Smeykal, H. (2004)

Self-ignition temperature EEC A16	Not auto-inflammable (below the melting point) (purity 99.9%)	None	Gonzalez, M.B. (2004b and 2004c)
Oxidising properties	Structural aspects and estimated thermodynamic properties indicated that 8-hydroxyquinoline has no oxidising properties	None	Tiemann, J. (2004)

3.1 Physical chemical properties

3.1.1 Summary and discussion of physical chemical properties

8-hidroxyquinoline is solid without flammability and explosive or oxidising properties.

3.1.2 Comparison with criteria

8-hidroxyquinoline does not fulfil the criteria for flammability and explosive or oxidising properties according to CLP Regulation

3.1.3 Conclusions on classification and labelling

8-hydroxyquinoline does not require classification for physico-chemical as flammable, auto-flammable, explosive or oxidizing.

RAC general comment
Quinolin-8-ol is refered to as 8 hydroxyquinoline throughtout this opinion.

4 HUMAN HEALTH HAZARD ASSESMENT

Taking into account all the information, a detailed review of the Spanish Authority concluded that the batches used in the toxicological studies are considered to be representative of the technical specification (discussed in PRAPer 81 expert meeting in September 2010).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

For non-human toxicokinetics information on 8-hydroxyquinoine, please see paragraph 4.1.3.

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

All the toxicokinetic data available were obtained from rat studies.

Absorption: Rapidly absorbed from gastrointestinal tract after single oral administration of 10 mg/kg bw of 8-hydroxyquinoline in all animals at 8 h (>80%) based on urinary excretion.

Excretion: Most of the radioactivity was eliminated by urine (80.0-79.6%) and faeces (3.7-4.0%) at 8 h after a single oral administration of 10 mg/kg bw in males-females respectively. At 120 h recovery for both sexes was almost complete. The administered radioactivity was excreted with a half-life of 28 min after oral administration and of 34 min after intravenous administration.

After intravenous administration of 15 mg/kg bw of 8-hydroxyquinoline to male rats with bile fistula, 82.8% of the test material was eliminated in urine and 8.7% in bile within 8 h.

Distribution: Radioactivity in the tissues was observed at marginal levels only at 72 h after oral administration. The greatest concentration found in tissues was in spleen (0.152%), kidneys (0.055%) and liver (0.033%). Mean plasma radioactivity concentrations was observed in all animals after single oral and intravenous administrations of 10 mg/kg bw at 15 min and at 5 min respectively. All available data indicate that there was no accumulation in tissues.

The systemic bioavailability of plasma radioactivity following oral administration of [^{14}C]-8-hydroxyquinoline was 63.4% of that following intravenous administration.

Metabolism: Two metabolites were found in male rat urine and bile after intravenous administration of 15 mg/kg bw of 8-hydroxyquinoline within 8 hours. 8-hydroxyquinoline glucuronide conjugate was collected in urine (59.9%) and bile (8.7%) and 8-hydroxyquinoline sulphate accounted for only in urine (22.9%). Unmetabolized 8-hydroxyquinoline was hardly detected both in urine and bile.

Enterohepatic circulation of 8-hydroxyquinoline was confirmed when the bile of one rat was infused to the duodenum of another one and both main metabolites were present in urine showing reabsorption of glucuronide conjugate.

The fate of the *in vivo* glucuronide conjugate was followed after intravenous administration to rats and about 90% and 10% of the dose were excreted in urine as the same conjugate. In the same way the sulphate metabolite was administered intravenously *in vivo* and 95% was detected in urine but not in bile. These results showed that no hydrolysis of the 8-hydroxyquinoline main metabolites occurred *in vivo*.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

<table>
<tr><th colspan="4">Acute Oral</th></tr>
<tr><th>Method</th><th>Observations</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>The study is pre-guideline
GLP: No
Study acceptable[1]
Wistar rats
10/sex/dose
14-days observation
Single doses of 600, 756, 953 and 1200 mg/kg bw of undiluted test material grounded into a fine powder and suspended in 1% Tylose and Tween</td>
<td>Mortality: Most of rats at higher dose rates died within 24 hours after administration.
<table>
<tr><th>Sex</th><th>Dose (mg/kg bw)</th><th>24 h</th><th>7 days</th><th>14 days</th></tr>
<tr><td>(M)</td><td>600</td><td>0/10</td><td>0/10</td><td>0/10</td></tr>
<tr><td></td><td>756</td><td>2/10</td><td>3/10</td><td>3/10</td></tr>
<tr><td></td><td>953</td><td>8/10</td><td>9/10</td><td>9/10</td></tr>
<tr><td></td><td>1200</td><td>10/10</td><td>10/10</td><td>10/10</td></tr>
<tr><td>(F)</td><td>600</td><td>0/10</td><td>1/10</td><td>1/10</td></tr>
<tr><td></td><td>756</td><td>3/10</td><td>3/10</td><td>3/10</td></tr>
<tr><td></td><td>953</td><td>7/10</td><td>9/10</td><td>9/10</td></tr>
<tr><td></td><td>1200</td><td>9/10</td><td>10/10</td><td>10/10</td></tr>
</table>
Clinical signs:
▪ In all dosage groups abdominal pain syndrome,</td>
<td>LD_{50} females: 790 mg/kg (705-885)
LD_{50} males: 800 mg/kg (714-896)
H302 Cat.4</td>
<td>Dickhaus, S., Heisler, E.; 1981a</td>
</tr>
</table>

<table>
<tr><td>and administered with a rigid bulb headed cannula
Purity: not specified</td><td>exophthalmia, gasping breathing, ataxia and disturbed coordination was observed within 1 hour after administration. Sedation (at all dose levels) and coma (at 756 and 1200 mg/kg bw) were noted after that. The surviving rats also displayed increased nervousness.
▪ Dose related reduction of the bodyweight gains.
<u>Necropsy findings:</u>
▪ Prematurely deceased animals revealed hyperaemia in the stomach and the small intestine (in all groups).
▪ The final dissection of surviving rats revealed distinct lobule marking in the liver and slightly gritty or mottled kidneys (in all groups). A few rats displayed increased localised infiltration in the small intestine (doses not specified).</td><td></td><td></td></tr>
<tr><td>The study is pre-guideline
GLP: No
Study acceptable[1]
CFI mice
5 animals/sex/dose
14-days observation
Single doses of 120, 151, 190 and 240 mg/kg bw suspended in 1% pharmacologically neutral Tylose with a few drops of Tween 20 and administered by a rigid stomach tube
Purity: not specified</td><td><u>Mortality:</u> Mortality occurred up to 24 hours after administration.
<table><tr><th>Sex</th><th>Dose (mg/kg bw)</th><th>24 h</th><th>7 days</th><th>14 days</th></tr><tr><td>(M)</td><td>120</td><td>0/5</td><td>0/5</td><td>0/5</td></tr><tr><td></td><td>151</td><td>0/5</td><td>0/5</td><td>0/5</td></tr><tr><td></td><td>190</td><td>4/5</td><td>4/5</td><td>4/5</td></tr><tr><td></td><td>240</td><td>5/5</td><td>5/5</td><td>5/5</td></tr><tr><td>(F)</td><td>120</td><td>0/5</td><td>0/5</td><td>0/5</td></tr><tr><td></td><td>151</td><td>1/5</td><td>1/5</td><td>1/5</td></tr><tr><td></td><td>190</td><td>3/5</td><td>3/5</td><td>3/5</td></tr><tr><td></td><td>240</td><td>5/5</td><td>5/5</td><td>5/5</td></tr></table><u>Clinical signs:</u>
▪ Up to 24 hours the mice displayed dose-related reduced activity, a decrease in respiratory rate, spasm and diminished reflex response.
▪ During the rest of the follow-up observation period, the surviving mice displayed sedation and reduced reactions and a dose-dependent decrease in bodyweight gains.
<u>Necropsy findings:</u>
▪ Hyperaemia of the small/large intestine, pale parenchyma in kidneys and swelling of the liver were observed in some cases at 151 and 190 mg/kg bw.
▪ Hyperaemia and dilatation of the small/large intestine was observed in some cases at 240 mg/kg bw.</td><td>LD_{50} (both sexes): 177 mg/ kg bw (155-202)
H301 Cat.3</td><td>Dickhaus, S., Heisler, E.; 1981b</td></tr>
<tr><td colspan="4">Acute Dermal</td></tr>
<tr><td>Method</td><td>Observations</td><td>Results</td><td>Reference</td></tr>
<tr><td>The study is pre-guideline
GLP: No
Study acceptable[1]
SPF Wistar rats
5 animals/sex/dose
14-days observation
Test substance was applied under occlusive dressing as a</td><td><u>Mortality:</u> No mortality occurred during the treatment.
<u>Clinical signs:</u> No clinical symptoms were observed.</td><td>LD_{50} > 10000 mg/kg bw</td><td>Dickhaus, S., Heisler, E.; 1981c</td></tr>
</table>

paste at dose levels of 0, 5000 and 10000 mg/ kg bw during 24 hours. Purity: not specified			

[1] These studies were pre-guideline, not GLP compliant and the purity of the test substance was not specified. However, in the absence of other available studies, they are considered acceptable to assess acute toxicity.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

LD_{50} for females: 790 mg/kg bw (705-885) and LD_{50} for males: 800 mg/kg bw (714-896) based on Dickhaus and Heisler (1981a) rat study.

LD_{50} 177 mg/kg bw (155-202) based on Dickhaus and Heisler (1981b) mice study.

Data reported in the EMEA document (EMEA/MRL/464/98-Final): oral LD_{50} values in a range of 1200 to 2300 mg/kg bw in rats and in a range of 220 to 280 mg/kg bw in mice.

4.2.1.2 Acute toxicity: inhalation

No available information about acute inhalation of 8-hydroxyquinoline since it was not required for the inclusion in the Annex I of Directive 91/414 (currently repealed by Regulation 1107/2009).

4.2.1.3 Acute toxicity: dermal

LD_{50} > 10000 mg/kg bw (Dickhaus and Heisler, 1981c).

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

Acute oral toxicity

One acute oral toxicity study in rat and another one in mice were carried out with 8-hydroxiquinoline. LD_{50} in rat study (Dickhaus and Heisler, 1981a) for male and female rats were 790 and 800 mg/kg bw respectively and LD_{50} in mice study (Dickhaus and Heisler, 1981b) was 177 mg/kg bw for both sexes. In both studies purity of the test substance was not stated. Besides, in the EMEA document for 8-hydroxiquinilone (EMEA/MRL/464/98-FINAL) it was reported oral LD_{50} values in a range of 1200 to 2300 mg/ kg bw in rats and in a range of 220 to 280 mg/kg bw in mice.

Acute oral toxicity was discussed during the peer review of 8-hydroxyquinoline. In Reporting Table, 8-hydroxyquinoline rev. 1-1 (08.04.2010), it was explained that results of the acute oral toxicity study in rat were supported by the results of the oral short-term toxicity studies [LD_{50}: 790 mg/kg bw (Dickhaus and Heisler, 1981a; Wistar rats); NOAEL/LOAEL : 97.7/300 mg/kg bw/d (Fascineli, 2006a; 90 days Wistar rats), 168/324 mg/kg bw/d (NTP, 1985; 90 days F344 rats) or 118/321 mg/kg bw/d (Bulnes Goicochea, 2004: 14 days Wistar rats)] while that does not occur in mouse study [LD_{50}: 177 mg/kg bw (Dickhaus and Heisler, 1981b; CFI mice), NOAEL/LOAEL: 405/774 mg/kg bw/d (NTP, 1985; 90 days B6C3F1 mice)]. Results in mice were ruled out and only rat data was taken into account to propose a classification for 8-hydroxyquinoline as R22 (EFSA Conclusion on the Peer Review of 8-hydroxyquinoline,

2011). However, the short term toxicity study in mice (NTP, 1985) was considered acceptable only as additional information and only mortality and variations in bodyweights and food consumption were evaluated. Furthermore, this study was carried out with B6C3F1 mice and the test substance was administered in the diet, while the acute oral toxicity study was performed with CFI mice and the administration was via stomach rigid tube. Besides, oral LD_{50} values in mice in EMEA document seem to support the oral LD_{50} value obtained in Dickhaus and Heisler mice study. MSCA considers that acute oral and short term toxicity studies in mice are not comparable and therefore, LD_{50} in mice cannot be discarded. According to the Guidance on the Application of the CLP Criteria (November 2013), in general terms, when there are data from several species, "classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested".

Consequently, considering the most sensitive species, the MSCA regards the LD_{50} of 177 mg/kg bw obtained with CFI mice (Dickhaus and Heisler, 1981b) to propose a classification as H301, Category 3 according to CLP.

Acute inhalation toxicity

For the inclusion of 8-hydroxyquinoline in the Annex I of Directive 91/414/EEC no inhalation study was required. This was agreed in the PRAPeR Expert Meeting 81 (30 August - 3 September 2010), since the active substance has a vapour pressure of 6.7×10^{-3} Pa at 20°C and there was no concern for inhalation exposure (drip irrigation application only). Therefore, no information about inhalation toxicity was available in the Assessment Report of the active substance.

According to the Guidance Document on Acute Inhalation Toxicity Testing (July 21, 2009) corresponding to OECD Document no 39 for inhalation and the Guidance on the Application of the CLP Criteria (November 2013), in the case of solids, an inhalation acute toxicity study in rat is required with a respirable atmosphere (1-4 μm) and if not, a robust consideration should be provided. No justification about impossibility of generation of a respirable atmosphere has been provided beyond Directive 91/414/EEC exemptions.

Regulation (EU) No 544/2011, setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market has been recently replaced by the Commission Regulation (EU) no 283/2013, modifying the data requirements in order to take into account current scientific and technical knowledge. According to this Regulation, for acute inhalation toxicity a study should be required when any of the following apply: the active substance has a vapour pressure $> 1 \times 10^{-2}$ Pa at 20 °C, is a powder containing a significant proportion of particles of a diameter < 50 μm (> 1 % on weight basis) or is included in products that are powders or are applied by spraying. Under these circumstances the realization of an inhalation acute toxicity study cannot be excluded since 8-hydroxyquinoline is a solid and its granulometry is not known.

It also has to be taken into consideration that apart from the use as PPP, this substance is used as laboratory reagent.

MSCA regards insufficient the available data and no conclusion about inhalation toxicity can be drawn due to the lack of data.

Acute dermal toxicity

One acute dermal toxicity study was carried out with 8-hydroxiquinoline. LD_{50} in rat study (Dickhaus and Heisler, 1981c) for male and female rats was > 10000 mg/kg bw. No classification is required for the active substance for acute dermal toxicity.

4.2.4 Comparison with criteria

Based on the reported acute oral LD_{50} value of 177 mg/kg bw in mice, 8-hydroxyquinoline should be classified as Acute Tox. 3 - H301 (Toxic if swallowed) according to CLP (oral LD_{50} guidance values for this category from 50 to 300 mg/kg bw) due to data in mice.

The LD_{50} value (>10000 mg/kg bw) in rat for dermal acute toxicity is clearly above the threshold value of 2000 mg/kg bw for triggering classification and labelling in CLP. Accordingly no acute classification is proposed for the dermal route.

A conclusion about classification according to inhalation could not be drawn due to the lack of data.

4.2.5 Conclusions on classification and labelling

CLP: Acute Tox. 3 - H301.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify 8-hydroxyquinoline as Acute Tox. 3 - H301 based on the reported acute oral LD_{50} value of 177 mg/kg bw obtained in a study on CFI mice (Dickhaus and Heisler, 1981b).

The DS proposed not to classify for the dermal route since no mortality or clinical signs were observed at the tested dose of 10 000 mg/kg bw (Dickhaus and Heisler, 1981c).

No data were available for the inhalation route.

Comments received during public consultation

One Member State Competent Authority (MSCA) expressed their general agreement with the classification(s) proposed for health hazards. Another Member State agreed with the classification as Acute Tox. 3 (H301) based on the reported oral LD_{50} of 177 mg/kg bw in mice (Cat. 3: Oral LD_{50} > 50 but ≤ 300 mg/kg bw).

Comments received from Industry considered the oral LD_{50} value estimated in the Dickhaus & Heisler (1981b) study to be incorrect and argued that impurities in the test item may have been responsible for the acute toxic effect in mice, as no specification or analysis was provided. A classification as Acute Tox. 4 - H302 (Harmful if swallowed) was suggested. In their response, the DS indicated that the oral LD_{50} value of 177 mg/kg bw obtained in mice by Dickhaus and Heisler (1981b) is in the same range (220 to 280 mg/kg bw) as that observed in a mouse study reported by EMEA.

Assessment and comparison with the classification criteria

The CLH report contains two oral acute toxicity studies. The rat and mouse studies were conducted in 1981, before GLP and test guidelines were developed. In neither study was the purity of the test substance specified; the DS considered the studies as acceptable.

In the absence of newer studies, RAC agrees with the DS to base the classification on the lowest LD_{50} in the most sensitive species and strain used. The LD_{50} values in rats were higher than for mice (females: 790 mg/kg bw, males: 800 mg/kg bw based on a study by the same authors, Dickhaus and Heisler (1981a)).

The comment of Industry that lack of specification on test material purity and impurities reduces the validity of the mouse study is reasonable. However, an argument for a difference in validity between the rat and mouse studies cannot be sustained, since there is also a lack of specification of test material and impurities in the rat study.
8-Hydroxyquinoline (purity 99.9%) was orally administered to NMRI mice in the *in vivo* mammalian spermatogonial chromosome aberration test (August, 2007). At 300 mg/kg bw slightly reduced motility and reduced muscle tone, slight ataxia and slight dyspnoea were noted in 7 of 7 animals in the 24 h sampling time group and slightly reduced motility and reduced muscle tone and slight ataxia were noted in 5 of 7 animals in the 48 h sampling time group from immediately after dosing to 6 h after administration. At 300 mg/kg bw mortality occurred in one animal of the 24 h sampling group and in one animal of the 48 h sampling time group (two days after administration). As all animals were killed at 24 h or 48 h after treatment, no information on the full 14 d observation time is available and an LD_{50} could not be calculated. However, this study demonstrated that mortalities occured at 300 mg/kg bw when the pure substance was applied.
RAC agrees with the DS proposal that based on the reported acute oral LD_{50} value of 177 mg/kg bw in mice, 8-hydroxyquinoline should be classified as Acute Tox. 3 - H301 (Toxic if swallowed) according to CLP (oral LD_{50} guidance values for this category range from 50 to 300 mg/kg bw).
RAC considers that for the available dermal acute toxicity study the LD_{50} was above the cut-off value and agrees with the DS proposal not to classify for the dermal route.

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

The available data indicates that 8-hydroxyquinoline does not need to be classified for specific target organ toxicity.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS concluded that no indication is given that 8-hydroxyquinoline should be classified for STOT SE.

Comments received during public consultation

One MSCA considered that there is enough information to enable a classification of 8-hydroxyquinoline as STOT SE 3 for narcotic effects and indicated that neurotoxic effects of 8-hydroxyquinoline and halogenated hydroxyquinoline derivates were observed both in animals and in humans. The findings are summarised below.

In developmental toxicity studies, transient nervous excitation followed by lethargy after the administration of 8-hydroxyquinoline were observed both in rats and rabbits. In rats, the effects observed were noted at doses of 300 and 600 mg/kg bw/d (Fascineli, 2006c) and in rabbits at 15 and 60 mg/kg bw/d (Fascineli, 2006d).

In an acute oral study in Wistar rats (Dickhaus and Heisler, 1981a), all treated animals (600, 756, 953 and 1200 mg/kg bw) showed ataxia, gasping breathing and disturbed coordination within 1 h after administration. Sedation (at all dose levels) and coma were noted at later time points. Although LD_{50} values of 790 mg/kg bw (females) and 800 mg/kg bw (males) were set, the surviving rats also displayed increased nervousness.

In a second CFI mouse acute oral study (Dickhaus and Heisler, 1981b), animals dosed at 120, 151, 190 and 240 mg/kg bw displayed dose related reduced activity, a decrease in respiratory rate, spasms and diminished reflex response within 24 h. An LD_{50} was set at 177 mg/kg bw (both sexes). During the rest of the follow-up observation period, the surviving mice displayed sedation and reduced reactions.

Furthermore, some symptoms of acute intoxication with 8-hydroxyquinoline were described in mice during the determination of the intraperitoneal LD_{50}. Although the signs were reported at lethal doses (death within 5 to 10 min after administration) they included confusion, respiratory difficulty, occasional hind leg paralysis and terminally, violent convulsion. Doses leading to delayed death (later than 6 h post administration) resulted in anorexia, malaise, slow protective reflex action and general indifference to optical and acoustical stimuli. In dogs, after a single intravenous dose of 10 mg/kg bw and above, significant central nervous system toxicity, presenting as anxiety or convulsion were noted (EMEA/MRL/464/98-FINAL; July, 1998).

The neurotoxic effects observed in animals after administration of 8-hydroxyquinoline are supported by human data on another halogenated hydroxyquinoline derivative, 5-chloro-7-iodo-8-hydroxyquinoline. Indeed, encephalopathy was related to the ingestion of a high dose of clioquinol over a short period. The neurotoxic effect consisted of drowsiness, mental confusion, disorientation, hallucinations, and headache with subsequent amnesia for events occurring during the episode (Baumgartner *et al.*, 1979).

In their response to the MSCA's comments, the DS explained that according to the CLP criteria for STOT SE, if lethality occurred at relevant doses, then a classification for acute toxicity would take precedence and STOT SE would not be assigned. Data mentioned in the comment about acute oral toxicity studies in mice and rats (Dickhaus, 1981a and 1981b) and for the intraperitoneal LD_{50} (EMEA/MRL/464/98-FINAL) should be taken with care since the effects were observed at dose levels close to or above the LD_{50} and they can be considered as clear signs of toxicity that have the potential to cause lethality. The most appropriate classification, either acute oral toxicity or STOT SE 3, should then be assigned to avoid a double classification.

Effects in the developmental studies observed in the absence of lethality were transient signs of nervous system excitation followed by lethargy. However, evaluation of the available information on the repeated dose toxicity of 8-hydroxyquinoline indicated that most of the studies showed no effects after test item administration.

In addition, the DS noted the severe neurotoxic effects observed after ingestion of clioquinol, a

halogenated derivative of 8-hydroxyquinoline (Baumgartner *et al.*, 1979). However, 8-hydroxyquinoline and clioquinol have different chemical structures and therefore the DS was of the opinion that data from this compound are not conclusive for the hazard assessment of 8-hydroxyquinoine and accordingly for the STOT SE 3 classification (narcotic effects).

Assessment and comparison with the classification criteria

Clinical symptoms indicating neurotoxicity observed in the oral acute toxicity studies (rats and mice), in the oral *in vivo* mammalian spermatogonial chromosome aberration test, and in the developmental studies (in rats and rabbits) may be considered to be related to the bolus administration, as these were gavage studies. Exceptions were the dog study where animals received the test substance in a capsule and the repeated dose studies in rats and mice, which were negative for CNS symptoms and which were diet studies.

It is the view of RAC that the observed effects after single oral exposure were related to the conditions at dose levels at or near the LD_{50}. These effects should be considered as covered by the adopted oral acute toxicity classification.

The symptoms in the developmental studies were described as transient: 10 min nervous excitation followed by (20 min) lethargy during the postadministration period wich were not followed by lethality or any other significant nonspecific toxicity.

The excitation observed in rats and rabbits in the developmental studies does not clearly match the nature of a narcotic effect, which is mainly a central nervous system depression.

RAC agrees with the DS conclusion that the available data are **not** sufficient to classify 8-hydroxyquinoline for STOT SE 3 for narcotic effects.

4.4 Irritation

4.4.1 Skin Irritation

Table 11: Summary table of relevant skin irritation studies

<table>
<tr><th>Method</th><th>Main results</th><th>Remarks</th><th>Reference</th></tr>
<tr>
<td>The study is pre-guideline
GLP: No
Study non acceptable
8 New Zealand White rabbits (sex not specified)
7-days observation
0.5 g of the test material applied heavily moistened to 2 intact and 2 scarified sites of each animal in occlusive conditions during 24 hours
Purity: not specified</td>
<td><u>Mean skin irritation scores</u>
<table><tr><th></th><th>Intact Skin</th><th>Scarified Skin</th></tr><tr><td>Erythema</td><td>0</td><td>0</td></tr><tr><td>Oedema</td><td>0</td><td>0</td></tr></table>Up to day 7 (end of the study), no irritation was observed on either intact or scarified skin.</td>
<td>The study is considered unacceptable:
Besides intact skin, scarified skin was used.
The period exposure was 24 hours instead of 4 hours.
The animals were not examined at 24 and 72 hours.</td>
<td>Dickhaus S., Dey-Hazra; 1981a</td>
</tr>
</table>

<table>
<tr>
<td>The study follows the EC guideline B.4.
GLP: Yes
Study acceptable
3 female New Zealand White rabbits
72 hours observation period
0.5 g of the test material (moistened) applied by a semi-occlusive dressing removed after 4 hours
Purity: 99.7%</td>
<td><u>Individual and mean skin irritation scores:</u>

<table>
<tr><th></th><th colspan="3">Erythema</th><th colspan="3">Oedema</th></tr>
<tr><td>Animal</td><td>1</td><td>2</td><td>3</td><td>1</td><td>2</td><td>3</td></tr>
<tr><td>After 24 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 48 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>After 72 h</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Mean score</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>

Overall mean score was 0 for both erythema and oedema.</td>
<td></td>
<td>Stelter, D.; 2008a</td>
</tr>
</table>

4.4.1.1 Non-human information

See table above.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

In Stelter (2008a) skin irritation study in rabbits, the overall mean score following grading at 24, 48 and 72 hours were 0 for erythema and oedema respectively. No signs of skin irritation were observed.

4.4.1.4 Comparison with criteria

The erythema and oedema irritation scores are below the trigger values for classification as irritating to the skin according to CLP (values ≥ 2.3 for erythema or oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal). Therefore, 8-hydroxyquinoline does not require a classification as irritating to the skin.

4.4.1.5 Conclusions on classification and labelling

CLP: Not classified based on available data.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

The DS did not propose classification as a skin irritant since a relevant guideline-conforming study (Stelter, 2008a) with 99.7% 8-hydroxyquinoline revealed no indication of skin irritation.

Comments received during public consultation

One MSCA expressed agreement with no classification for skin irritation.

Assessment and comparison with the classification criteria

No potential for skin irritation was identified in a skin irritation study that was conducted according to OECD TG 404 and GLP.

4.4.2 Eye Irritation

Table 12: Summary table of relevant eye irritation studies

<table>
<tr><th>Method</th><th>Main results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>The study is pre-guideline
GLP: No
Study non acceptable
8 New Zealand White rabbits (sex not specified)
72 h observation period.
The eye was treated with 0.1 ml of the test substance in the form of a 10% solution
Purity: not specified</td><td><u>Individual and mean eye irritation scores</u>
<table>
<tr><th rowspan="2">Effect</th><th colspan="8">Mean scores for each rabbit (24, 48 and 72 hours)</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th></tr>
<tr><td>Conjunctival erythema</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>Chemosis</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>Corneal opacity</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>Iris lesions</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></td><td>▪ The test substance was applied in form of 10% solution and the vehicle was not reported.
▪ Purity of the test substance not specified.</td><td>Dickhaus, S, Dey-Hazra; 1981b</td></tr>
<tr><td>The study follows the EC guideline B.5.
GLP: Yes
Study acceptable
New Zealand White rabbits (3 female)
20 days observation period for animal no. 1 and 7 days for animal no. 2 and 3
0,1 ml of undiluted test material
Purity: 99.7%</td><td><u>Mean values of eye irritation scores (24, 48 and 72 hours):</u>
<table>
<tr><th>Effect</th><th colspan="3"></th></tr>
<tr><td>Rabbit</td><th>1</th><th>2</th><th>3</th></tr>
<tr><td>Conjunctival erythema</td><td>1.00</td><td>1.33</td><td>1.33</td></tr>
<tr><td>Chemosis</td><td>0.33</td><td>0.33</td><td>0.67</td></tr>
<tr><td>Corneal opacity</td><td>1.00</td><td>0</td><td>0</td></tr>
<tr><td>Iris lesions</td><td>0</td><td>0</td><td>0</td></tr>
</table></td><td>▪ Animal no. 1 showed a corneal lesion that persisted until day 20 (end of the study).
▪ Lesions in animal no. 2 and 3 were fully reversible within 7 days.
H318</td><td>Stelter, D.; 2008b</td></tr>
</table>

4.4.2.1 Non-human information

See table above.

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

In Stelter (2008a) skin irritation study in rabbit, corneal opacity or iritis score were ≤ 1 and conjunctival redness or edema score were ≤ 2. However one animal showed a corneal lesion that persisted until day 20 (end of the study).

4.4.2.4 Comparison with criteria

The individual and group mean eye irritation scores do not meet the criteria for classification as irritating to the eyes according to CLP (corneal opacity or iritis score equal to or higher than 1 or conjunctival redness or oedema score equal to or higher than 2). However one animal showed a corneal lesion that persisted until the end of the study on day 20. According to CLP, substances which seriously damage the eyes are classified in Category 1 when

produce in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days. Therefore, the not reversible corneal lesion present in one animal at the end of the study meets the criteria for classification of 8-hydroxyquinoline as Eye Damage 1 – H318.

4.4.2.5 Conclusions on classification and labelling

CLP: Eye Dam. 1 – H318

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS based their conclusion on a guideline-consistent eye irritation study using 99.5% pure 8-hydroxyquinoline. In the Stelter (2008a) eye irritation study in the rabbit, corneal opacity or iritis scores were ≤ 1 and conjunctival redness or oedema scores were ≤ 2. However one animal showed a corneal lesion that persisted until day 20 (end of the study).
The DS concluded that the individual and group mean eye irritation scores do not meet the criteria for classification as irritating to the eyes according to CLP (corneal opacity or iritis score equal to or higher than 1 or conjunctival redness or oedema score equal to or higher than 2). However one animal showed a corneal lesion that persisted until the end of the study on day 20. According to CLP, substances which seriously damage the eyes are classified in Category 1 when they produce in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of (normally) 21 days. Therefore, the not reversible corneal lesion present in one animal at the end of the study meets the criteria for classification of 8-hydroxyquinoline as Eye Dam. 1; H318.

Comments received during public consultation

One MSCA agreed with the classification for Eye Dam. 1.

Assessment and comparison with the classification criteria

RAC agrees with the proposal to classify as Eye Dam. 1; H318 (Causes serious eye damage) based on the observation that a corneal lesion in one animal persisted until day 20.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

According to section 2.4.1, in the ECHA inventory, one notifier purposed a classification for 8-hydroxyquinoline as STOT SE 3 (H335). However, there is no information regarding the ability of 8-hydroxyquinoline to cause irritation to the respiratory tract.

4.4.3.4 Conclusions on classification and labelling

CLP: Not classified based on available data.

4.5 Corrosivity

4.5.1 Non-human information

No corrosive effects have been seen in data available.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

8-hydroxyquinoline does not present corrosive effects in data available.

4.5.4 Comparison with criteria

8-hydroxyquinoline does not require classification due to corrosivity.

4.5.5 Conclusions on classification and labelling

CLP: Not classified based on available data.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

No skin sensitisation studies in animals were provided for the inclusion in the Annex I of the Directive 91/414/EEC since it is known that 8-hydroxyquinoline induces skin sensitisation in humans.

4.6.1.2 Human information

The following three articles compiling data in humans of the sensitisation potential of 8-hydroxyquinoline and quinoline derivatives are available:

"Sensibilisierungen und Gruppensensibilisierungen durch Chinolinderivate"
(Sensitisation and group sensitisations caused by quinoline derivates)
Pevny I. and Schellenberg J. 22. Jg. Heft 1, 1971
Dermatologische Klinik and Poliklinik der Universitat Würburg

MSCA comments: contradictions on the identity of Chinosol® were observed. This substance was referenced in the first paragraph of the article as a chlorine hydroxyquinoline derivative while in other parts of the text appeared as 8-hydroxyquinoline. MSCA regards Chinosol® corresponds to 8-hydroxyquinoline. Besides, the human studies lack some information: grade of exposition (concentration of the applied test substance is unknown), duration of some studies and specific details about the mode of application.

The document consists of a wide revision of the allergic properties of quinoline derivatives, including 8-hydroxyquinoline (Chinosol®). It takes into consideration information from literature and the results of three studies provided by the reporters. These three studies through topical application were carried out using quinoline derivatives, including 8-hydroxyquinoline (Chinosol®).

- The first study was carried out in 1967/68 with 127 patients. Sensitisation rate for 8-hydroxyquinoline was 4.7%.
- A second study was carried out in 1969 with 100 patients. Subjects also included patients for whom previous topical use was not certain but only probable. Sensitivity to 8-hydroxyquinoline was confirmed in 8%.
- Another study was carried out simultaneously in 1969 with 100 patients. Sensitivity to 8-hydroxyquinoline was confirmed in six patients (6%).

Authors concluded that the group-allergic reactions which take place most frequently are those between compounds containing an OH group or an OH group and a halogen group, being the chlorinated compound the most frequent followed by the fluorinated, bromated and then, much less frequently the pure iodated chinoline derivatives. This was also supported by information collected in literature.

A wide range of quinoline derivatives, including 8-hydroxyquinoline, caused allergen reactions to humans.

"Hydroxychinoline — ein schwaches Kontaktallergen" (Hydroxyquinoline — a weak contact allergen)
Rothe, A. Medicamentum, Berlin (1978), 366-367
Central Institute for Occupational Medicine of the GDR, Berlin

MSCA comments: the epidermal test lacks information: grade of exposition (concentration of the applied test substance is unknown) and specific details about the mode of application.

The author estimates that information provided by Pevny (1971) cannot be taken into consideration since most of the patients of the studies were examined because suspicion of hypersensitivity to 8-hydroxyquinoline or its derivatives already existed.

In an epidermal test series for the determination of contact eczema caused by drugs within a period of six years, hypersensitivity of 8-hydroxyquinoline was observed only in three patients out of 450. He states that 8-hydroxyquinoline is a weak allergen.
"Kontaktsensibilisierungen durch Tosylchloramidnatrium (Chloramin®) and Hydroxychinolin (Sulphachin®)" (Contact Dermatitis caused by Tosylchloramide® and Hydroxyquinoline (Sulfachin®)
Metzner, H.-H. Dermatol. Mon.schr. 173 (1987) 674-676

MSCA comments: only one subject was evaluated.

The document compiles two cases with severe eczema reactions appeared after exposition to 8-hydroxyquinoline sulphate (Sulphachin®) and tosylchloramide (Chloramin®). The case with 8-hydroxyquinoline sulphate is explained below.

32 year-old subject with dermatitis was treated with 0.1% aqueous solution of 8-hydroxyquinoline and an ointment containing 0.02% of the same substance. However, the eczema was exacerbated and the therapy was changed to treatment to oxytetracycline. After delayed improvement of the eczema, an epidermal patch test with aqueous solutions with 8-hydroxiquinoline sulphate yielded to positive skin reactions with symptoms of inflammation. A clear reaction of infiltration was observed at concentrations above 0.01%.

Therefore, it was concluded that 8-hydroxyquinoline can act as a powerful skin sensitiser.

4.6.1.3 Summary and discussion of skin sensitisation

Topical application of 8-hydroxyquinoline resulted in positive sensitisation responses in three studies carried out in humans (Pevny, 1971). The indexes of sensitivity (percentage of positive responses) were 4.7%, 8% and 6% respectively. The study lacks some information (grade of exposition, duration of some studies and specific details about the mode of application). 8-hydroxyquinoline was considered an allergen in humans.

In an epidermal test series (Rothe, 1977) for the determination of contact eczema caused by drugs within a period of six years, hypersensitivity to 8-hydroxyquinoline was observed only in three patients out of 450. Neither the grade of exposition nor more detail on the mode of application was provided. In this case 8-hydroxyquinoline was found to be a weak contact allergen.

8-hydroxyquinoline sulphate showed a positive response in only one subject at concentrations of the test compound (aqueous solutions) above 0.01% (Metzner, 1987) and was considered a strong sensitiser.

4.6.1.4 Comparison with criteria

There is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of people. Regarding available data in humans, 8-hydroxyquinoline should be classified as skin sensitiser: H317 (May cause an allergic skin reaction).

According to CLP criteria, sub-categorisation has been introduced in the 2nd ATP. In the case of human data, sub-categorisation has to follow the criteria given in section 3.4.2.2.2. The absence of data about the grade of exposure, the duration of the studies in some cases and the mode of application in Pevny and Rothe studies complicates the evaluation of the information in order to differentiate between sub-categories 1A and 1B. Results in Metzner study in one subject with 8-hydroxyquinoline sulphate gave a threshold value of 0.01% for skin sensitisation which means a strong incidence in the response but the frequency in the population was not evaluated since only one subject was observed. Therefore, the MSCA considers that information available in not sufficient for sub-categorisation.

Taking into consideration data provided in human studies for 8-hydroxyquinoline, the MSCA has concluded that it is not feasible to set sub-categories. Therefore, 8-hydroxyquinoline shall be classified in Category 1 since there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons and data are not sufficient for sub-categorisation. Following these criteria, 8-hydroxyquinoline should be classified as Skin Sens. 1 - H317 (May cause an allergic skin reaction).

4.6.1.5 Conclusions on classification and labelling

CLP: Skin Sens. 1 - H317.

4.6.2 Respiratory Sensitisation

No data on respiratory sensitisation available.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal The DS indicated that skin sensitisation studies in animals were not provided to support the inclusion of 8-hydroxyquinoline in Annex I of Dir 91/414/EEC, as it is known that 8-hydroxyquinoline is a skin sensitiser in humans.

The CLH report documented skin sensitisation in three human studies published in 1971-1987 (Pevny, 1971, Rothe, 1977, Metzner, 1987). The highest percentages of positive response to 8-hydroxyquinoline was observed in 4.7%, 8% and 6% of patients after topical application in three studies published by Pevny (1971). However the DS found that the study reports lack some information (grade of exposure, duration of some studies and specific details about the mode of application).

Comments received during public consultation

Two MSCA agreed with the proposed classification as a skin sensitiser (Skin Sens. 1 - H317). One MSCA explained that it agreed with the proposed classification because sensitisation in humans studies was reported in 3 studies with sensitisation rates of 4.7%, 8% and 6%; all considered high frequency (≥ 0.2% of general population, ≥ 1% of selected dermatitis patients and ≥ 2% selected dermatitis patients). Sub-categorisation was considered to not be possible due to lack of information with regards to grade of exposure, duration of studies (in some cases) and mode of application.

Assessment and comparison with the classification criteria

There is evidence from historical data that the substance can lead to sensitisation by skin contact in humans. There are no data from animal studies.

To reflect the potency of a skin sensitiser, sub-categorisation should be proposed if data allow this. The sensitisation rates of 4.7%, 8% and 6% in the three studies of the Pevny publication were above the threshold for high frequency in Table 3.4.2-b of the CLP Guidance which is ≥ 1% of unselected dermatitis patients and ≥ 2% of selected dermatitis patients. From the available information it is not clear whether the patients were unselected or selected dermatitis patients. The guidance defines selected dermatitis patients as those on which aimed testing or a special test series was conducted. As a single diagnostic standard epicutan testing (patch test) was conducted in groups of ≥ 100 patients of the dermatologic clinic for each of the three studies of the Pevny publication, the groups could be identified as selected dermatitis patients. The test material was identified as Chinosol® solution. Currently available Chinosol® -containing medical solutions (e.g. for antimicrobial/antifungal disinfection) contain up to 0.25% hydroxyquinoline sulphate. The uncertainties identified by the DS regarding the duration of the studies and mode of application are reported in the Pevny studies. The remaining uncertainties are the lack of information on the concentration of the Chinosol® solution used as test material for the diagnostic patch tests in the 1970's and the lack of details of the testing (e.g. observation time). As the subcategorisation requires information on the frequencies (once) and on the level of exposure (unknown), RAC supports the view of the DS that subcategorisation is not feasible based on this study.

Some information on the concentration that provoked a positive skin reaction was given by the Metzner (1987) study, which documented one case report. Eczema appeared after exposure to 8-hydroxyquinoline sulphate (Sulphachin®) and this was exacerbated when treated with 0.1% aqueous solution of 8-hydroxyquinoline and an ointment containing 0.02% of the same substance. After delayed improvement of the eczema, an epidermal patch test with aqueous solutions with 8-hydroxyquinoline sulphate yielded positive skin reactions with symptoms of inflammation from concentrations of 0.01% which would correspond to a relatively low exposure.

Conclusion
Taking into consideration data provided in human studies for 8-hydroxyquinoline that were published between 1978-1987, RAC agrees that it is not feasible to set sub-categories. Therefore, 8-hydroxyquinoline shall be classified in Category 1 since there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons and data are not sufficient for sub-categorisation. Following these criteria, 8-hydroxyquinoline should be classified as Skin Sens. 1 - H317

(May cause an allergic skin reaction).

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 13: Summary of short-term studies

Method	Target organ/ Main effect	LOAEL mg/kg/day	NOAEL mg/kg/day	Reference
Rat toxicity studies				
Range finding 14 days oral in rat (diet) Wistar rats (*Rattus novergicus*) (BIOAGRI Laboratories DI/Brazil) Purity: 99.68% 5 rats sex/dose level Dose: 0, 1000, 3000, 8000 ppm ▪ (m): 0, 117.8, 321.5, 724.4 mg/kg bw/d ▪ (f): 0, 120.5, 344.1, 864.4 mg/kg bw/d OECD 407 Guideline (1995) GLP: Yes Rats were only observed for mortality, signs of toxicity, bodyweight variations and daily intake. Haematology, clotting and clinical chemistry were only performed at 0 and 1000 ppm. Study acceptable	**At 8000 ppm:** Decreased bodyweight gain in both sexes. Decreased food consumption during the first week in males (54.7%) **At 3000 ppm:** Decreased bodyweight gain in both sexes. Decreased food consumption during the first week in males (19.3%)	3000 ppm 321.5 (m) 344 (f)	1000 ppm 117.8 (m) 120.5 (f)	***Bulnes Goicochea, C. (2004)***
Range finding 15 days oral in rat (diet) Rat F344/N (Labs. Charles River) Purity: approximately of 99% 5 rats sex/dose level Dose: 0, 3000, 6000, 12000, 25000, 50000 ppm OECD 407 Guideline (1998) GLP: No Rats were only observed for mortality, signs of toxicity and bodyweight variations. Study acceptable as additional information	Target organs were not identified At 50000 ppm 2/5 males died, one on day 12 and the other on day 13. Decreased bodyweight in males from 12000 ppm and in females from 25000 ppm.	12000 ppm	6000 ppm	***National Toxicology Program (1985)***
90 days oral in rat (diet) Rat F344/N (Labs. Charles River) Purity: approximately of 99% 10 rats by sex/ group doses Dose: 0, 800, 1500, 3000, 6000 y 12000 ppm ▪ (m): 0, 48, 87, 168, 342, 660 mg/kg bw/d ▪ (f): 0, 66, 128, 180, 324, 660 mg/kg bw/d OECD 408 Guideline (1998) GLP: No Individual data were not reported. No haematology, clinical chemistry or urinalysis were performed. Rats were	Target organs were not identified. **At 12000 ppm:** Bodyweights decreases of 18% in males and 9.5% in females Bodyweights gain decreases of 38.7% in males and 28% in females Decrease food consumption of 30.4% in females. **At 6000 ppm:** Bodyweights decreases of 10.5% in females. Bodyweights gain decreases of 30.7% in females.	6000 ppm 342 (m) 324 (f)	3000 ppm 168 (m) 180 (f)	***National Toxicology Program (1985)***

Method	Target organ/ Main effect	LOAEL mg/kg/day	NOAEL mg/kg/day	Reference
only observed for mortality, signs of toxicity, bodyweight and food consumption. Histopathology was only observed in controls and at 12000 ppm. Study acceptable as additional information	Decrease food consumption of 31.7% in females.			
90 days oral in rat (diet) Wistar rats (*Rattus novergicus*). (BIOAGRI Laboratories DI/Brazil) Purity: 99.68% 10 rats by sex/ group doses in principal and satellite groups Principal group : Duration: 13 weeks Dose: 0, 1000, 3000, 6000 ppm ▪ (m): 0, 97.7, 300, 547.7 mg/kg bw/d ▪ (f): 0, 114.2, 348.1, 623.7 mg/kg bw/d Satellite group: Duration: 17 weeks (for recovery period) Dose: 0, 6000 ppm ▪ (m): 0, 547.7 mg/kg bw/d ▪ (f): 0, 623.7 mg/kg bw/d OECD 408 Guideline (1998) GLP: Yes Study acceptable	**At 6000 ppm**: decreases in bw, bw gain and food consumption in males and food consumption in females. Reduced RBC and HCT and increased MCH and MCHC in females during treatment and recovery period. Increased MCV in males during treatment and recovery period. Decreases in protombine time and total protein in males. Increased relative left kidney, heart, brain, testes and spleen weights **At 3000 ppm**: reduced RBC and HCT and increased MCH and MCHC in females. Increased MCV in males. Increased relative left kidney and spleen weights in males.	3000 ppm 300 (m) 348 (f)	1000 ppm 97.7 (m) 114 (f)	***Fascineli, M.L. (2006a)***
Mice toxicity studies				
Range finding 15 days oral in mouse (diet) Mice B6C3F1 (Labs. Charles River) Purity: approximately of 99%. 5 mice sex/dose Dose: 0, 3000, 6000, 12000, 25000, 50000 ppm. OECD 407 Guideline (1998) GLP: No Mice were only observed for mortality, signs of toxicity and bodyweight variations. Study acceptable as additional information	Target organs were not identified All animals that received 25000 ppm or more died. Decreased bodyweight in males treated at 12000 ppm.	12000 ppm	6000 ppm	***National Toxicology Program (1985)***
90 days oral in mouse (diet) Mice B6C3F1 (Labs. Charles River) Purity: approximately of 99% 10 mices by sex/ group doses Dose: 0, 400, 800, 1500, 3000, 6000 ppm ▪ (m): 0, 60, 113, 195, 405, 774 mg/kg bw/d ▪ (f): 0, 77, 166, 275, 1176, 888 mg/kg bw/d OECD 408 Guideline (1998) GLP: No Individual data were not reported. No haematology, clinical chemistry or urinalysis were performed. Animals were only observed for mortality, signs of	Target organs were not identified. **At 6000 ppm:** Decreases in bw of 11.3 and 10.4 % in males and females respectively. Decreases in bw gain of 33.6 and 38.6% in males and females respectively. Decreases in food consumption of 17.8 and 26 % in males and females respectively	6000 ppm 774 (m)	3000 ppm 405 (m)	***National Toxicology Program (1985)***

Method	Target organ/ Main effect	LOAEL mg/kg/day	NOAEL mg/kg/day	Reference
toxicity, bodyweight and food consumption. Histopathology was only observed in controls and at 6000 ppm. Study acceptable as additional information				
Dog toxicity studies				
90 days oral in dog (via capsules) Beagle dogs (*Canis familiaris*) Purity: 99.68% 4 beagle dogs by sex/ group doses Based on the EMEA document (EMEA/MRL/464/98), which describes the NOAEL as 6 mg/kg bw/d in a 104 weeks oral study, and data generated in the preliminary test (NOAEL lower than 100 mg/kg bw/d in 28-day exposure period) the selected doses were 0, 10, 50, 100 mg/kg bw/d. OECD 409 Guideline (1998) GLP: Yes Study acceptable	Target organs were not identified. Adverse effects were not observed.	Not applicable	> 100	***Marcondes de França, A. (2005)***

Rat studies

Oral 14-days toxicity study in rat. (*Bulnes Goicochea, 2004)*

Title	**14 day oral toxicity in rats**
Author (s) (year):	*Bulnes Goicochea, C. (2004)*
Administration	Oral (dietary) for 14 days
Guideline (year)	OECD 407 (1995)
Specie	Wistar rats
GLP	Yes
Purity:	99.68%
Groups	5 rats/sex/dose level
Dose levels	0, 1000, 3000, 8000 ppm equivalent to: Males: 0, 117.8, 321.5, 724.4 mg/kg bw/d Females: 0, 120.5, 344.1, 864.4 mg/kg bw/d
Study acceptable	

Table 14: Main findings in 14-days study in rat. *(Bulnes Goicochea, 2004)*

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Mean compound intakes (mg/kg bw/d)	-	117.8	321.5	724.4	-	120.5	344.1	864.4
Bodyweight gain (g)								
0-14 days	74.4	81.4	55.8 (-25.0%)	32.4 (-56.5%)	30.8	29.0 (-5.9%)	26.4 (-14.3%)	24.8 (-19.5%)
Food consumption (g/animal/day)								
0-7 days	129.5	123.1	104.5 (-19.3%)	58.7 (-54.7%)	108.7	124.8	122.3	102.7
7-14 days	113.4	112.6	109.8	122.4	118.4	116.3	107.1	113.4

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Hematology								
Red bood cells								
RBC ($x10^6/nm^3$)	7.81	8.05			7.37	7.60		
HB (g/dl)	16.1	16.8			16.3	16.2		
HCT (%)	52.9	54.8			49.2	49.3		
MCV (μ^3)	67.7	68.1			66.7	65.0		
MCH (µg)	22.6	20.9			22.2	21.6		
MCHC (%)	30.4	30.6			33.2	33.3		
Platelets ($x10^3/mm^3$)	834	989			1031	924		
Mean blood coagulation time								
Prothombin time (s)	14.5	13.3			13.2	12.8		
APTT (s)	21.9	18.9			14.6	18.2		
White blood cells								
Leukocyte ($/mm^3$)	5600	6220			6960	6160		
Lymphocyte ($/mm^3$)	4791	5570			5976	5501		
Band ($/mm^3$)	14	32			45	13		
Segmented ($/mm^3$)	673	455			782	497		
Eosinophil ($/mm^3$)	56	86			81	87		
Basophil ($/mm^3$)	0	0			0	0		
Monocyte ($/mm^3$)	65	77			76	62		
Clinical biochemistry								
AST (U/L)	148.2	111.8			240.6	157		
ALT (U/L)	81.0	76.2			77.6	71.4		
ALP (U/L)	225.6	250.8			187.2	180.8		
Albumin (g/dL)	3.63	3.64			3.94	4.0		
Total protein(g/dL)	6.13	6.18			6.57	6.38		
Cholesterol(mg/dL)	67.2	67.0			75.6	76.0		
Glucose (mg/dL)	182.8	159.0			150.4	185.0		
BUN (mg/dL)	52.8	55.8			66.2	65.0		
Creatinine (mg/dL)	1.0	0.93			1.17	1.16		
Ca (mEq/L)	10.9	11.1			10.6	10.9		
Na (mEq/L)	135.0	134.2			138.6	136.6		
K (mEq/L)	7.96	8.02			7.88	7.54		
Pathology								
No examined	5	5	5	5	5	5	5	5
Hydronephrosis (right)	0	0	1	0				
Hydronephrosis (Bilateral)	1	1	0	1	1	1	0	0
Decreased testes (Bilateral)	1	0	0	0				

Oral 15-day toxicity study in rat [National Toxicology Program (NTP, 1985)]

Title	**Fifteen-day studies in rats**
Reference (year):	National Toxicology Program. (1985)
Administration	Diet for 15 days
Specie	Rats F344/N
Guideline (year)	OECD 407 Guideline (1998)
GLP	No
Purity:	Approximately 99%
Groups	5 F rats by sex and dose level
Dose levels	0, 3000, 6000, 12000, 25000 and 50000 ppm
Desviations	Dose levels in mg/kg bw/day were not established.
This study was accepted as additional information	

Table 15: Main findings in 14-days study in rats (NTP, 1985)

Parameter	**Dose level (ppm)**											
	Males						**Females**					
	0	**3000**	**6000**	**12000**	**25000**	**50000**	**0**	**3000**	**6000**	**12000**	**25000**	**50000**
Mortality												
Mortality	0	0	0	0	0	2/5	0	0	0	0	0	0
Bodyweight												
Mean initial bw (g)	152	151	152	152	151	152	124	123	123	124	124	123
Mean final bw (g)	225	222	218	**192** (-14.7%)	**145** (-35.6%)	**105** (-53.3%)	153	149	152	152	**131** (-14.4%)	**103** (-32.7%)
Change bw (g)	+73	+71	+66	**+40**	**-6**	**-47**	+29	+26	+29	+28	**+7**	**-20**

Oral thirteen-weeks studies in rats [National Toxicology Program (NTP, 1985)].

Title	**Thirteen-weeks studies in rats**
Reference (year):	National Toxicology Program. (1985)
Administration	diet for 13 weeks
Specie	Rats F344/N
Guideline (year)	OECD 408 Guideline (1998)
GLP	No
Purity:	Approximately 99%
Groups	10 F344/N rats by sex and dose level
Dose levels	0, 800, 1500, 3000, 6000 or 12000 ppm equivalent to Males: 0, 48, 87, 168, 342, 660 mg/kg bw/d Females: 0, 66, 128, 180, 324, 660 mg/kg bw/d
Desviations	Individual data were not reported. No hematology, clinical chemistry or urinalysis were performed.
This study was accepted as additional information	

Table 16: Main findings in 13-week study in rats (NTP, 1985)

Parameter	**Dose levels (ppm)**											
	Males						**Females**					
	0	**800**	**1500**	**3000**	**6000**	**12000**	**0**	**800**	**1500**	**3000**	**6000**	**12000**
Mean compound intakes (mg/kg bw/d)	0	48	87	168	342	660	0	66	128	180	324	660
Mortality												
Mortality	0	0	0	0	0	0	0	0	0	0	0	0
Bodyweights												

Parameter	Dose levels (ppm)											
	Males						Females					
	0	**800**	**1500**	**3000**	**6000**	**12000**	**0**	**800**	**1500**	**3000**	**6000**	**12000**
Mean initial bw (g)	181	182	183	182	181	182	135	136	135	135	136	136
Mean final bw (g)	344	333	338	324	327	282 (-18.0%)	210	207	203	198	188 (-10.5%)	190 (-9.5%)
Change bw (g)	+163	+151	+155	+142	+146	+100 (-38.7%)	+75	+71	+68	+63	+52 (-30.7%)	+54 (-28%)
Food consumption (week 12)												
Food consumption (g/kg bw/day)	53	60	58	56	57	55	79	83	85	60	54 (-31.7%)	55 (-30.4%)
Pathology												
Hyperplasia in the pancreatic lymph nodes	-					-	-					2/10

Oral 90-day toxicity study in rat. *(Fascineli, 2006a).*

Title	**90 day oral toxicity in rats**
Author (s) (year):	*Fascineli, M.L. (2006a)*
Administration	Oral (dietary) for 13 weeks
Specie	Wistar rats
Guideline (year)	OECD 408 (1998)
GLP	Yes
Purity:	99.68%
Groups	Principal group: 10 rats/sex/dose level Satellite group: 10 sex/ group control and highest dose (were maintained for more 4 weeks days the end of the treatment for observation of reversibility or persistence of toxic effects)
Dose levels	0, 1000, 3000, 6000 ppm equivalent to: Males: 0, 97.7, 300, 547.7 mg/kg bw/d Females: 0, 114.2, 348.1, 623.7 mg/kg bw/d
	Study acceptable

Table 17: Main findings in 90-day study in rat. *(Fascineli, 2006a)*

Parameter	Dose level (ppm)							
	Males				Females			
	0	**1000**	**3000**	**6000**	**0**	**1000**	**3000**	**6000**
Mean compound intakes (mg/kg bw/d)	-	97.7	300	547.7	-	114.2	348.1	623.7
Bodyweight (g)								
Week 2	244.5	243.6	236.1	**233.4*** **(-4.5%)**	166.5	176.2*	172.1	171.8
Week 13	398.4	393.2	373.7	**357.1*** **(-10.4%)**	220.1	236.4*	232.1*	229.4*
Week 17[a]	380.8	-	-	376.9	214.6	-	-	225.1*
Bodyweight gain (g)								
Weeks (0-13)	236.3	229.8	220.4	**190.9*** **(-19.2%)**	84.4	95.3*	95.4*	92.3
Weeks (13-17)[a]	0.3	-	-	13.0*	-3.7	-	-	-3.9
Food consumption (g/animal/day)								
Week 0-1	177.7	158.7	162.3	**139.9*** **(-21.3%)**	170.4	156.2	163.3	**152.6*** **(-10.4%)**
Week 12-13	75.3	**64.0*** **(-15.0%)**	65.9 (-12.5%)	**58.0*** **(-23.0%)**	111.1	**86.0*** **(-22.6%)**	**89.5*** **(-19.4%)**	**71.3*** **(-35.8%)**
Week 16-17[a]	76.7	-	-	**65.0*** **(-15.3%)**	133.1	-	-	**88.5*** **(-33.5%)**

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	6000	0	1000	3000	6000
Hematology								
Red bood cells								
RBC ($x10^6/nm^3$)	8.63	8.81	8.50	8.51	7.98	7.73	**7.07*** **(-11.4%)**	**6.69*** **(-16.2%)**
RBC ($x10^6/nm^3$) Recovery period					9.16	-	-	**8.04*** **(-12.2%)**
Hb (g/dl)	16.7	16.8	16.9	16.9	16.2	16.0	15.6	15.7
Hct (%)	47.7	48.8	48.5	48.6	43.0	41.5	**38.6*** **(-10.2%)**	**36.8*** **(-14.4%)**
Hct (%) Recovery period					48.7	-	-	**43.8*** **(-10.1%)**
MCV (μ^3)	55.2	55.3	**57.3*** **(+3.8%)**	**57.2*** **(+3.6%)**	53.9	53.8	54.6	54.9
MCV (μ^3) Recovery period	51.1	-	-	**53.1*** **(+3.9%)**				
MCH (µg)	19.4	19.1	19.9	19.9	20.3	20.8	**22.1*** **(+8.9%)**	**23.5*** **(+15.8%)**
MCH (µg) Recovery period					18.7	-	-	**21.2*** **(+13.4%)**
MCHC (%)	35.1	34.5	34.9	34.8	37.7	38.7	**40.5*** **(+7.4%)**	**42.7*** **(+13.3%)**
MCHC (%) Recovery period					35.3	-	-	**38.9** **(+10.2%)**
Platelets ($x10^3/mm^3$)	764	744	772	751	817	895	**989***	**1128***
Mean blood coagulation time								
Prothombin time (s)	14.5	14.3	14.0	13.7*	13.2	12.9	13.1	13.0
APTT (s)	14.7	15.8	14.8	15.9	14.6	11.6	14.1	14.6
White blood cells								
Total White Blood cell count ($/mm^3$)	6890	6060	6900	6730	4440	4020	4140	4480
Lymphocyte ($/mm^3$)	5911	4746	5576	5525	3809	3416	3458	3718
Band Neutrophils ($/mm^3$)	92	96	85	93	65	46	57	52
Segmented Neutrophils ($/mm^3$)	671	936	972	834	400	424	506	531
Eosinophil ($/mm^3$)	116	99	132	136	89	71	51	92
Basophil ($/mm^3$)	0	0	0	0	0	0	0	0
Monocyte ($/mm^3$)	101	183	136	142	76	63	68	88
Clinical biochemistry								
AST (U/L)	93.7	105.5	92.8	114.0	113.7	122.0	108.6	114.1
ALT (U/L)	57.3	62.8	62.5	69.5	46.6	39.4	63.1	49.2
ALP (U/L)	104.4	114.6	117.5	79.8	66.1	46.8	72.4	42.3
Albumin (g/dL)	3.48	3.52	3.43	3.41	3.67	3.72	3.72	3.53
Total protein (g/dL)	6.92	6.87	6.97	**6.58*** **(-4.9%)**	6.75	6.82	7.01	6.67
Total protein Recovery period	6.35	-	-	6.17				
Cholesterol (mg/dL)	61.5	59.6	71.8	75.4	71.6	72.5	82.1	73.0
Glucose (mg/dL)	184.1	153.7	178.6	166.0	154.9	146.4	157.4	167.1
BUN (mg/dL)	55.5	59.3	61.2	56.4	46.1	41.6	48.4	43.6
Creatinine (mg/dL)	0.93	0.93	0.93	0.91	0.97	0.99	0.98	0.99
Ca (mg/dL)	11.11	10.62	11.34	10.72	12.49	11.75	12.57	12.21

Parameter	Dose level (ppm)							
	Males				Females			
	0	**1000**	**3000**	**6000**	**0**	**1000**	**3000**	**6000**
Na (mEq/L)	144.0	143.7	143.3	144.0	145.6	145.6	147.0	144.3
K (mEq/L)	8.58	8.99	8.91	7.92	7.19	7.20	7.17	7.95
Organ weights (g)								
Heart								
Absolute	1.402	1.400	1.363	1.356	1.000	1.047	0.995	0.947
Relative	0.337	0.358	0.365	**0.386*** **(+14.8%)**	0.452	0.443	0.429	0.412
Relative (recovery period)	0.361	-	-	0.363				
Liver								
Absolute	13.452	12.198	13.113	**11.317*** **(-15.9%)**	7.268	7.292	**8.526*** **(-17.3%)**	7.444
Absolute (recovery period)	11.170	-	-	12.447				
Relative	3.235	3.107	**3.500***	3.226	3.297	3.090	3.676	3.244
Right Kidney								
Absolute	1.456	1.411	1.330	**1.290*** **(-11.7%)**	0.775	0.805	**0.857***	0.794
Absolute (recovery period)	1.316	-	-	1.356				
Relative	0.349	0.359	0.356	0.368	0.350	0.341	0.369	0.346
Left Kidney								
Absolute	1.361	1.326	1.320	**1.228*** **(-10.3%)**	0.746	0.785	0.804	0.757
Absolute (recovery period)	1.279	-	-	1.298				
Relative	0.327	0.338	**0.353*** **(+9.2%)**	**0.351*** **(+9.2%)**	0.337	0.332	0.346	0.330
Relative (recovery period)	0.335	-	-	0.345				
Spleen								
Absolute	0.664	0.645	0.689	0.678	0.480	0.499	0.499	0.493
Relative	0.159	0.164	**0.185*** **(+18.9%)**	**0.195*** **(+25.2%)**	0.216	0.211	0.215	0.215
Relative (recovery period)	0.174	-	-	**0.215*** **(+28.7%)**				
Right Adrenal								
Absolute	0.035	0.037	0.031	0.033	0.035	0.040	0.039	0.042
Relative	0.008	0.009	0.008	0.009	0.016	0.017	0.017	0.018
Left Adrenal								
Absolute	0.036	0.035	0.032	0.037	0.038	0.041	0.042	0.041
Relative	0.009	0.009	0.009	0.011	0.017	0.018	0.018	0.018
Thymus								
Absolute	0.422	0.407	0.356	0.354	0.337	0.357	0.316	0.337
Relative	0.101	0.104	0.095	0.101	0.152	0.151	0.137	0.146
Brain								
Absolute	2.071	2.066	1.997	1.998	1.849	1.890	1.882	1.889
Relative	0.499	0.529	0.537	**0.572*** **(+16.0%)**	0.835	0.800	0.813	0.825
Relative (recovery period)	0.519	-	-	0.555				
Right Testis								
Absolute	1.893	1.837	1.818	1.762				
Relative	0.455	0.471	0.487	**0.505*** **(+11.0%)**				
Relative (recovery period)	0.465	-	-	0.462				

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	6000	0	1000	3000	6000
Left Testis								
Absolute	1.891	1.819	1.736	1.774				
Relative	0.455	0.466	0.466	**0.508*** **(+13.2%)**				
Relative (recovery period)	0.468	-	-	0.487				
Right Epididymis								
Absolute	0.648	0.605	0.659	0.621				
Relative	0.156	0.155	0.177	0.178				
Left Epididymis								
Absolute	0.668	0.594	0.653	0.616				
Relative	0.161	0.152	0.175	0.177				
Uterus								
Absolute					0.596	**0.883*** **(+48.7%)**	0.693	**0.839*** **(+42.0%)**
Absolute (recovery period)					0.848	-	-	1.024
Relative					0.267	**0.374***	0.299	0.371
Right Ovary								
Absolute					0.076	0.086	0.074	0.080
Relative					0.034	0.036	0.032	0.035
Left Ovary								
Absolute					0.070	0.085	0.083	0.080
Relative					0.031	0.036	0.036	0.035
Pathology								
Right Kidney								
Hydronephrosis								
Principal group	0/10	0/10	0/10	0/10				
Satellite group	1/10	-	-	1/10				
Uterus								
Hydrometra								
Principal group					0/10	1/10	0/10	2/10
Satellite group					1/10	-	-	2/10
Left ovary								
Cyst								
Principal group					0/10	0/10	1/10	0/10
Satellite group					0/10	-	-	1/10

[a] *Satellite groups*
** Significantly different from the control group (p<0.05)*

Mice studies

Fifteen-day toxicity study in mice [(NationalToxicology Program (NTP, 1985)].

Title	**Fifteen-day studies in mice.**
Reference (year):	National Toxicology Program. (1985)
Administration	Diet for 15 days
Specie	Mice B6C3F1
Guideline (year)	OECD 407 Guideline (1998)
GLP	No
Purity:	Approximately 99%
Groups	5 mice by sex and dose level
Dose levels	0, 3000, 6000, 12000, 25000 and 50000 ppm
Desviations	Dose levels in mg/kg bw/day were not established.
This study was accepted as additional information	

Table 18: Main findings in fifteen-day study in mice (NTP, 1985)

Parameter	Dose level (ppm)											
	Males						Females					
	0	3000	6000	12000	25000	50000	0	3000	6000	12000	25000	50000
Mortality												
Mortality	0	0	0	0	5/5	5/5	0	0	0	0	5/5	5/5
Bodyweight												
Mean initial bw (g)	26.6	26.6	26.4	26.7	26.5	26.5	19.8	20.2	20.2	20.1	19.7	19.5
Mean final bw (g)	29.0	26.6	26.8	25.7 (-11.4%)	-	-	21.6	21.4	20.9	20.8	-	-
Change bw (g)	+2.4	0.0	+0.4	**-1.0**	-	-	+1.8	+1.2	+0.7	+0.7	-	-

Oral thirteen-weeks study in mice [National Toxicology Program (NTP, 1985)].

Title	**Thirteen-weeks studies in mice**
Reference (year):	National Toxicology Program. (1985)
Administration	diet for 13 weeks
Specie	Mice B6C3F1
Guideline (year)	OECD 408 Guideline (1998)
GLP	No
Purity:	Approximately of 99%
Groups	10 mice by sex and dose level
Dose levels	0, 400, 800, 1500, 3000 or 6000 ppm equivalent to: males: 0, 60, 113, 195, 405, 774 mg/kg bw/d females: 0, 77, 166, 275, 1176, 888 mg/kg bw/d
Desviations	Individual data were not reported. No haematology, clinical chemistry or urinalysis were performed.
This study was accepted as additional information	

Table 19: Main findings in 13-week study in mice (NTP, 1985)

Parameter	**Doses levels (ppm)**											
	Males						**Females**					
	0	**400**	**800**	**1500**	**3000**	**6000**	**0**	**400**	**800**	**1500**	**3000**	**6000**
Mean compound intakes (mg(kg bw/d)	0	60	113	195	405	774	0	77	166	275	**1176**[a]	888
Mortality												
Mortality	1/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10
Bodyweight												
Mean initial bw (g)	24.4	24.7	24.3	24.6	24.8	24.1	18.6	18.6	18.7	18.8	18.8	19.0
Mean final bw (g)	35.4	36.7	35.6	34.3	34.8	**31.4 (-11.3%)**	26.9	26.8	27.2	27.2	26.3	**24.1 (-10.4%)**
Change bw (g)	11.0	12.0	11.3	9.7	10.0	**7.3 (-33.6%)**	8.3	8.2	8.5	8.4	7.3	**5.1 (-38.6%)**
Feed consumption (week 12)												
Feed consumption (g/kg bw/day)	157	149	141	130	135	**129 (-17.8%)**	200	192	207	183	**392**[a]	**148 (-26%)**

[a] *Mean feed consumption in female mice treated at 3000 ppm was abnormaly elevated (almost two times the mean consumption to the other female groups) therefore intake of active substance in (mg/kg/bw/day) was higher than the theoretical high dose level.*

Dog studies

Oral 90-day toxicity study in dog. (*Marcondes de Franca, A., 2005)*

Title	**90-day Oral Toxicity Study in dogs**
Author (s) (year):	*Marcondes de Franca, A. (2005)*
Administration	Oral for 90 days via capsules
Specie	Beagle dogs
Guideline (year)	OECD 409 (1998).
GLP	Yes
Purity:	99.68%
Groups	4 dogs/sex/dose/level
Dose levels	0, 10, 50, 100 mg/kg bw/day
Study acceptable	

Table 20: Main findings in 90-day study in dog. ***(Marcondes de Franca, 2005)***

Parameter	Dose level (mg/kg bw/d)							
	Males				Females			
	0	**10**	**50**	**100**	**0**	**10**	**50**	**100**
Bodyweight (g)								
Week 0 (Beginning)	9.12	8.45	8.90	9.02	8.35	7.81	7.75	7.34
Week 13 (Final)	11.25	11.05	10.61	10.92	10.08	9.39	8.99	8.85
Bodyweight gain (g)								
Absolute gain (g)	2.13	2.60	1.71	1.90	1.73	1.59	1.25	1.51
Food consumption (g/animal/day)								
Week 1	250	250	250	250	250	250	234	233
Week 8	250	250	240	250	250	250	**214*** **(-14.4%)**	**224*** **(-10.4%)**
Week 9	250	250	238	250	250	250	**210*** **(-16%)**	**228*** **(-8.8%)**
Week 10	250	250	232	250	250	250	**220*** **(-12%)**	**226*** **(-9.6%)**
Week 11	250	250	233	250	250	250	**204*** **(-18.4%)**	**231*** **(-7.6%)**
Week 13	250	250	233	231	230	250	186	219
Hematology (week 13)								
Red bood cells								
Not determinated								
Mean blood coagulation time								
Prothombin time (s)	7.46	7.39	7.73	7.09	7.69	7.35	7.58	7.09
APTT (s)	20.3	26.1	16.7	14.9	14.7	26.8	**36.8***	31.2
White blood cells								
Total White Blood cell count (/mm^3)	14025	16300	13475	16925	11750	11650	9500	11775
Lymphocyte (/mm^3)	4978	**7287** **(+46.4%)**	**5621** **(+12.9%)**	**7036** **(+41.4%)**	4020	4033	3716	5011
Band Neutrophils (/mm^3)	173	286	126	50	289	286	108	197
Segmented Neutrophils (/mm^3)	8265	8050	7091	9234	6863	6693	5281	5938
Eosinophil (/mm^3)	469	514	503	437	462	498	301	512
Basophil (/mm^3)	0	0	0	0	0	0	0	0
Monocyte (/mm^3)	140	163	135	169	118	140	95	118
Clinical biochemistry (week 13)								
AST (U/L)	35	39	32	42	39	34	39	37
ALT (U/L)	70	55	71	106	68	54	91	115
GGT (U/L)	3	7	6	4	5	**3***	3	3
AP (U/L)	75	96	63	75	97	79	62	75

Parameter	Dose level (mg/kg bw/d)							
	Males				Females			
	0	**10**	**50**	**100**	**0**	**10**	**50**	**100**
Albumin (g/dL)	3.6	3.6	3.4	**3***	3.6	3.5	3.8	3.6
Total protein (g/dL)	5.8	5.6	**5.3***	**4.7***	5.6	5.5	5.7	5.5
Cholesterol (mg/dL)	187	**153***	**148***	173	178	180	198	220
Glucose (mg/dL)	111	118	114	122	107	113	102	108
BUN (mg/dL)	28	33	34	32	33	33	33	32
Creatinine (mg/dL)	1	1	1	1	1	1	1	1
Total Bilirr (mg/dL)	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.3
Ca (mg/dL)	11	11	11	11	11	11	11	11
Phos (mg/dL)	5.4	5.4	6.3	6.0	5.6	6.0	6.4	5.8
Cl (mmol/L)	115	116	117	118	117	116	115	117
Na (mmol/L)	135	134	135	134	135	**132***	135	136
K (mmol/L)	3.9	3.7	3.7	3.8	3.9	3.6	3.8	4.0
Urinalysis (week 13)								
Density	1.023	1.018	1.021	1.020	1.028	1.023	**1.019***	1.019
pH	6.3	6.5	6.6	6.0	5.3	5.9	6.8	6.9
Organ weights (g)								
Heart								
Absolute					77	80	73	67
Relative	0.760	0.765	0.816	0.779				
Liver								
Absolute					340	359	286	337
Relative	3.522	3.572	3.784	3.383				
Right Kidney								
Absolute					21	21	20	18
Relative	0.224	0.238	0.253	0.211				
Left Kidney								
Absolute					22	21	20	21
Relative	0.230	0.247	0.253	0.221				
Spleen								
Absolute					74	61	54	51
Relative	0.735	0.728	0.860	0.538				
Right Adrenal								
Absolute					0.46	0.50	0.49	0.51
Relative	0.004	0.004	0.005	0.004				
Left Adrenal								
Absolute					0.43	0.46	0.47	0.46
Relative	0.004	0.005	0.004	0.005				
Thymus								
Absolute					19	17	11	13
Relative	0.131	0.112	0.152	0.184				
Brain								
Absolute					79	81	73	82
Relative	0.714	0.707	0.790	0.792				
Right Thyroid								
Absolute	0.39	0.37	0.48	**0.62***	0.53	0.46	0.36	0.39
Relative	0.0034	0.0033	**0.0048***	**0.0056***	0.0052	0.0049	0.0040	0.0044
Left Thyroid								
Absolute	0.37	0.38	0.43	**0.53***	0.48	0.44	0.35	0.38
Relative	0.0032	**0.0034***	0.0043	**0.0048***	0.0047	0.0047	0.0039	0.0043
Right Testis								
Absolute								
Relative	0.074	0.081	0.085	0.063				

Parameter	Dose level (mg/kg bw/d)							
	Males				Females			
	0	10	50	100	0	10	50	100
Left Testis Absolute Relative	0.072	0.081	0.084	0.067				
Right Epididymis Absolute Relative	0.017	0.018	0.018	0.019				
Left Epididymis Absolute Relative	0.018	0.018	0.021	0.017				
Uterus Absolute Relative					5.6	4.3	1.2	3.7
Right Ovary Absolute Relative					1.0	0.8	0.6	1.1
Left Ovary Absolute Relative					0.8	1.4	0.7	1.1
Pathology								
Right Thyroid Gland Hyperplasia of C-cells	2/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4
Adenoma of C-cells	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Left Thyroid Gland Hyperplasia of C-cells	0/4	0/4	0/4	1/4	1/4	0/4	0/4	1/4
Right Parathyroid Gland Hyperplasia	1/4	0/4	0/4	2/4	1/4	0/4	0/4	2/4
Left Parathyroid Gland Hyperplasia	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4

** Significantly different from the control group (p<0.05)*

In males, the statistically significant increase in absolute and relative thyroid weights in the high dose and also in the relative right thyroid weight of the mid dose is based on the incidentally low thyroid weights of the control males rather than on a thyroid stimulating effect of 8-hydroxyquinoline. Moreover, the thyroid weight mean value in mid and high dose levels in males are well within the historical control range of laboratory concurrent studies, whereas the thyroid weight mean value of the control males are at the low end of this range (Table 21). In addition, the increase in thyroid weight was not accompanied by histopathological changes of the thyroid.

Table 21: Thyroid weights in the 90-day study in the beagle dogs in comparisons with historical control data

	Data from study Dose level (mg/kg bw/d)				Historical control data Range (28 animals)
	0	10	50	100	
Thyroid weight (g)					
Right thyroid	0.39	0.37	0.48	0.62	0.390-0.729
Left thyroid	0.37	0.38	0.43	0.53	0.367-0.813

4.7.1.2 Repeated dose toxicity: inhalation

For the inclusion in the Annex I of Directive 91/414/EEC no inhalation studies were required for the active substance. This was accepted in the PRAPeR Expert Meeting 81 (30 August - 3 September 2010) for 8-hydroxyquinoline since the active substance has a vapour pressure of 6.7×10^{-3} Pa at 20°C and considering that there was no concern for inhalation exposure due to the use of the product (drip irrigation). Therefore, no information about inhalation toxicity is available in the Assessment Report of the active substance.

4.7.1.3 Repeated dose toxicity: dermal

For the inclusion in the Annex I of Directive 91/414 no dermal studies were supplied for the active substance. This was accepted in the PRAPeR Expert Meeting 81 (30 August - 3 September 2010) for 8-hydroxyquinoline based on the low acute dermal toxicity (dermal LD_{50} > 10000 mg/kg bw).

4.7.1.4 Repeated dose toxicity: other routes

No data available

4.7.1.5 Human information

No data available

4.7.1.6 Other relevant information

No data available

4.7.1.7 Summary and discussion of repeated dose toxicity

Three studies have been supplied and sponsored by Probelte S.A. (one range finding study in rat and two 90-days oral studies in rat and dog respectively) to assess the short-term toxicity of 8-hydroxyquinoline. The studies were reported over the period 2004 to 2006, all of them were GLP and guideline compliant and all were accepted.

A revision of the toxicological and carcinogenesis studies of 8-hydroxyquinoline in rats and mice performed by the National Toxicology Program (NTP, 1985) was also provided and considered as additional information. In this revision, dietary studies at 15 days and 13 weeks conducted on years 1978 and 1979 were included.

Oral short-term toxicity studies in rats:

A range-finding dietary study (**14 days oral in rats**) was presented *(Bulnes Goicochea, 2004)*. Rats were only observed for mortality, signs of toxicity, bodyweight variations and daily intake. Haematology, clotting and clinical chemistry were only performed at 0 and 1000 ppm. No mortality or clinical signs or disturbances of the general behaviour were detected after treatment. A decrease of bodyweight gain in both sexes at 3000 and 8000 ppm groups were observed. Males from 3000 ppm showed food consumption decrease during the first week of treatment, and returned to the normal values during the subsequent week. Food consumption of females was not affected by treatment. There were no differences between control group and test group of 1000 ppm in the haematological and clotting parameters in males and females. Clinical biochemistry parameters were not affected by treatment in males and females. No alterations that could be related to the treatment were observed at necropsy.

A range-finding dietary study (**15 days oral in rats**) was presented in the *NTP publication, 1985*, in which mortality was observed at 50000 ppm (two male rats). None of the female rats

died. Apart from mortality, the only evaluated adverse effects were signs of toxicity and bodyweight variations. Decrease in bodyweights respect to the controls in rats males treated from 12000 ppm and in females from 25000 ppm was observed. At 50000 ppm animals of both sexes appeared emaciated. Althoug feed consumption was not measured, rats of each sex that received 12000 ppm or more appeared to eat less that did the controls.

In a **13-week dietary study in rats** (*NTP, 1985)* the only observed adverse effects were mortality, signs of toxicity and bodyweight variations. Besides, histopathology was evaluated in controls and the high dose group. At 12000 ppm there was a decrease of the bodyweights (18%-9.5% in males and females respectively), bodyweight gain (38.7%-28% in males and females respectively) and food consumption (30.4% in females). At 6000 ppm in females a decrease of bodyweight (10.5%), bodyweight gain (30.7%) and food consumption (31.7%) was observed.

The histopathological examination of 27 organs/tissues of the animals of the high dose group revealed no substance related findings. Lymphoid hyperplasia in the pancreatic lymph nodes was found in 2/10 females that received the highest dose but not in the controls. This lesion, however, was not considered to be compound related and was not seen in Fascineli 90-day rat study.

In the **90-days dietary study in rats** (*Fascineli, 2006a)*, there were no deaths during the study. Statistically significant findings observed were:

In <u>males</u> at 6000 ppm a decrease in mean body weight, body weight gain and food consumption were seen from week 2 to the end of the treatment. Besides, a decrease of the protombin time and total protein was seen at this dose, although there was a recovery for total protein. At dose of 3000 ppm and above, it was found an increased Mean Corpuscular Volume (MCV) during the treatment period that remained increased at 6000 ppm during the recovery period. At dose of 3000 ppm and above the mean relative organ weight of left kidney and spleen was increased during treatment period. Spleen weight remained increased during the recovery period at the top dose level. At 6000 ppm the mean relative organ weight of heart, brain and testis was also increased during treatment period.

In <u>females</u> at dose of 1000 ppm and above a decrease in mean food consumption from week 2 to the end of the treatment period (week 13) was seen, that remained decreased during recovery period at 6000 ppm. Food consumption reduction was not concomitant to bodyweight reduction so it was not considered as an adverse effect. A decrease of mean red cells count (RBC) and hematocrit (Hct) and an increase of mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were observed at dose of 3000 ppm and above during treatment period, that remained during the recovery period at 6000 ppm

There were no macroscopic or microscopic alterations that could be attributed to the treatment.

<u>Oral short-term toxicity studies in mouse:</u>

A range-finding dietary study (**15 days oral in mouse**) was presented in the *NTP publication* in which mortality was observed from 25000 ppm (all animals died from day 4 to 12 of the study). Apart from mortality, mice were only observed for signs of toxicity and bodyweight variations. Decrease in bodyweights respect to the controls in males at 12000 ppm were seen. Although feed consumption was not measured, mice that received 12000 ppm or more appeared to eat less than the controls. Five out of five females that received 50000 ppm and four out of five female that received 25000 ppm were emaciated according to necropsy.

A **13-weeks dietary study** was presented in the *NTP publication, 1985,* in which one control male and three females treated at 3000 ppm were found dead, but it was considered accidental. Apart from mortality, mice were only observed signs of toxicity and bodyweight variations. Besides, histopathology was evaluated in controls and at 6000 ppm. Statistically significant decrease in final body weight (11.3 and 10.4% in males and females respectively), bodyweight gain (33.6 and 38.6% in males and females respectively) and food consumption (17.8 and 26 % in males and females respectively) was observed at 6000 ppm. No compound-related histopathologic effects were observed at the high dose male or female mice.

Oral short-term toxicity studies in dogs:

In the **90-days oral study** *(Marcondes de Franca, 2005),* no deaths were recorded during the test. At dose ≥50 mg/kg bw/d females showed statistically significant decrease in food consumption, on weeks 8 to 11 (up to 18%). Males of all dose groups showed statistically significant increase of lymphocyte level. An increase in the relative weight of right thyroid gland from 50 mg/kg bw/day and left thyroid gland at 100 mg/kg bw/d was observed in males but they fall into the range of historical controls data of laboratory.
There were no alterations on serum chemistry, urinalysis, gross and histopathology that could be attributed to test item.

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

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

Data for classification after repeated exposure according to CLP are available in paragraph 4.7.1.7.

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

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity in humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes.This can be on the basis of human data or evidence from studies in animals that cause such effects at or below given guidance values (≤ 10 mg/kg bw/day or ≤ 100 mg/kg bw/day in a 90 day oral study in the rat). All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included under this classification.

As noted above, there are no toxicological data available on 8-hydroxyquinoline in humans for STOT RE.

No significant effects were observed in the repeated dose toxicity studies in animals with 8-hydroxyquinoline below the guidance values for classification with STOT RE. Only some variations in body weights and food consumption and small changes with minimal toxicological importance in clinical biochemistry, haematology parameters and organ weights were observed below the limits for classification. These effects are those considered in section 3.9.2.8 of CLP Regulation not to support classification for specific target organ toxicity following repeated exposure.

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

Effects observed in the repeated dose toxicity studies do not trigger the criteria for classification and labelling with STOT RE according to Regulation (EC) No 1272/2008.

CLP: No classification is required based on the available data

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

No classification as STOT RE was proposed by the DS as no human data were available and as no evidence on specific or target organ toxicity effects at the doses relevant for classification (≤ 100 mg/kg bw/d in a 90 day oral study) resulted from the available studies (see Table 13 in the CLH report).
Four repeated dose (diet) studies in rats (14 day and 15 day range finding studies, two 90 day studies), two diet studies in mice (15 day range finding study and a 90 day study) and one 90 day (capsule) study in dogs were available.
No studies on other routes were available.

Comments received during public consultation

Two MSCAs agreed with the proposal for no classification for STOT RE.

Assessment and comparison with the classification criteria

RAC concludes, in agreement with the proposal of the DS, that no classification for STOT RE is warranted.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 22: 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) ***8-Hydroxyquinoline (Batch no. 070723-1 and purity 99.7%).***	***S. typhimurium* TA98, TA100, TA1535, TA 1537 and TA102 S9-mix from livers of rats induced with phenobarbital and β-naphtoflavone.**	**1st experiment: 0, 1.0, 3.16, 10.0, 31.6, 100, 316 and 1000 µg/plate (±S9) 2nd experiment: 0, 0.5, 1.58, 5.0, 15.8, 50, 158 and 500 µg/plate (±S9)**	**Negative in all strains (±S9)**	**1st experiment: Cytotoxicity from dose level of 100 µg/plate (-S9) and from dose level of 316 µg/plate (+S9). 2nd experiment: Cytotoxicity from dose level of 158 µg/plate (±S9).**	**Donath, C., 2008 (Report No. 082379). Acceptable**
Bacterial reverse mutation assay Not guideline and GLP *8-Hydroxyquinoline sulphate (purity and batch not reported).* Disolvent: 0,05 M phosphate buffer.	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 S9 from livers of rats induced with aroclor 1254 or with phenopharbital	One experiment: 0,10, 20, 50, 75 and 100 µg/plate (±S9)	Positive but non dose-response with metabolic activation induced with aroclor in TA1537 at 50 µg/plate and TA100 at doses ≥ 20 µg/plate. Negative in all strains in the absence or presence of metabolic activation with phenobarbital.	Cytotoxicity from dose level of 75 µg/plate (±S9).	Epler, J.L., et al., 1977. Published study. Only Supplementary information
Bacterial reverse mutation assay Not guideline and GLP *8-Hydroxyquinoline (purity and batch not reported).*	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 S9 from livers of rats with Aroclor.	One experiment: Five dose levels in the range of 0-0.3 µmole/plate (±S9)	Positive in TA98 and TA100 (+S9). Negative in TA1535, TA1537, TA1538 (+S9) Negative in all strains (-S9).	Study poorly described. Cytotoxicity assessment was not included in the report.	Gocke, E., et al., 1981. Published study. Only Supplementary information
Bacterial reverse mutation assay Not guideline and GLP *8-Hydroxyquinoline (purity and batch not reported).* *8-Hydroxyquinoline sulphate (purity 99%, batch not reported).*	*S. typhimurium* TA1535, TA97, TA98 and TA100 S9 from livers of rats and hamsters.	One experiment: 8-Hydroxyquinoline: 0, 1, 3, 10, 16, 33, 66 and 100 µg/plate (±S9) 8-Hydroxyquinoline sulphate: 0, 1, 3, 10, 16, 33, 66, 100 and 166 µg/plate (±S9).	8-Hydroxyquinoline: Positive in TA100 and TA97 (+S9). Negative in TA100 and TA97 (-S9). Negative in TA98 and TA1535 (±S9). 8-Hydroxyquinoline sulphate: Positive in TA100 and TA98 (+S9). Negative in TA100 and TA98 (-S9).	Test protocol: Initial testing was in TA100 and TA98. If a positive result was obtained in one of these two strains it was repeated and the other strains were not used. If the tests were negative, the other strains were used.	Zeiger. E., et al., 1988. Published study. Only Supplementary information

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
***In vitro* gene mutation in mammalian cells**					
In vitro mammalian cell gene mutation assay Not guideline and GLP *8-Hydroxyquinoline sulphate (purity and batch not reported).*	L5178Y (tk^+/ tk^-)mouse lymphoma cells Treatment: 4 h	-S9: 1st experiment: 0, 0.2, 0.4, 0.8, 1.6 and 3.2 µg/ml 2nd experiment: 0, 0.1, 0.2, 0.4, 0.8, 1.5 and 2.2 µg/ml	Positive (-S9). The colonies aren´t scored using the criteria of normal growth (large) and slow growth (small) colonies.	1st experiment: Cytotoxicity from dose level of 3.2 µg/ml 2nd experiment: Cytotoxicity from dose level of 1,5 µg/ml In the presence of S9 it was not tested.	McGregor, D.B., et al., 1988. Published study. Only supplementary information
***In vitro* chromosome aberrations in mammalian cells**					
In vitro* mammalian chromosome aberration assay GLP compliant (OCDE 473)** ***8-Hydroxyquinoline (Batch no. 070723-1 and purity 99.7%)	**V79 cells of Chinese hamster without / With metabolic activation S9-mix from liver of rats induced with phenobarbital and β-naphtoflavone.**	**(-S9) 31.3, 62.5, and 125 µg/ml** **(+S9) 2, 4, 5, 6.5 and 8 µg/ml** **Exposure: 4h** **Fixation period: 20 h**	**-S9: Positive only at the high dose (125 µg/ml) with manifested toxicity.** **+S9: Positive and dose-dependent increase of aberrant cells at 4 µg/ml and above.**	**Toxicity:** **-S9: relative MI decreased to 44% at 125 µg/ml** **+S9: relative MI decreased to 51% and 31% at doses of 6.5 and 8 µg/ml respectively.**	**Becker, T., 2008** **(Report 082380)** **Acceptable**
In vitro mammalian chromosome aberration assay Not guideline and GLP *8-Hydroxyquinoline sulphate (purity and batch not reported).*	Human leukocyte cultures peripheral blood	5 µM (14 h treatment, 2.5 h recovery) 10 µM (14 h treatment, 2.5 h recovery) 10 µM (2 h treatment, 3 h recovery) 10 µM (2 h treatment, 14 h recovery)	Positive	Study poorly described. There is not indication in the report if S9 was used or not.	Epler, J.L., et al, 1977. Published study. Only Supplementary information
In vivo, somatic cells					
In vivo* mammalian erythrocyte micronucleus test GLP compliant (OECD 474)** ***8-Hydroxyquinoline (Bath no. 070723-1 and purity 99.7%)	**Peripheral blood cells from male and female NMRI mice.**	**Single dose levels of 0, 7, 17.5 and 35 mg/kg bw by ip route (44 h and 68 h exposure).**	**Negative**	**MTD was 35 mg/kg bw due to the toxicity observed in a pre-experiment, in 3 males and 3 females.**	**Hofman-Hüther, H., 2008** **(Report 082381)** **Acceptable**
In vivo mammalian bone marrow micronucleus test *Not guideline and GLP* *8-Hydroxyquinoline (purity and batch not reported).*	Bone marrow cells from male and female NMRI mice.	7.3, 21.8 or 43.5 mg /kg bw by ip route, two times at 0 and 24 h. Bone marrow samples at 30h.	Negative	Study poorly described.	Gocke, E., et al., 1981. Published study. Only Supplementary information

Test *Test substance*	System	Dosage	Results	Comments	Reference Acceptability
In vivo mammalian erythrocyte micronucleus test *Not guideline and GLP* *8-Hydroxyquinoline (purity and batch not reported).*	Bone marrow cells from male CD-1 mice.	Single dose levels of 0, 25, 50 and 100 mg/kg by ip route. Bone marrow samples at 24, 48, and 72 h.	Positive: Slight increment of MPCEs. At 24h for the high dose(100 mg/kg) and at 48h for the low and medium does (25 and 50 mg/kg)	Overt toxicity and high mortality rate in animals treated with 100 mg/kg.	Hamoud, M.A., et al., 1989. Published study. Only Supplementary information
In vivo mammalian chromosome aberration assay. *Not guideline and GLP* *8-Hydroxyquinoline (purity and batch not reported).*	Bone marrow cells from male B6C3F1 mice.	Single dose levels of 0, 25, 50 and 100 mg/kg bw by ip route (17 h exposure). Single dose levels of 0, 17.5, 35, and 70 mg/kg by ip route (36 h exposure).	Negative	Overt toxicity at 70 and 100 mg/kg bw.	McFee, A.F., 1989 Only Supplementary information
In vivo SCE assay *Not guideline and GLP* *8-Hydroxyquinoline (purity and batch not reported)*		Single dose levels of 0, 25, 50 and 100 mg/kg bw by ip route (23 h exposure). Single dose levels of 0, 17.5, 35, and 70 mg/kg by ip route (42 h exposure).	Negative		
In vivo UDS assay *Not guideline and GLP* *8-Hydroxyquinoline (purity and batch not reported)*	Hepatocytes from male Alderley Park (Alpk AP_fSD) rats	Single dose levels of 100, 150, and 250 mg/kg bw (12h) and of 225 mg/kg bw (24h) and 500 mg/kg bw (2h) by gavage.	Negative	Data about cytotoxicity, acute toxicity and clinical signs were not reported.	Ashby, J., et al., 1989 Published study. Only Supplementary information
In vivo, germ cells					
In vivo* mammalian spermatogonial chromosome aberration assay GLP compliant (OECD 483)** ***8-Hydroxyquinoline, (Batch no. 051114-A/601502 and purity 99.8%)	**Spermatogonial germ cells from NMRI mice.**	**Single dose levels 75, 150 and 300 mg/kg bw by gavage.** **Sampling times: 24 and 48 h.**	**Negative**	**A reduction of 50, 71 and 60 % in the MI was observed at 75, 150 and 300 mg/kg bw, respectively.**	**August, M., 2007** **(Amended by Leuschner J., 2008)** **Acceptable**

ip: intraperitoneal administration.

4.9.1 Non-human information

The mutagenic potential of 8-hydroxyquinoline has been assessed by *in vitro* studies (gene mutations in bacterial and mammalian cells and chromosomal aberrations in mammalian cells) and by *in vivo* studies in somatic cells (micronucleus assay and chromosomal aberrations in mice and UDS–test in rats) and in germ cells (spermatogonial chromosome aberrations in mice).

Fourteen studies were available for evaluating the genotoxicity of 8-hydroxyquinoline. Only four of them were performed with an analytical 8-hydroxyquinoline (99.7-99.8%) and according to test guidelines and GLP-compliant; and consequently considered acceptable.

The other ten studies were published as scientific literature. Most of these studies were performed to compare the effects between quinolone and its derivatives, included the 8-hydroxyquinoline. Chemicals with similar structures frequently demonstrate a wide variety of characteristics and effects when getting in contact with organic systems. This is what occurs with quinolone, a carcinogenic substance, and 8-hydroxyquinoline, without any evidence of carcinogenic potential. Although the structural analogy of these two substances, they seem to have two different mechanisms of action.

The interpretation and assessment of the data collected in these assays was difficult due to the lack of information, including unknown purity and batch specifications. Thus, these ten studies were considered only supplementary information.

These studies are summarised in the Table 22

4.9.1.1 *In vitro* data

***In vitro* gene mutation in bacterial**

One study and three scientific publications about bacterial mutagenicity were available:

The study (Donath, 2008) was more recent and presented defined batch specifications, known purity of 8-hydroxyquinoline (99.7%) and was performed according to OECD TG 471 (B13/14 UE). In two consecutive experiments no mutagenic activity was reported both in the presence or absence of rat liver S9 in tested *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 at dose levels of up to 1000 µg of 8-hydroxyquinoline per plate.

The bacterial mutagenicity assays reported in the three scientific publications (Epler et al., 1977; Gocke et al., 1981; Zeiger et al., 1988) were non-GLP compliant and pre-guideline. Purity of 8-hydroxyquinoline and batch specifications were also unknown.

In the 1st scientific publication (Epler et al., 1977), positive results but non-dose dependent were obtained in *Salmonella typhimurium* strains TA1537 (at 50µg/plate) and TA100 (at 20µg/plate and above) with metabolic activation induced with aroclor 1254. Negative results were observed following treatment with 8-hydroxiquinoline sulphate, either in the absence or presence of metabolic activation with phenobarbital.

In the 2nd scientific publication (Gocke et al., 1981), mutagenicity was reported in the presence of rat liver S9 in *Salmonella typhimurium* strains TA98 and TA100. In the 3rd scientific publication (Zeiger et al., 1988), 8-hydroxyquinoline induced positive results in *Salmonella typhimurium* strains TA97 and TA100 in the presence of S9 and 8-hydroxyquinoline sulphate induced positive results in TA98 and TA100 with metabolic activation. No mutagenic activity was noted in the absence of metabolic activation.

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

Mammalian cell *in vitro* mutagenicity assays compiled in a scientific publication (McGregor, 1988) were conducted with 8-hydroxyquinoline sulphate in L5178Y tk+/tk- mouse lymphoma cells. The assays were non-GLP compliant and pre-guideline. Purity of 8-hydroxyquinoline and batch specifications were also unknown. Mutagenic activity was noted in the absence of rat liver S9. No mutagenicity assay in the presence of metabolic activation system was performed with 8-hydroxyquinoline (see Table 24).

Table 23: Responses of the L5178Y tk+/tk- mouse lymphoma cells forward mutation assay with 8-hydroxyquinoline sulphate (McGregor, 1988)

Without S9 mix Trial 1						**Without S9 mix Trial 2**					
Conc. µg/ml	**CE**	**RTG**	**MC**	**MF**	**AVE MF**	Conc. µg/ml	**CE**	**RTG**	**MC**	**MF**	**AVE MF**
DMSO	101	116	154	51		DMSO	46	68	135	98	
0.0	95	97	162	57		0.0	89	117	145	54	
	76	87	96	42			64	114	120	63	
	78	100	128	55	51		72	100	155	72	72
0.2	73	80	119	55		0.1	73	92	205	94	
	72	80	107	50	52		73	100	160	73	84
0.4	68	88	120	59		0.2	86	116	145	56	
	64	93	78	41	50		103	132	165	54	55
0.8	98	78	197	67		0.4	85	92	273	106	
	69	71	140	68	68		67	104	206	102	104*
1.6	81	31	283	116		0.8	70	70	268	128	
	93	30	305	110	113*		82	72	251	102	115*
3.2	76	12	342	149		1.5	63	5	419	222	
	58	10	292	166	158*		68	5	460	227	224*
MMS 15µg/ml	26	15	488	626		2.2	TOX	-	-	-	-
	22	14	370	556	591*		TOX	-	-	-	-
						MMS 15µg/ml	30	22	382	417	
							31	26	274	290	354*

CE=cloning efficiency %. RTG=relative total growth; MC=mutant colony count; MF=mutant fraction (mutant colonies per 10^6 clonable cells); AVE MF= group average mutant fraction
TOX = toxic; MMS= methyl methanesulphonate
*= P< 5%

Toxic effect of the test item was observed at concentrations of 3.2 µg/ml in trial 1 and from 1.5 µg/ml in trial 2.

8-hydroxyquinoline sulphate induced significant increases in both number and fraction of mutants at very low concentrations. The LOED (lowest observed effective dose) without metabolic activation was 1.6 µg/ml in one experiment and 0.4 µg/ml in the other, corresponding RTGs (relative total growth) were 30% and 98%.

Conclusions

8-hydroxyquinoline sulphate induced significant increases in the number and fraction of mutants in the absence of S9. It was not tested in the presence of S9.

***In vitro* chromosome aberrations in mammalian cells**

Chromosome aberrations in V79 Chinese hamster cells

There is an available study with 8-hydroxyquinoline (Becker, 2008) performed with Chinese hamster V79 cells according to OECD guideline 473 and GLP compliant. Purity (99.7%) and batch specifications of the test substance were well defined. Main study doses were: without metabolic activation, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 250, and 350 µg/ml; with metabolic activation: 0.5, 1, 2, 4, 5, 6.5, 8, and 10 µg/ml. Only the following concentrations were selected for the microscopic analyses: without metabolic activation: 31.3, 62.5 and 125 µg/ml; with metabolic activation: 2, 4, 5, 6.5 and 8 µg/ml.

Table 24: Percentage of cells with chromosomal aberrations in cultured V79 cells treated with 8-hydroxyquinoline (Becker, 2008)

Dose Group	Concent. (µg/ml)	Treatment Time	Fixation Interval	Cell Density Relative (%)	MI Relative (%)	Mean % aberrant cells	
						Incl. gaps	Excl. gaps
Without metabolic activation							
C	0	4h	20h	122	118	3.0	0.5
S	0	4h	20h	100	100	5.5	2.0
4	31.3	4h	20h	81	96	6.0	3.0
5	62.5	4h	20h	100	91	5.0	3.0
6	125	4h	20h	98	44	10.0	**6.5**
EMS	900	4h	20h	94	44	14.0	9.5
With metabolic activation							
C	0	4h	20h	130	97	2.0	1.0
S	0	4h	20h	100	100	7.5	3.0
3	2	4h	20h	137	103	4.5	1.5
4	4	4h	20h	128	96	9.0	**5.0**
5	5	4h	20h	78	105	8.0	**6.0**
6	6.5	4h	20h	72	51	10.0	**8.0**
7	8	4h	20h	68	31	18.6	**13.7**
CPA	0.83	4h	20h	98	108	15.5	9.5

C: Negative control (culture medium)
MI: Mitotic Index
S: Solvent control (DMSO)
EMS: Positive control (without metabolic activation: Ethylmethanesulfonate)
CPA: Positive control (with metabolic activation: Cyclphosphamide)

Toxic effects with the test substance were observed in the main experiment with and without metabolic activation. The highest dose group evaluated (125 µg/ml) without metabolic activation induced a decrease of the relative mitotic index down to 44%. The cell density was not decreased. With metabolic activation, the relative mitotic index was decreased to 51% and 31% at the highest dose groups evaluated (6.5 and 8 µg/ml) respectively). The cell density relative was decreased down to 68% only at the dose of 8 µg/ml (see Table 24).

Without metabolic activation, at the concentration of 125 µg/ml, the aberration rate was clearly increased (6.5%) compared to the negative and solvent controls. With metabolic activation, a biologically relevant increase of aberrant cells was noted at all concentrations evaluated (4, 5, 6.5 and 8 µg/ml), except for the lowest one (2 µg/ml). Mean values of 5.0, 6.0, 8.0 and 13.7% aberrant cells were found respectively. The resultant aberration rates were above the historical control data. In addition, a dose-effect relationship was observed in the presence of metabolic activation (see Table 24).

No biologically relevant increase in the frequencies of polyploid cells was observed after the treatment with the test substance.

EMS and CPA were used as positive controls and induced distinct and biologically relevant increases in cells with structural chromosomal aberration.

Conclusion: Under the experimental conditions reported, 8-hydroxquinoline induced chromosomal aberrations in the V79 Chinese hamster cell line.

Chromosome aberrations in human leukocytes

There is only data from a scientific publication (Epler et al., 1977) that contains a poorly described study non-GLP compliant and pre-guideline, carried out with 8-hydroxyquinoline sulphate.

8-hydroxyquinoline sulphate was assayed for the potential to induce chromosomal aberrations in human leukocyte cultures at dose levels of 5 µM and 10 µM. It was not reported if the substance was tested in the presence or absence of S9.

Under the conditions of this study, 8-hydroxyquinoline sulphate induced chromosome aberrations in human leukocytes.

4.9.1.2 *In vivo* data

<u>*In vivo* studies in mammalian somatic cells</u>

In vivo mammalian erythrocyte micronucleus test

One study and two scientific publications about *in vivo* mammalian micronucleus were available.

In a recent GLP compliant study (Hofman-Hüther, 2008) conducted according to OECD guideline 474 with well-defined batch specification and purity (99.7%), no clastogenic activity was reported to 8-hydroxyquinoline at dose levels up to 35 mg/kg bw administered intraperitoneally to male and female NMRI mice. Detailed data from this study is detailed below.

In the preliminary experiment different concentrations of the test item were evaluated. One male and one female mouse received a single dose of 48 mg/kg bw (intraperitoneal route), according the LD_{50} published in the EMEA document for 8-hydroxiquinilone (EMEA/MRL/464/98-FINAL), and showed high toxic symptoms after application of the test item. Three female and three male mice received a single dose of 35 mg/kg bw intraperitoneally and showed toxic symptoms but survived 72 h after the treatment. This dose was selected as maximum tolerable dose (MTD). In the main experiment three dose levels were used (7, 17.5 and 35 mg/kg bw). Peripheral blood samples were collected for micronuclei analysis in polychromatic erythrocytes, 44 h and 68 h after a single administration of the test item. The results of this study are shown in the Table 25.

Table 25: Summary of results of micronuclei test with 8-hydroxyquinoline (Hofman-Hüther, 2008)

Dose Group	Dose (mg/kg bw)	Time after administration (h)	% cells with Micronuclei ± SD		Rel. PCE	
			Male	Female	Male	Female
NC	0	44	0.20±0.08	0.10±0.04	2.43	1.76
0.2MTD	7	44	0.23±0.04	0.18±0.05	2.67	2.41
0.5MTD	17.5	44	0.18±0.07	0.12±0.05	2.33	1.96
1MTD	35	44	0.21±0.06	0.14±0.05	2.18	1.71
CPA	40	44	2.12±1.61	1.33±0.46	1.10	1.14
NC	0	68	0.18±0.05	0.18±0.06	2.65	1.87
1MTD	35	68	0.22±0.04	0.21±0.06	1.96	1.41
Hist. NC (2007-2008)	0	ND	0.27±0.07 (0.14-0.43)	0.23±0.05 (0.14-0.34)	2.45±0.49 (1.46-3.97)	1.94±0.47 (1.19-2.86)
Hist. PC (2007-2008)	ND	ND	2.32±0.54 (1.05-3.33)	1.72±0.41 (1.09-2.52)	0.98±0.32 (0.30-1.97)	0.89±0.28 (0.30-1.56)

Rel. PCE: proportion of polychromatic (immature) erythrocytes among total erythrocytes.
CPA: Cyclophosphamide.
NC: negative control.
PC: positive control.

All animals treated with the highest dose (35 mg/kg bw) showed toxic effects. The animals treated with 17.5 and 7 mg/kg bw showed slight or no toxic effects after the treatment with the test substance.

Proportion of polychromatic erythrocytes (PCE): the PCE values observed in the groups treated with 7 and 17.5 mg/kg bw were comparable to negative control at 44 h. The animals treated with 35 mg/kg bw showed PCE values at 44 h and 68 h that were slightly reduced

compared to the corresponding negative controls, but the reductions were no statistically significant.

Micronucleated polychromatic erythrocytes: all mean values of micronuclei formation observed in the male groups after treatment with the test item were within the range of the corresponding negative control. The mean values observed in the female groups were slightly increased, but the increases were not statistically significant, except for the 7 mg/kg bw group (44 h). The value observed for this group was increased compared to the corresponding negative control. However, all observed values were within the historical negative control data, and additionally it has to be pointed out that the obtained value for females of the negative control group (0.10%) was low and fell out of the range of the historical controls (0.14%-0.34%).

It can be concluded that under the reported experimental conditions, 8-hydroxyquinoline did not induce structural and/or numerical chromosomal damage in the immature erythrocytes of the mouse.

Two scientific publications non-GLP compliant and pre-guideline (Hamoud et al., 1989, Gocke et al., 1981) compiled *in vivo* micronucleus assays in the bone marrow of different strains of mice (male CD-1, male and female NMRI). Purity or batch specifications of 8-hydroxyquinoline were unknown. These studies are considered as supplementary information.

A poorly-described published study (Gocke et al., 1981) reported negative results in the bone marrow micronucleus test when NMRI mice were treated by intraperitoneal route two times at 0 and 24 hours at dose levels ranged from 7.3 to 43.5 mg/kg bw.

However, the other *in vivo* study (Hamoud et al., 1989) reported positive results in the erythrocyte micronucleus test when male CD-1 mice were treated by single intraperitoneal route at dose levels ranged from 25 to 100 mg/kg bw. The high dose (100 mg/kg bw) was very toxic and resulted in death of many mice several hours after the injection and therefore only a sampling time of 24 h was available for this dose level. The results of this study are shown in the Table 26.

Table 26: Micronuclei induced in bone marrow erythrocytes of CD-1 male mice after single intraperitoneal treatment with 8-hydroxyquinoline (Hamoud et al., 1989)

Sampling time (h)	Dose (mg/kg bw)	MPCE/1000 PCE ± S.E[a]	MNCE/1000 NCE ± S.E[b]	PCN/NCE[c]
24	0	2.5±0.2	1.5±0.2	1.04
	25	2.9±0.4	3.0±0.3**	0.64
	50	3.6±0.4	3.2±0.4**	0.69
	100[d]	4.7±08*	5.3±0.6**	0.59
48	25	4.0±0.6*	3.6±0.1**	0.85
	50	3.4±0.3*	3.5±0.2**	1.08
72	25	2.5±0.3	2.2±0.2*	0.85
	50	3.0±0.2	3.0±0.2**	0.92
24[e]	0	2.7±0.3	1.6±0.1	1.04
	25	2.7±0.3	2.7±0.2**	0.69
	50[f]	3.0±0.2	4.4±0.4**	0.59

[a]MPCE: Number of micronucleated polychromatic erythrocytes. PCE: Number of polychromatic erythrocytes. The number is based on 10000 PCEs (2000 PCEs were scored for each of 5 animals).
[b]MNCE: Number of micronucleated normochromatic erythrocytes. NCE: Number of normochromatic erythrocytes. The number is based on 12500 NCEs (2500 NCEs were scored for each of 5 animals).
[c]Based on 5000 PCEs
[d]Only 3 animals were analyzed
[e]Results from repeat experiment
[f]Only 4 animals were analyzed
*p<0.05 ; ** p<0.01 a: Results from repeated experiment.

There was a slight increase in the number of micronucleated polychromatic erythrocytes (MPCEs) in treated animals over controls. The increases were statistically significant at 24 h for the high dose and at 48 h for the low and medium doses. This was not confirmed with the repeat 24 h data.

The numbers of MNCEs induced by all doses over all three sampling times were small but significantly different from the controls, and there was also a significant dose-related trend at 24 h which was confirmed with the repeat 24 h data. The peak incidence of induced MNCEs was at or close to 48 h after treatment. The PCE to NCE ratios for treated animals were much lower than controls, particularly at 24 h sampling time. In this study, 8-hydroxyquinoline induced a significant number of MNCEs, although it did not produce high numbers of MPCEs over the three sampling times tested. However, this study is considered as supplementary information.

In vivo* mammalian bone marrow chromosome aberration test and sister chromatid exchanges (SCE) in mouse marrow cell *in vivo

8-hydroxyquinoline was assayed for the potential to induce chromosomal aberrations in bone marrow cells of B6C3F1 mice by single dose levels ranged from 17.5 to 100 mg/kg bw (McFee, 1989). Sister chromatid exchanges in the marrow cells were also quantified. The study was non-GLP compliant and pre-guideline. Purity and batch specifications of the test compound were unknown. In a first trial, 8-hydroxyquinoline was injected intraperitoneally at doses of 25, 50 and 100 mg/kg bw. Due to lethality of the high dose, doses were reduced to 17.5, 35 y 70 mg/kg bw in the second trial. High mortality, 42 and 40%, was observed at the high dose level in the first and second trial, respectively.

Under the conditions of this study, 8-hydroxyquinoline did not induce chromosomal aberrations in bone marrow cells of mice. Besides, no increase in the rate of sister chromatid exchange was observed.

Unscheduled DNA-synthesis in rat hepatocytes *in vivo*

A scientific publication (Ashby et al., 1989) compiles an *in vivo* study in rat that assesses the potential of 8-hydroxyquinoline to induce unscheduled DNA synthesis (UDS) in hepatocytes. The study was non-GLP compliant, pre-guideline and the purity of the test compound was unknown.

Test substance was administered orally at single doses of 100, 150 and 250 mg/kg bw (exposure for 12 h), 225 mg/kg bw (exposure for 24 h) and 500 mg/kg bw (exposure for 2 h). Hepatocytes were isolated 2, 12 and 24 h after administration and assessed for unscheduled DNA synthesis. Data about cytotoxicity, acute toxicity study and clinical signs were not reported.

The results of this study indicate that 8-hydroxyquinoline does not induce unscheduled DNA synthesis in rat hepatocytes treated up to 500 mg/kg bw.

***In vivo* studies in mammalian germ cells**

Mammalian spermatogonial chromosome aberration in NMRI mouse *in vivo*

In a recent study (Leuschner, 2008) conducted according to OECD guideline 483, 8-hydroxyquinoline was assayed in an *in vivo* mammalian spermatogonial chromosome aberration test in NMRI mouse. The study was GLP compliant and the purity (99.8%) and the batch specifications of the test compound were well defined.

Three dose levels were employed (75, 150 and 300 mg/kg bw) by single oral administration. The dose level of 300 mg/kg bw was considered to be the maximum tolerated dose level. Two sampling level times were employed in this study: 24 hours after administration (all doses); 48 hours after administration (high dose).

The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with 8-hydroxyquinoline ranged from 0.3% to 0.7%. These results were within the normal range, and hence no significant difference was observed compared to negative control (0.3%). The number of cells with gaps was also within the range of the negative control (treated groups: 0.6% to 3.3%; control: 2.0 %). The positive control, mitomycin C, induced significant levels of chromosomal aberrations.

A reduction of 50, 71 and 60 % in the MI was observed at 75, 150 and 300 mg/kg bw.

No polyploid was noted.

In conclusion, 8-hydroxyquinoline tested up to the maximum tolerated dose of 300 mg/kg bw by single oral administration to mice showed no mutagenic properties in the mammalian spermatogonial chromosome aberration test.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Most of the genotoxicity studies (10 out of 14) submitted were scientific literature, not GLP compliant, non-guideline and with purity and batch specifications unknown. These studies were evaluated and included in DAR but considered only as supplementary information. Therefore, the weight of the evaluation fell on the well conducted studies (4 out of 14) with test substance well characterized (known purity and batch), performed according to OECD guidelines and GLP compliant.

A recent study (Donath, 2008) with 8-hydroxyquinoline (batch and purity known) performed according OECD guideline 471, revealed negative results in *S. typhimurium* in the absence and presence of metabolic activity. However, in three publications (Epler et al., 1977; Gocke et al., 1981; Zeiger et al., 1988) carried out with 8-hydroxyquinoline and 8-hydroxyquinoline sulphate positive results in four strains of *Salmonella typhimurium* with metabolic activation (TA 1537, TA 100, TA 97 and TA98) were observed. No mutagenic activity was noted in the absence of metabolic activation. Purity or batch specifications of 8-hydroxyquinoline were unknown. These studies are considered as supplementary information.

Considering the data from the first study, it can be concluded that 8-hydroxyquinoline is not mutagenic in *Salmonella*.

Assays on gene mutation and chromosomal aberrations *in vitro* showed positive results. In a new study (Becker, 2008) with 8-hydroxyquinoline (batch and purity known and considered appropriate) performed according to OECD guideline 473, test substance induced structural chromosomal aberrations in the V79 Chinese hamster cell line without and with metabolic activation. Only the increases were dose-dependent in the presence of metabolic activation.

Furthermore, 8-hydroxyquinoline sulphate induced gene mutations at the thymidine kinase in

L5178Y tk^+/tk^- mouse lymphoma cells, in the absence of metabolic activation (McGregor, 1988). It was not tested in the presence of metabolic activation. In a poorly described published study (Epler et al., 1977), 8-hydroxyquinoline induced chromosome aberrations in human leukocytes. Considering all data, the weight of evidence suggests that 8-hydroxiquinoline is mutagenic in the assays *in vitro*.

Six *in vivo* genotoxicity assays in mammalian somatic cells are available including chromosomal aberration and DNA damage.

A new study (Hofman-Hüther, 2008) with 8-hydroxyquinoline (batch and purity known and considered appropriate) performed according to OECD guideline 474, showed negative results in the micronucleus test in the peripheral blood cells of mouse at dose levels ranged from 7 to 35 mg/kg bw. A dose of 35 mg/kg bw was considered the MTD due to the signs of toxicity noted. The relative PCE values at this dose were slightly reduced but not statistically significant compared to the negative control.

Therefore the assay is to be considered as a valid assessment of *in vivo* clastogenic activity, inasmuch as the study was carried out at dose levels approaching the MTD.

Other published assay, poorly described, showed that 8-hydroxyquinoline does not induce micronucleus and chromosome aberrations in the bone marrow of the mouse (Gocke et al., 1981 and McFee, 1989).

However, Hamoud et al. (1989) observed positive results in the micronucleus test in the bone marrow cells of mouse at dose levels ranged from 25 to 100 mg/kg bw. In this study, 8-hydroxyquinoline induced a significant number of MNCEs, although it did not produce high numbers of MPCEs over the three sampling times tested. This may indicate that 8-hydroxyquinoline could affect the later stages of cell cycle. This study, non GLP-compliant and pre-guideline, is included in a scientific publication. Purity of 8-hydroxyquinoline and batch specifications were also unknown. Therefore, this study is considered as supplementary information.

Other two assays about DNA damage compiled in scientific literature, carried out with *in vivo* somatic cells, showed negative results and were considered as supplementary information. In an *in vivo* SCE assay in bone marrow cells from mice (McFee, 1989) the results were negative and in another *in vivo* UDS assay in primary rat hepatocytes (Ashby et al., 1989) 8-hydroxyquinoline did not induce unscheduled DNA synthesis under the used test conditions.

Finally, the effect in mammalian germ cells *in vivo*, was investigated in an oral gavage study in mouse (Leuschner, 2008). The assay on spermatogonial chromosome aberration was fully acceptable and provided negative results.

Accordingly, the weight of evidence suggests that 8-hydroxyquinoline is mutagenic *in vitro* but not mutagenic *in vivo* in either somatic or germ cells, based on well conducted studies.

4.9.5 Comparison with 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.

The weight of evidence suggests that 8-hydroxyquinoline is not mutagenic in bacteria. A positive result for gene mutations was observed in mouse lymphoma cells (L5178Y tk^+/tk^-) *in vitro* (in the absence of S9). Chromosomal aberration *in vitro* studies showed positive results to chromosomal damage (leukocytes human, V79 cells of Chinese hamster). With respect to chromosomal aberrations in *in vivo* studies, one out of four *in vivo* studies showed positive results indicating an increase in the micronuclei of bone marrow cells in mice. However the results of a new well-conducted study on micronucleus test in peripheral blood cells from mice did not confirm the potential of 8-hydroxyquinoline to produce chromosomal damage. Furthermore, negative results were found in both *in vivo* UDS and SCE assays. In addition, the *in vivo* assay on spermatogonial chromosomes aberrations gave clear negative results.

Based on the results of all studies provided, the weight of evidence suggests no *in vivo* genotoxic potential of by 8-hydroxyquinoline. Therefore 8-hydroxyquinoline does not warrant classification for mutagenicity according to CLP criteria.

4.9.6 Conclusions on classification and labelling

CLP: A classification is not required

RAC evaluation of germ cell mutagenicity
Summary of the Dossier submitter's proposal
The DS concluded on the basis of the available genotoxicity data that classification of 8-hydroxyquinoline for mutagenicity was not justified. *In vitro* tests Regarding the induction of gene mutations in bacteria, a negative result (Donath, 2008) as well as positive results (Gocke *et al*., 1981; Zeiger *et al*., 1988) were available for 8-hydroxyquinoline. After assessing the evidence, the DS suggested that 8-hydroxyquinoline is not mutagenic in bacteria. In a mammalian cell culture test with V79 cells, a positive result was observed for 8-hydroxyquinoline in a guideline-compliant

chromosomal aberration test (Becker, 2008).

For 8-hydroxyquinoline sulphate, only flawed positive studies are available (non guideline-compliance; poorly described studies lacking key information; no specification of purity of the tested substance; no GLP-certification). Positive results were reported from bacterial gene mutation tests (Epler *et al*., 1977; Zeiger *et al*., 1988), from a mouse lymphoma test (McGregor *et al*., 1988) as well as from a chromosomal aberration test with human leukocytes (Epler *et al*., 1977).

In vivo tests
With respect to induction of clastogenic effects by 8-hydroxyquinoline *in vivo*, one study with methodological deficiencies showed a positive result, indicating an increase in the micronuclei of bone marrow cells in mice (Hamond *et al*., 1989).

However the negative result of a guideline-compliant micronucleus test in peripheral blood cells from mice (Hofman-Hüther, 2008) did not confirm the potential of 8-hydroxyquinoline to produce chromosomal damage. Furthermore, negative micronucleus chromosomal aberration tests in bone marrow cells of mice (Gocke *et al*., 1981 McFee, 1989 respectively) () were availablebut provided only supplementary information due to deficiencies in their study design.

A guideline-compliant *in vivo* assay on spermatogonial chromosome aberrations in mice with 8-hydroxyquinoline was clearly negative (August, 2007) as were tests of unscheduled DNA synthesis (Ashby *et al*., 1989) and sister-chromatid exchange (McFee, 1989).

Summary
On the basis of this analysis and assessment of all available studies with 8-hydroxyquinoline and 8-hydroxyquinoline sulphate, the DS came to the conclusion that 8-hydroxyquinoline induces no classification-relevant *in vivo* effects. Therefore, no classification as a germ cell mutagen is required.

Comments received during public consultation

One MSCA agreed with the proposal for no classification for 8-hydroxyquinoline.

Assessment and comparison with the classification criteria

Robust studies as well as studies with deficiencies are available for 8-hydroxyquinoline.
For the assessment of germ cell mutagenicity RAC gives the greatest weight to those studies performed in accordance with the corresponding OECD test guidelineand where the purity of the test substance as well as a GLP-certification was available (See Table A below). Studies with deficiencies in reporting and/or methodology regarding the current guideline standards as well as studies with 8-hydroxyquinoline sulphate (CAS: 134-31-1) were considered for the assessment of the genotoxicity of 8-hydroxyquinoline, but they were less relevant as sufficient information from valid guideline compliant studies was available.

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Table – Overview of reliable tests with 8-hydroxyquinoline for the toxicological endpoint germ cell mutagenicity

Type of Study	**Test system**	**Dose***	**Results**	**Reference**
Bacterial gene mutation test (OECD TG 471)	S. typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 102	0 – 1000 µg/plate	Negative (+/- S9-mix)	Donath, 2008
In vitro chromosomal aberration test (OECD TG 473)	V79 cells	0 – 125 µg/mL (- S9-mix) 0 – 8 µg/mL (+ S9-mix)	Positive (+/- S9-mix)	Becker, 2008
In vivo micronucleus test (OECD TG 474)	Peripheral blood cells (mice)	0 – 35 mg/kg bw (MTD) Single i.p injection	Negative	Hofman-Hüther, 2008
In vivo mammalian spermatogonial aberration assay (OECD TG 483)	Spermatogonial germ cells (mice)	0 – 300 mg/kg bw Single oral gavage	Negative	August, 2007

* In all tests, the highest tested doses are justified due to the induction of toxic effects.

All in all, four studies gave the following reliable information about mutagenicity of 8-hydroxyquinoline:
- The substance did not induce gene mutations in bacteria (Donath, 2008).
- In proliferating V79 cells of a directly exposed cell line, clastogenic effects were detected with and without S9-mix (Becker, 2008).
- The ability to induce clastogenic effects *in vitro* was confirmed neither in soma cells nor in germ cells. An *in vivo* micronucleus assay with peripheral blood cells as target cells (Becker 2008) as well as an *in vivo* mammalian spermatogonial chromosome aberration test (Hofman-Hüther 2007) were negative.

In summary: based on the negative *in vivo* guideline studies no mutagenicity was induced in soma cells (criterion for Category 2) or in germ cells (criterion for Category 1B). Taking into account its systemic availability, 8-hydroxyquinoline is considered to be non-mutagenic *in vivo*. Accordingly, RAC concludes that no classification for germ cell mutagenicity is warranted for 8-hydroxyquinoline.

4.10 Carcinogenicity

Long-term studies have been performed with 8-hydroxyquinoline in the diet of rats and mice for two years.

Table 27: Summary table of relevant carcinogenicity studies

Method	Main Results and Remarks	Reference
2-year oral study in rats (Toxicology and carcinogenesis studies of 8-HQ in F344/N rats and B6C3F$_1$ mice, feed studies) Testing Laboratory: EG&G Mason Research Institute Guidelines: not stated GLP: No F344/N rats 50/sex/dose 103 weeks Purity: 99% Doses: 0, 1500 and 3000 ppm in the diet, equivalent to: ▪ males: 0, 73 and 143 mg/kg b.w./d ▪ females: 0, 89 and 166 mg/kg b.w./d Deficiences: Only two dose levels were assayed. No haematology, urinalysis, clinical chemistry or organ weights were performed.	*Survival*: no significant differences in survival were observed between any groups of either sex. *Mean body weights:* ↓ in high dose rats of each sex compared with the controls (throughout most of the study), getting to a -10.1% at termination for males, and -8.3% at termination for females (reaching a -13.5% on week 92). *Feed consumption:* ↓average daily feed consumption per rat in both dose groups. **Non-neoplastic findings** *Lung:* The incidence of epithelial hyperplasia in female rats was slightly increased in the high dose group, while it was slightly decreased in the high dose males. **Neoplastic findings** *Lung*: Statistically significant positive trend of combined alveolar/bronchiolar adenomas and carcinomas in male rats. The incidence of this lesion in males of the high dose group was significantly greater than that in the controls by a survival-adjusted statistical test. *Thyroid Gland:* Statistically significant positive trend of C-cell carcinomas and combined C-cell adenomas and carcinomas in male rats and C-cell adenomas in female rats. The incidences in the dosed groups of either sex were not significantly different from those in the controls, by the survival-adjusted tests performed. However, according to Fisher's exact test (which doesn't adjust for survival differences), the incidence of combined C-cell adenomas and carcinomas was significantly greater in male rats of the high dose group, compared with controls.	U.S. National Toxicology Program. Technical Report Series no. 276, 1985. (NTP TR 276) NIH Publication No. 85-2532 NTP-83-029
2-year oral study in mice (Toxicology and carcinogenesis studies of 8-HQ in F344/N rats and B6C3F$_1$ mice, feed studies) Testing Laboratory: EG&G Mason Research Institute Guidelines: not stated GLP: No B6C3F$_1$ mice 50/sex/dose 103 weeks Purity: 99%	*Survival*: no significant differences in survival were observed between any groups of either sex. *Mean body weights:* ↓mean body weights of female mice of both dose groups, compared with controls, reaching the lowest point of -8.3% on week 96 for the low dose, and -18.4% on week 84 for the high dose females. Slight ↓ in high dose male mice throughout most of the study (reaching the lowest point of -6.8% on week 92). *Feed consumption:* ↓average daily feed consumption in both dosed groups of either sex. **Non-neoplastic findings** *Necrotizing inflammation of multiple organs:* found in female mice that died before the end of the study (primarily after week 80). These lesions were consistent with *Klebsiella* infection. **Neoplastic findings** *Lung:* ↑ incidences of lung alveolar/bronchiolar adenomas in	U.S. National Toxicology Program. Technical Report Series no. 276, 1985. (NTP TR 276) NIH Publication No. 85-2532 NTP-83-029

Doses: 0, 1500 and 3000 ppm in the diet, equivalent to: ▪ males: 0, 217 and 396 mg/kg b.w./d ▪ females: 0, 349 and 619 mg/kg b.w./d <u>Deficiences</u>: Only two dose levels were assayed. No haematology, urinalysis, clinical chemistry or organ weights were performed.	dosed male and female mice. No clear dose-effect relationship was observed for these increments and, according with the study report, these increases were within the range of historical values. No statistical significance was observed for these findings. <u>*Circulatory System:*</u> ↑ combined incidence of hemangiomas and hemangiosarcomas in low dose female mice, statistically significant by the Fisher's exact test (which doesn't adjust for survival differences), but not statistically significant by methods that adjusted for survival. No dose-response relationship was observed for this finding.	

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.1.1 Oral carcinogenesis in rats

Oral carcinogenesis of 8-hydroxyquinoline was studied in a 2-year oral toxicology and carcinogenesis study in rats *(U.S. National Toxicology Program. Technical Report Series no. 276. NIH Publication No. 85-2532. NTP-83-029).*

This study presents several deficiencies, such as the testing of only two dose levels or the lack of important measurements (like haematology, clinical chemistry, urinalysis or organ weights). The lack of important data in this study does not allow a complete evaluation of both chronic toxicity and carcinogenicity of 8-hydroxyquinoline.

<u>Findings:</u>

Statistical analysis of primary tumours included 2 survival-adjusted methods, used to evaluate tumour incidence. The *Life Table Analyses* assumed that all tumours of a given type observed in animals dying before the end of the study were "fatal", and the *Incidental Tumor Analyses* assumed that such tumours were "incidental". Reported P values for these survival-adjusted tests were either associated with the trend test or corresponded to pairwise comparisons between each dose group and the controls.

In addition, another 2 methods that don't adjust for survival incidences were used. These tests are the *Fisher's exact test* for pairwise comparisons and the *Cochran-Armitage linear trend test*, and both are based on the overall proportion of tumour-bearing animals.

No significant differences in survival were observed between any groups of either sex.

Mean body weights of high dose rats of each sex were lower than those of the controls throughout most of the study, getting to a -10.1% at termination for males, and -8.3% at termination for females (reaching a -13.5% on week 92). Table 28 summarizes the mean body weights and survival of the rats over the study period.

Average daily feed consumption per rat decreased in both dosed groups of either sex. Compared with control values, feed consumption was -7% and -11% for males and females of the low dose group, respectively; and -12% and -22% for males and females of the high dose group, respectively.

Approximate daily chemical consumption for low dose and high dose rats was 73 and 143 mg/kg bw for males and 89 and 166 mg/kg bw for females, respectively.

Table 28: Mean body weights and survival (rats).

Weeks on study	Control		1500 ppm			3000 ppm		
	Av. Wt (grams)	No. of survivors	Av. Wt (grams)	Wt (percent of controls)	No. of survivors	Av. Wt (grams)	Wt (percent of controls)	No. of survivors
MALE RATS								
0	160	50	159	99.4	50	159	99.4	50
15	353	50	357	101.1	50	355	100.6	50
39	430	49	434	100.9	50	422	98.1	50
63	477	48	484	101.5	48	452	94.8	50
83	472	45	480	101.7	47	445	94.3	48
92	462	37	478	103.5	42	434	93.9	41
99	450	32	443	98.4	38	413	91.8	37
104	465	28	443	95.3	34	418	89.9	33
FEMALE RATS								
0	125	50	125	100.0	50	124	99.2	50
16	211	50	204	96.7	50	200	94.8	50
40	246	50	237	96.3	50	225	91.5	50
64	307	50	294	95.8	50	273	88.9	49
84	339	48	329	97.1	49	306	90.3	46
92	347	47	325	93.7	49	300	86.5	44
100	335	45	333	99.4	43	302	90.1	40
104	336	37	344	102.4	40	308	91.7	37

Non-neoplastic findings (see Table 29):

Lung: The incidence of epithelial hyperplasia in female rats was slightly increased in the high dose group (0/50; 0% for the control and low dose groups; and 2/50; 4% for the high dose group), while it was slightly decreased in the high dose males (5/50; 10% for the control and low dose groups; and 3/50; 6% for the high dose group).

Thyroid Gland: The incidence of C-cell hyperplasia was greater in the controls than in the male or female dosed groups (incidences for males were 4/50; 8% for the controls; 3/49; 6% for the low dose group, and 1/47; 2% for the high dose group. The incidences for females were 9/48; 19% for the controls; 6/50; 12% for the low dose group, and 1/49; 2% for the high dose group).

Neoplastic findings (see Table 29 and Table 30):

Lung: In female rats, the incidences of alveolar/bronchiolar adenomas were only slightly higher in dosed groups (1/50; 2% for the control group; and 2/50; 4% for the low and high dose groups). No carcinomas were observed in the lungs of female rats. No statistical analysis was presented for pulmonary tumours in female rats.

In male rats, there was a dose-dependent increase in the incidences of alveolar/bronchiolar adenomas, but no statistical significance was observed (0/50; 0% for the control group; 2/50; 4% for the low dose group and 3/50; 6% for the high dose group). The incidences observed at either dose group of male rats were within the range of the historical control data for male F344/N rats at this laboratory (EG&G Mason Research Institute) [(0/50 - 3/49); (0% - 6.1%)] and the overall historical incidence range of the NTP Carcinogenesis Program [(0/89 - 3/47); (0% - 6.4%)].

A slight increase was also observed in the incidence of alveolar/bronchiolar carcinomas observed in dosed male rats, compared with the controls (0/50; 0% for the control group; and 1/50; 2% for the low and high dose groups). These results were within the range of the historical control data for male F344/N rats at this laboratory [(0/50 – 1/50); (0% - 2%)] and the overall historical incidence range of the NTP Carcinogenesis Program [(0/50 - 3/50); (0% - 6 %)]. No statistical analysis was presented for this finding.

An increase in the incidence of combined alveolar/bronchiolar adenomas and carcinomas in male rats occurred with a statistically significant positive trend. The incidence of this lesion in the high dose group male rats (4/50; 8%) was significantly greater by a survival-adjusted test (*Incidental tumor test*) compared with the concurrent controls (0/50; 0%), and was slightly out of the range of the historical control data for male F344/N rats at this laboratory [(0/50 - 3/49); (0% - 6.1%)], although it was within the overall historical incidence range of the NTP Carcinogenesis Program [(0/50 - 4/49); (0% - 8.2%)].

Thyroid Gland: Incidences of C-cell adenomas in female rats and C-cell carcinomas and combined C-cell adenomas and carcinomas in male rats showed a statistically significant positive trend.

The incidence of C-cell adenomas observed in female rats showed a statistically significant and dose-related positive trend (1/48; 2% for the controls; 2/50; 4% for the low dose group, and 5/49; 10% for the high dose group). The incidence observed in the high dose female group (5/49; 10%) was slightly over the range of the historical control data for female F344/N rats at this laboratory [(0/49 - 3/46); (0% - 6.5%)], although it was within the overall historical incidence range of the NTP Carcinogenesis Program [(0/86 – 8/52); (0% - 15.4%)]. However, no statistical significance was observed comparing each dosed group and the controls.

Thyroid gland C-cell carcinomas in male rats also showed a statistically significant positive trend (the incidences were: 0/50; 0% for the controls, 0/49; 0% for the low dose group and 4/47; 9% for the high dose group), but no statistical significance was observed comparing each dosed group and the controls. In this case, all the results were within the range of historical control data for male F344/N rats at this laboratory [(0/50 – 6/49); (0% - 12.2%)] and the overall historical incidence range of the NTP Carcinogenesis Program [(0/52 – 6/49); (0% - 12.2%)].

In the case of the combined C-cell adenomas and carcinomas observed in male rats of the high dose group (6/47; 13%), the incidence was greater compared with the concurrent controls (1/50; 2%) but was within the range of the historical control data for male F344/N rats at this laboratory [(1/50 - 10/49); (2% - 20.4%)] and the overall historical incidence range of the NTP Carcinogenesis Program [(0/47 – 10/49); (0% - 20.4%)]. The difference between high dose and control group incidences was statistically significant according to Fisher's exact test (which doesn't adjust for survival differences), but no statistical significance was found by either of the survival-adjusted tests performed.

Table 29: Incidence of microscopic lessions in F344/N rats.

	Males			**Females**		
	Control	**1500 ppm**	**3000ppm**	**Control**	**1500 ppm**	**3000ppm**
Lungs						
Epitelial Hyperplasia	5/50(10%)	5/50(10%)	3/50(6%)	0/50(0%)	0/50(0%)	2/50(4%)
Alveolar/Bronchiolar Adenoma	0/50(0%)	2/50(4%)	3/50(6%)	1/50(2%)	2/50(4%)	2/50(4%)
Alveolar/Bronchiolar carcinoma	0/50(0%)	1/50(2%)	1/50(2%)	0/50(0%)	0/50(0%)	0/50(0%)

	Males			Females		
	Control	**1500 ppm**	**3000ppm**	**Control**	**1500 ppm**	**3000ppm**
Lungs						
Combined alveolar/bronchiolar adenoma and carcinoma	**0/50(0%)**[†]	3/50(6%)	**4/50(8%)***			
Thyroid gland						
C-cell Hyperplasia	4/50(8%)	3/49(6%)	1/47(2%)	9/48(19%)	6/50(12%)	1/49(2%)
C-cell Adenoma	1/50(2%)	1/49(2%)	2/47(4%)	**1/48(2%)**[†]	2/50(4%)	5/49(10%)
C-cell carcinoma	**0/50(0%)**[†]	0/49(0%)	4/47(9%)	2/48(4%)	0/50(0%)	1/49(2%)
Combined C-cell adenoma and carcinoma	**1/50(2%)**[†]	1/49(2%)	**6/47(13%)**[F]	3/48(6%)	2/50(4%)	6/49(12%)

***** statistically significant by survival-adjusted method (pairwise comparisons), $p \leq 0.05$

[F] statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), $p \leq 0.05$

[†] statistically significant positive trend ($p \leq 0.05$)

Table 30: Historical control data of neoplastic lesions in F344/N rats.

Neoplastic Lesion		**Alveolar/Bronchiolar adenoma**	**Alveolar/bronchiolar carcinoma**	**Alveolar/bronchiolar adenoma or carcinoma**
Males	Historical Incidence at EG&G Mason Research Institute [a]			
	TOTAL SD	12/696 (1.7%) 2.07%	1/696 (0.1%) 0.53%	13/696 (1.9%) 2.01%
	Range			
	High	3/49 (6.1%)	1/50 (2%)	3/49 (6.1%)
	Low	0/50 (0%)	0/50 (0%)	0/50 (0%)
	Overall Historical Incidence at All Laboratories (NTP) [c]			
	TOTAL SD	36/2357 (1.5%) 2.05%	23/2357 (1.0%) 1.71%	57/2357 (2.4%) 2.35%
	Range			
	High	3/47 (6.4%)	3/50 (6%)	4/49 (8.2%)
	Low	0/89 (0%)	0/50 (0%)	0/50 (0%)
Neoplastic Lesion		**C-Cell Adenoma**	**C-Cell Carcinoma**	**C-Cell Adenoma or carcinoma**
Males	Historical Incidence at EG&G Mason Research Institute [a]			
	TOTAL SD	27/664 (4.1%) 3.31%	27/664 (4.1%) 3.54%	54/664 (8.1%) 5.16 %
	Range			
	High	5/44 (11.4%)	6/49 (12.2%)	10/49 (20.4%)
	Low	0/50 (0%)	0/50 (0%)	1/50 (2%)
	Overall Historical Incidence at All Laboratories (NTP) [c]			
	TOTAL SD	121/2282 (5.3%) 4.49%	84/2282 (3.7%) 3.31%	203/2282 (8.9%) 4.99%
	Range			
	High	9/50 (18%)	6/49 (12.2%)	10/49 (20.4%)
	Low	0/89 (0%)	0/52 (0%)	0/47 (0%)
Females	Historical Incidence at EG&G Mason Research Institute [b]			
	TOTAL SD	21/724 (2.9%) 2.22%	25/724 (3.5%) 3.01%	46/724 (6.4%) 2.88%
	Range			
	High	3/46 (6.5%)	5/50 (10%)	6/50 (12%)
	Low	0/49 (0%)	0/49 (0%)	1/48 (2.1%)

	Overall Historical Incidence at All Laboratories (NTP) [c]			
	TOTAL SD	119/2317 (5.1%) 4.34%	81/2317 (3.5%) 2.99%	197/2317 (8.5%) 4.74%
	Range			
	High	8/52 (15.4%)	6/48 (12.5%)	9/50 (18%)
	Low	0/86(0%)	0/52(0%)	0/50 (0%)

Data as of March 16, 1983, for studies of at least 104 weeks. (The National Toxicology Program (NTP) was established in 1978).

[a] Data based in 14 studies performed at this laboratory (included 8-hydroxyquinoline).

[b] Data based in 15 studies performed at this laboratory (included 8-hydroxyquinoline).

[c] Number of studies or laboratories: not specified.

4.10.1.1.2 Oral Carcinogenesis in mice

Oral carcinogenesis of 8-hydroxyquinoline was studied in a 2-year oral toxicology and carcinogenesis study in mice *(U.S. National Toxicology Program. Technical Report Series no. 276. NIH Publication No. 85-2532. NTP-83-029).*

This study presents several deficiencies, such as the testing of only two dose levels or the lack of important measurements (like haematology, clinical chemistry, urinalysis or organ weights). The lack of important data in this study does not allow a complete evaluation of both chronic toxicity and carcinogenicity of 8-hydroxyquinoline.

Findings:

Statistical analysis of primary tumours included 2 survival-adjusted methods, used to evaluate tumour incidence. The *Life Table Analyses* assumed that all tumours of a given type observed in animals dying before the end of the study were "fatal", and the *Incidental Tumor Analyses* assumed that such tumours were "incidental". Reported P values for these survival-adjusted tests were either associated with the trend test or corresponded to pairwise comparisons between each dose group and the controls.

In addition, another 2 methods that don't adjust for survival incidences were used. These tests are the *Fisher's exact test* for pairwise comparisons and the *Cochran-Armitage linear trend test,* and both are based on the overall proportion of tumour-bearing animals.

No significant differences in survival were observed between any groups of either sex.

Mean body weights of female mice of both dosed groups were lower than those of the controls, getting to a -2.2% and -11.1% at termination for low and high doses, respectively (reaching the lowest point of -8.3% on week 96 for low dose females, and -18.4% on week 84 for the high dose).

Table 31 summarizes the mean body weights and survival of the mice over the study period.

Regarding male mice mean body weights, high dose values were slightly lower than those of the controls throughout most of the study (reaching the lowest point of -6.8% on week 92). Low dose values of males were closer to those of the controls throughout most of the study (although the first week decreased to a 85.2% of controls).

Average daily feed consumption decreased in both dosed groups of either sex. Compared with control values, feed consumption was -19% and -14% for males and females of the low dose group, respectively; and -28% and -29% for males and females of the high dose group, respectively.

Approximate daily chemical consumption for low dose and high dose mice was 217 and 396 mg/kg bw for males and 349 and 619 mg/kg bw for females, respectively.

Table 31: Mean body weights and survival (mice).

Weeks on study	Control		1500 ppm			3000 ppm		
	Av. Wt (grams)	No. of survivors	Av. Wt (grams)	Wt (percent of controls)	No. of survivors	Av. Wt (grams)	Wt (percent of controls)	No. of survivors
MALE MICE								
0	25	50	25	100	50	25	100	50
1	27	50	23	85.2	50	26	96.3	50
10	33	49	33	100	50	32	97	49
24	38	48	38	100	49	37	97.4	48
56	45	44	43	95.6	48	42	93.3	47
76	45	40	45	100	48	43	95.6	44
92	44	35	43	97.7	45	41	93.2	41
100	42	31	42	100	36	41	97.6	36
104	42	29	41	97.6	35	42	100	35
FEMALE MICE								
0	19	50	19	100	50	19	100	50
10	25	50	25	100	50	24	96	50
24	31	50	31	100	50	29	93.5	50
56	44	48	42	95.5	50	38	86.4	49
76	50	47	46	92	50	42	84	48
84	49	42	46	93.9	47	40	81.6	41
96	48	29	44	91.7	36	40	83.3	37
100	47	28	44	93.6	30	39	83	34
104	45	24	44	97.8	27	40	88.9	30

Non-neoplastic findings (see Table 32):

Necrotizing inflammation of multiple organs (utero-ovarian and thoracic or abdominal cavities) was found in female mice that died before the end of the study (primarily after week 80). These lesions were microscopically consistent with *Klebsiella* infection, and overall 22/50 control, 13/50 low dose, and 12/50 high dose female mice were infected.

Neoplastic findings (see Table 32 and Table 33)*:*

Lung: Dosed male and female mice showed increased incidences of lung alveolar/bronchiolar adenomas with no clear dose relationship (incidences for males were 5/50; 10% for the controls; 9/49; 18% for the low dose group, and 9/50; 18% for the high dose group. For females, the incidences were 1/49; 2% for the controls; 5/50; 10% for the low dose group, and 4/50; 8% for the high dose group). However, these increases were within the range of historical values according to the study reporter, (although no historical control data was available for mice pulmonary lesions in the report). No statistical significance was observed for these findings.

Circulatory System: Low dose female mice showed an increased incidence of hemangiomas and combined hemangiomas and hemangiosarcomas. The incidence of hemangiomas in the low dose group (4/50; 8%) was greater than the incidence in the concurrent controls (0/50; 0%), and it was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory [(0/50 – 3/50); (0% - 6%)] and the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice [(0/51 - 3/47); (0% - 6.4%)]. However, no statistical significance was observed for this finding and there was no dose-response relationship (incidences: 0/50; 0% for the controls; 4/50; 8% for the low dose group, and 1/50; 2% for the high dose group).

As for the combined incidence of hemangiomas and hemangiosarcomas in low dose female mice (5/50; 10%), it was significantly greater than that in the controls (0/50; 0%) by the Fisher's exact test (which doesn't adjust for survival differences), but the difference was not significant by either of the methods that adjusted for survival. The incidence of this lesion in the low dose female mice (5/50; 10%), was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory [(0/50 – 4/50); (0% - 8%)], but was within the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice [(0/50 - 5/49); (0% - 10.2%)]. No dose-response relationship was observed for this finding (incidences: 0/50; 0% for the controls; 5/50; 10% for the low dose group, and 1/50; 2% for the high dose group).

In the case of male mice of the control group, it was remarkable the elevated incidences of circulatory system tumours (7/50; 14% for hemangiomas and 10/50; 20% for combined hemangiomas and hemangiosarcomas). These incidences became the upper limits of the historical incidence ranges in both, the laboratory were the test was performed and the NTP Carcinogenesis Program. According to the study reporter there was no explanation apparent for this increased incidence of the circulatory system tumours in the concurrent controls relative to NTP historical control values.

Probably as a result of this unusual high values in the control group, hemangiomas and combined hemangiomas and hemangiosarcomas in male mice occurred with a statistically significant negative trend, and the incidences in the dosed groups were significantly lower than those in the controls.

Table 32: Incidence of microscopic lessions in $B6C3F_1$ mice.

	Males			**Females**		
	Control	**1500 ppm**	**3000ppm**	**Control**	**1500 ppm**	**3000ppm**
Lungs						
Epitelial Hyperplasia	1/50(2%)	0/49(0%)	5/50(10%)	1/49(2%)	0/50(0%)	0/50(0%)
Alveolar/Bronchiolar Adenoma	5/50(10%)	9/49(18%)	9/50(18%)	1/49(2%)	5/50(10%)	4/50(8%)
Alveolar/Bronchiolar carcinoma	1/50(2%)	1/49(2%)	1/50(2%)	1/49(2%)	0/50(0%)	1/50(2%)
Combined alveolar/ bronchiolar adenoma and carcinoma	6/50(12%)	10/49(20%)	10/50(20%)	2/49(4%)	5/50(10%)	5/50(10%)
Circulatory System						
Hemangioma	7/50(14%) ^{N††}	1/50(2%) ^{N*}	0/50(0%) ^{N**FF}	0/50(0%)	4/50(8%)	1/50(2%)
Hemangiosarcoma	3/50(6%)	1/50(2%)	1/50(2%)	0/50(0%)	1/50(2%)	0/50(0%)
Combined hemangioma and hemangiosarcoma	10/50(20%) ^{N††}	2/50(4%) ^{N**F}	1/50(2%) ^{N**F}	0/50(0%)	**5/50(10%)^{F}**	1/50(2%)

^{N} Negative trend or lower incidence.
† statistically significant trend, p≤ 0.05.
†† statistically significant trend, p≤ 0.01.
***** statistically significant by survival-adjusted method (pairwise comparisons), p≤ 0.05
****** statistically significant by survival-adjusted method (pairwise comparisons), p≤ 0.01
^{F} statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), p≤ 0.05
^{FF} statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), p≤ 0.01

Table 33: Historical control data of neoplastic lesions in B6C3F$_1$ mice.

Neoplastic Lesion		Hemangioma	Hemangiosarcoma	Combined hemangioma and hemangiosarcoma
Males	Historical Incidence at EG&G Mason Research Institute [a]			
	TOTAL SD	17/745 (2.3%) 3.85%	31/745 (4.2%) 3.00%	47/745 (6.3%) 5.36%
	Range			
	High	7/50 (14%)	5/49 (10.2%)	10/50 (20%)
	Low	0/50 (0%)	0/50 (0%)	0/50 (0%)
	Overall Historical Incidence at All Laboratories (NTP) [b]			
	TOTAL SD	34/2395 (1.4%) [c] 2.43%	65/2395 (2.7%) [d] 2.55%	98/2395 (4.1%) 3.89%
	Range			
	High	7/50 (14%)	5/49 (10.2%)	10/50 (20%)
	Low	0/50 (0%)	0/50 (0%)	0/50 (0%)
Females	Historical Incidence at EG&G Mason Research Institute [a]			
	TOTAL SD	15/748 (2.0%) 2.14%	14/748 (1.9%) 2.33%	29/748 (3.9%) 2.56 %
	Range			
	High	3/50 (6%)	3/50 (6%)	4/50 (8%)
	Low	0/50 (0%)	0/50 (0%)	0/50 (0%)
	Overall Historical Incidence at All Laboratories (NTP) [b]			
	TOTAL SD	39/2537 (1.5%) [e] 1.87%	51/2537 (2.0%) [f] 2.37%	90/2537 (3.5%) 2.61%
	Range			
	High	3/47 (6.4%)	4/50 (8%)	5/49 (10.2%)
	Low	0/51 (0%)	0/50 (0%)	0/50 (0%)

Data as of March 16, 1983, for studies of at least 104 weeks.
[a] Data based in 15 studies performed at this laboratory (included 8-hydroxyquinoline).
[b] Number of studies or laboratories: not specified.
[c] Includes 1 diagnosis of angioma.
[d] Includes 17 diagnoses of angiosarcoma.
[e] Includes 3 diagnosis of angioma.
[f] Includes 8 diagnosis of angiosarcoma.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

Quinoline, the parent compound of 8-hydroxyquinoline, is classified as carcinogenic, category 1B according to Regulation (EC) No 1272/2008 (Annex VI Table 3.1) because it has been shown to be a hepatocarcinogen in rats and mice. On the contrary, no clear evidence of carcinogenicity could be derived from the available information of 8-hydroxyquinoline.

The target organ for the carcinogenic activity of quinoline is the liver, where it has been shown to increase the incidence of hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas in rats and mice. No such effects were observed in the dosed rats of either sex of the available carcinogenicity study with 8-hydroxyquinoline. In the study performed with 8-hydroxyquinoline in mice, an increase of the combined incidence of hemangiomas and hemangiosarcomas was observed only in low dose female mice, which was statistically significant by the Fisher's exact test (it doesn't adjust for survival differences), but not

statistically significant by methods that adjusted for survival. Besides, no dose-response relationship was observed for this finding. Therefore this finding was not considered related to the treatment with 8-hydroxyquinoline.

Quinoline is also classified as mutagenic, category 2 according to Regulation (EC) No 1272/2008 (Annex VI Table 3.1), as it has shown mutagenic activity *in vitro* and *in vivo*, while 8-hydroxyquinoline showed to be mutagenic *in vitro* but not mutagenic *in vivo* (based on well conducted studies).

Ten of the fourteen studies available for evaluating the genotoxicity of 8-hydroxyquinoline were published as scientific literature, and most of them were performed to compare the mode of action between quinoline and its derivatives, included the 8-hydroxyquinoline. Quinoline was found to be a powerful S-phase inducer, with an optimum response between 16 and 36 hours after oral dosing of 225-500 mg/kg, whereas the same doses of 8-hydroxyquinoline did not induce S-phase activity (Ashby et al., 1989). Chemicals with similar structures frequently demonstrate to have different characteristics and effects when getting in contact with organic systems. This is probably what occurs with quinoline (a carcinogenic substance) and 8-hydroxyquinoline: despite the structural analogy of these two substances, they seem to have two different mechanisms of action.

4.10.4 Summary and discussion of carcinogenicity

4.10.4.1 Summary and discussion of carcinogenicity. Carcinogenesis in rats

Due to the deficiencies of the study (testing of only two dose levels or the limited investigations of systemic toxicity parameters), a complete evaluation of the carcinogenicity of 8-hydroxyquinoline cannot be performed. However, as it is detailed below, the results do not suggest a carcinogenic effect since the neoplastic findings observed could not be associated with the treatment, or could not be considered enough evidence of carcinogenicity.

In male rats, combined alveolar/bronchiolar adenomas and carcinomas occurred with a statistically significant positive trend (mainly due to an increased incidence of adenomas). The incidence of this lesion in the high dose group male rats (8%) was significantly greater than that in the concurrent controls (0%) by a survival-adjusted test. However, it was only slightly out of the range of the historical control data for male F344/N rats at the testing facility (0% - 6.1%) and within the overall historical incidence range of the NTP Carcinogenesis Program (0% - 8.2%). This increase was not supported by an increase in the incidence of epithelial hyperplasia (10% for the control and low dose groups; and 6% for the high dose group). Besides, adenomas were lesions that were border-line between focal epithelial hyperplasia and small adenomas. Most of those neoplastic lesions observed in dosed animals did not appear to differ from lung tumors observed in control animals. Hence, due to these uncertainties, the concern for the low increase in the incidence of these tumour findings in male rats appear unrelated to treatment and are not considered an indication of carcinogenic hazard.

Thyroid gland C-cell adenomas observed in female rats showed a statistically significant and dose-dependent positive trend (2% for the controls; 4% for the low dose group, and 10% for the high dose group). The incidence observed in the high dose female group (10%) was only slightly over the range of the historical control data for female F344/N rats at this laboratory (0% - 6.5%), although it was within the overall historical incidence range of the NTP Carcinogenesis Program (0% - 15.4%). However, no statistical significance in pair-wise comparisons was observed. Besides, this increase was not supported by an increase in the incidence of C-cell hyperplasia (which decreased with dose: 19% for the controls; 12% for the

low dose group, and 2% for the high dose group). Overall, it appears questionable whether the non-significant, small increase in the incidence of adenomas in the high dose females should be interpreted to be treatment-related, but it doesn't seem enough evidence of carcinogenicity.

In male rats, thyroid gland C-cell carcinomas occurred with a statistically significant positive trend, but no statistical significance was observed comparing each dosed group and the controls (0% for the controls and the low dose group, and 9% for the high dose group). All the results were within the range of historical control data for male F344/N rats at this laboratory and the overall historical incidence range of the NTP Carcinogenesis Program (0% - 12.2%, in both cases).

A statistically significant positive trend was also observed for thyroid gland combined C-cell adenomas and carcinomas in male rats. In this case the incidence in the high dose group (13%) was statistically significant compared with the concurrent control (2%), according to Fisher's exact test (which doesn't adjust for survival differences), but no statistical significance was found by the survival-adjusted tests performed. However, the incidence of this finding was within the range of the historical control data for male F344/N rats at this laboratory (2% - 20.4%) and the overall historical incidence range of the NTP Carcinogenesis Program (0% - 20.4%).

The described increments of thyroid gland C-cell neoplasias observed in male rats were within the range of the historical control data of the testing facility and were not supported by an increase in the incidence of C-cell hyperplasia (which decreased with dose: 8% for the controls; 6% for the low dose group, and 2% for the high dose group). Therefore, these tumours are likely to be unrelated to the treatment.

Overall, the increases in the incidence of tumours observed in rats were unlikely to be treatment-related.

4.10.4.2 Summary and discussion of carcinogenicity. Carcinogenesis in mice

Due to the deficiencies of the study (testing of only two dose levels or the limited investigations of systemic toxicity parameters), a complete evaluation of the carcinogenicity of 8-hydroxyquinoline cannot be performed. However, as it is detailed below, the results do not suggest a carcinogenic effect since the neoplastic findings observed could not be associated with the treatment.

Dosed male and female mice showed increased incidences of lung alveolar/bronchiolar adenomas (incidences for males were 10% for the controls; and 18% for the low and high dose groups. For females, the incidences were 2% for the controls; 10% for the low dose group, and 8% for the high dose group). However, there was neither a statistically significant positive trend nor statistical significance in pair-wise comparison. Besides, data do not indicate a clear dose-response relationship and the incidences were reported to be within the range of historical control data. These increases are, therefore, not considered treatment related.

Low dose female mice showed an increased incidence of hemangiomas and combined hemangiomas and hemangiosarcomas. The incidence of hemangiomas in the low dose group (8%) was greater than the incidence in the concurrent controls (0%), and it was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory (0% - 6%) and the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice (0% - 6.4%). However, no statistical significance was observed for this finding and there was no dose-response relationship (incidences: 0% for the controls; 8% for the low dose group, and 2% for the high dose group). As for the increase of the combined incidence

of hemangiomas and hemangiosarcomas observed in low dose female mice (10%), it was statistically significant compared with controls (0%) by the Fisher's exact test (which doesn't adjust for survival differences), but no statistical significance was observed by methods that adjusted for survival. The incidence of this lesion was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory (0% - 8%), but was within the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice (0% - 10.2%). However, no dose-response relationship was observed for this finding either (incidences: 0% for the controls; 10% for the low dose group, and 2% for the high dose group).

Since there was no dose-response relationship for the increases in the incidence of hemangiomas and combined hemangiomas and hemangiosarcomas, these findings are assessed to be chance findings.

Therefore, none of the effects observed in mice were regarded as being associated with the administration of 8-hydroxyquinoline.

4.10.5 Comparison with criteria

As there is no epidemiological evidence of carcinogenicity to humans with 8-hydroxyquinoline, given that no human data is available, a classification in Category 1A does not apply.

Considering a reading-across approach, there is a structural analogy with quinoline (the parent compound of 8-hydroxyquinoline), for which there is good evidence of carcinogenicity, and it is classified as carcinogenic, category 1B. However, there is no substantial support to classify 8-hydroxyquinoline on the basis of such structural analogy, since there are considerable differences between both substances. Regarding their mutagenic activity, quinoline is mutagenic *in vitro* and *in vivo*, while 8-hydroxyquinoline is mutagenic *in vitro* but not mutagenic *in vivo*. As for the findings of the carcinogenicity studies performed with these substances, quinoline has been shown to increase the incidence of hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas in the liver of rats and mice, and no such effects were observed in rats or male mice treated with 8-hydroxyquinoline. Although hemangiomas and hemangiosarcomas were observed in female mice treated with 8-hydroxyquinoline, the finding is not considered treatment related, as there was no dose-response relationship. Therefore, despite the structural analogy of these two substances, they seem to have different mechanisms of action.

Moreover, classification of 8-hydroxyquinoline in Category 1B is not regarded appropriate as no clear evidence of carcinogenicity can be derived from the assessed studies. The tumour profile, in combination with the lack of *in vivo* genotoxic activity, does not allow for a Category 1B. Besides, there are important drawbacks related to limited investigations of systemic toxicity parameters in the carcinogenicity studies.

The remaining question is whether the strength of the evidence derived from the available data is enough for a classification in Category 2 or it is not enough to warrant the classification for carcinogenicity of 8-hydroxyquinoline.

The available information does not provide enough evidence to support a classification of 8-hydroxyquinoline in Category 2. The evidence of carcinogenicity is not substantial, with only equivocal evidence of induction of tumours in rats. Findings observed in the available studies could not be associated with the treatment with 8-hydroxyquinoline, mainly based on low incidence rates, rather weak dose-response relationship, lack of statistical significance and results being within the historical control range in most cases. Overall, the weight and

strength of the evidence is considered to be insufficient to justify a classification for carcinogenicity.

Therefore, based on the comparison of the available carcinogenicity data with CLP classification criteria it is proposed not to classify 8-hydroxyquinoline for carcinogenicity.

4.10.6 Conclusions on Classification and Labelling

CLP: Not classified based on available data.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS concluded, based on two oral toxicology and carcinogenicity studies (NTP, 1985), that the results did not suggest a carcinogenic effect, since the neoplastic findings observed could not be associated with the treatment, or could not be considered sufficient evidence of carcinogenicity. These studies (both in rats and mice) did not comply with the test guideline OECD TG 453 on chronic toxicity and carcinogenicity (testing on two doses only, lack of haematology and clinical chemistry, urinalysis and organ weights).

The main findings were summarised as follows:

Rat carcinogenicity study

Table 29 (of the CLH report): Incidence of microscopic lesions in F344/N rats.

	Males			**Females**		
	Control	**1500 ppm**	**3000 ppm**	**Control**	**1500 ppm**	**3000 ppm**
Lungs						
Epitelial Hyperplasia	5/50 (10%)	5/50 (10%)	3/50 (6%)	0/50 (0%)	0/50 (0%)	2/50 (
Alveolar/Bronchiolar Adenoma	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)	2/50 (4%)	2/50 (
Alveolar/Bronchiolar carcinoma	0/50 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (
Combined alveolar/bronchiolar adenoma and carcinoma	**0/50 (0%)†**	3/50 (6%)	**4/50 (8%)***			
Thyroid gland						
C-cell Hyperplasia	4/50 (8%)	3/49 (6%)	1/47 (2%)	9/48 (19%)	6/50 (12%)	1/49 (
C-cell Adenoma	1/50 (2%)	1/49 (2%)	2/47 (4%)	**1/48 (2%)†**	2/50 (4%)	5/49 (10%)
C-cell carcinoma	**0/50 (0%)†**	0/49 (0%)	4/47 (9%)	2/48 (4%)	0/50 (0%)	1/49 (
Combined C-cell adenoma and carcinoma	**1/50 (2%)†**	1/49 (2%)	**6/47 (13%)F**	3/48 (6%)	2/50 (4%)	6/49 (12%)

***** statistically significant by survival-adjusted method (pairwise comparisons), $p \leq 0.05$.
F statistically significant by Fisher's exact test for pairwise comparisons (not adjuste survival differences), $p \leq 0.05$. **†** statistically significant positive trend ($p \leq 0.05$)

(Doses: 0, 1500 and 3000 ppm in the diet, equivalent to: males: 0, 73 and 143 mg/kg bw/d, females: 0, 89 and 166 mg/kg bw/d)

In male rats, combined alveolar/bronchiolar adenomas and carcinomas occurred with a statistically significant positive trend (mainly due to an increased incidence of adenomas). The incidence of this lesion in the high dose group male rats (8%) was significantly greater than that in the concurrent controls (0%) based on a survival-adjusted statistical test. However, it was only slightly outside the range of the historical control data for male F344/N rats at the testing facility (0% - 6.1%) and was within the overall historical incidence range of the NTP Carcinogenesis Program (0% - 8.2%). This increase was not supported by an increase in the incidence of epithelial hyperplasia (10% for the control and low dose groups; and 6% for the high dose group). Besides, the adenomas were lesions that were border-line between focal epithelial hyperplasia and small adenomas. Most of those neoplastic lesions observed in dosed animals did not appear to differ from lung tumours observed in control animals. Hence, due to these uncertainties, the low increase in the incidence of these tumour findings in male rats appear unrelated to treatment and are not considered an indication of carcinogenic hazard.

Thyroid gland C-cell adenomas observed in female rats showed a statistically significant and dose-dependent positive trend (2% for the controls; 4% for the low dose group, and 10% for the high dose group). The incidence observed in the high dose female group (10%) was only slightly over the range of the historical control data for female F344/N rats at this laboratory (0% - 6.5%), although it was within the overall historical incidence range of the NTP Carcinogenesis Program (0% - 15.4%). However, no statistical significance in pair-wise comparisons was observed. Besides, this increase was not supported by an increase in the incidence of C-cell hyperplasia (which decreased with dose: 19% for the controls; 12% for the low dose group, and 2% for the high dose group). Overall, it appears questionable whether the non-significant, small increase in the incidence of adenomas in the high dose females should be interpreted to be treatment-related, and it doesn't seem sufficient to be considered evidence for carcinogenicity.

In male rats, thyroid gland C-cell carcinomas occurred with a statistically significant positive trend, but no statistical significance was observed comparing each dosed group and the controls (0% for the controls and the low dose group, and 9% for the high dose group). All the results were within the range of historical control data for male F344/N rats at this laboratory and the overall historical incidence range of the NTP Carcinogenesis Program (0% - 12.2%, in both cases).

A statistically significant positive trend was also observed for thyroid gland combined C-cell adenomas and carcinomas in male rats. In this case the incidence in the high dose group (13%) was statistically significant compared with the concurrent control (2%), according to Fisher's exact test (which doesn't adjust for survival differences), but no statistical significance was found by the survival-adjusted tests performed. However, the incidence of this finding was within the range of the historical control data for male F344/N rats at this laboratory (2% - 20.4%) and the overall historical incidence range of the NTP Carcinogenesis Program (0% - 20.4%).

The described increments of thyroid gland C-cell neoplasias observed in male rats were within the range of the historical control data of the testing facility and were not supported by an increase in the incidence of C-cell hyperplasia (which decreased with dose: 8% for the controls; 6% for the low dose group, and 2% for the high dose group). Therefore, these tumours were considered by the DS likely to be unrelated to the treatment.

Overall, the increases in the incidence of tumours observed in rats were unlikely to be treatment-related.

Mouse carcinogenicity study

Table 32 (of the CLH report): incidence of microscopic lessions in $B6C3F_1$ mice.

	Males			**Females**		
	Control	**1500 ppm**	**3000 ppm**	**Control**	**1500 ppm**	**3000 ppm**
Lungs						
Epitelial Hyperplasia	1/50 (2%)	0/49 (0%)	5/50 (10%)	1/49 (2%)	0/50 (0%)	0/50 (0%)
Alveolar/Bronchiolar Adenoma	5/50 (10%)	9/49 (18%)	9/50 (18%)	1/49 (2%)	5/50 (10%)	4/50 (8%)
Alveolar/Bronchiolar carcinoma	1/50 (2%)	1/49 (2%)	1/50 (2%)	1/49 (2%)	0/50 (0%)	1/50 (2%)
Combined alveolar/ bronchiolar adenoma and carcinoma	6/50 (12%)	10/49 (20%)	10/50 (20%)	2/49 (4%)	5/50 (10%)	5/50 (10%)
Circulatory System						
Hemangioma	7/50 (14%)^{N††}	1/50 (2%) $^{N}*$	0/50 (0%) $^{N}**^{FF}$	0/50 (0%)	4/50 (8%)	1/50 (2%)
Hemangiosarcoma	3/50 (6%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Combined hemangioma and hemangiosarcoma	10/50 (20%) ^{N††}	2/50 (4%) $^{N}**^{F}$	1/50 (2%) $^{N}**^{F}$	0/50 (0%)	**5/50 (10%)**^{F}	1/50 (2%)

^{N} Negative trend or lower incidence. † statistically significant trend, $p \leq 0.05$. †† statistically significant trend, $p \leq 0.01$. ***** statistically significant by survival-adjusted method (pairwise comparisons), $p \leq 0.05$ ****** statistically significant by survival-adjusted method (pairwise comparisons), $p \leq 0.01$ ^{F} statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), $p \leq 0.05$ $^{F F}$ statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), $p \leq 0.01$

(Doses: 0, 1500 and 3000 ppm in the diet, equivalent to: males: 0, 217 and 396 mg/kg bw/d, females: 0, 349 and 619 mg/kg bw/d)

Dosed male and female mice showed increased incidences of lung alveolar/bronchiolar adenomas. Incidences for males were 10% for the controls and 18% for the low and high dose groups. For females, the incidences were 2% for the controls, 10% for the low dose group, and 8% for the high dose group. However, there was neither a statistically significant positive trend nor statistical significance in the pair-wise comparison. Besides, the data did not indicate a clear dose-response relationship and the incidences were reported to be within the range of historical control data. These increases were, therefore, not considered by the DS to be treatment related.

Low dose female mice showed an increased incidence in hemangiomas and combined hemangiomas and hemangiosarcomas. The incidence of hemangiomas in the low dose group (8%) was greater than the incidence in the concurrent controls (0%), and it was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory (0% - 6%) and the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice (0% - 6.4%). However, no statistical significance was observed for this finding and there was no dose-response relationship (incidences: 0% for the controls; 8% for the low dose group, and 2% for the high dose group). As for the increase in the combined incidence of hemangiomas and hemangiosarcomas observed in low dose female mice (10%), it was statistically

significant compared with controls (0%) by the Fisher's exact test (which doesn't adjust for survival differences), but no statistical significance was observed by methods that adjusted for survival. The incidence of this lesion was slightly out of the range of the historical control data for female $B6C3F_1$ mice at this laboratory (0% - 8%), but was within the overall historical incidence range of the NTP Carcinogenesis Program for female $B6C3F_1$ mice (0% - 10.2%). However, no dose-response relationship was observed for this finding either (incidences: 0% for the controls; 10% for the low dose group, and 2% for the high dose group).

Since there was no dose-response relationship for the increases in the incidence of hemangiomas and combined hemangiomas and hemangiosarcomas, these findings were assessed to be chance findings.

Therefore, none of the effects observed in mice were regarded by the DS to be associated with the administration of 8-hydroxyquinoline.

Other information

The DS indicated that quinoline, the parent compound of 8-hydroxyquinoline, is classified as Carc. 1B because it has been shown to be a hepatocarcinogen in rats and mice. In contrast, no clear evidence of carcinogenicity could be derived from the available information on 8-hydroxyquinoline.

The target organ for the carcinogenic activity of quinoline is the liver, where it has been shown to increase the incidence of hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas in rats and mice. No such effects were observed in the dosed rats of either sex in the available carcinogenicity study with 8-hydroxyquinoline. In the study performed with 8-hydroxyquinoline in mice, an increase in the combined incidence of hemangiomas and hemangiosarcomas was observed only in low dose female mice, which was statistically significant by the Fisher's exact test (which doesn't adjust for survival differences), but was not statistically significant by methods that adjusted for survival. Besides, no dose-response relationship was observed for this finding. Therefore this finding was not considered related to the treatment with 8-hydroxyquinoline.

A structurally related substance, quinoline, is classified as Muta. 2, , as it has shown mutagenic activity *in vitro* and *in vivo*, while 8-hydroxyquinoline showed evidence of mutagenicity *in vitro* but not *in vivo* (based on well conducted studies).

Ten of the fourteen studies available for evaluating the genotoxicity of 8-hydroxyquinoline were published in the scientific literature, and most of them were performed to compare the mode of action between quinoline and its derivatives, including 8-hydroxyquinoline. Quinoline was found to be a powerful S-phase inducer, with an optimum response between 16 and 36 h after oral dosing of 225-500 mg/kg, whereas the same doses of 8-hydroxyquinoline did not induce S-phase activity (Ashby *et al*., 1989).

Comments received during public consultation

Two MSCA ageed with no classification for carcinogenicity. One considered the increases in male rat C-cell tumours and alveolar/bronchiolar tumours marginal and regarded them as not related to the test substance.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the evidence of carcinogenicity is not substantial, with equivocal evidence of induction of tumours in rats. There is uncertainty whether findings observed in the available studies could be associated with the treatment with 8-hydroxyquinoline, mainly based on low incidence rates, rather weak dose-response

relationship, and the lack of statistical significance (in particular for the single tumour types observed). RAC places more weight on these facts than on the argument of the DS that the results were within the historical control range in most cases as supportive for the lack of a causal relationship to the treatment. RAC notes that this comparison was only based on the upper limits of the observed ranges.

Overall RAC agrees that the weight and strength of the evidence is considered to be insufficient to justify a classification for carcinogenicity. Therefore, based on the comparison of the available carcinogenicity data with CLP classification criteria RAC concludes that 8-hydroxyquinoline need not be classified for carcinogenicity.

Supplemental information - In depth analyses by RAC

The DS indicated that a structurally related substance, quinoline, the parent compound of 8-hydroxyquinoline, is classified as Carc. 1B because it has been shown to be a hepatocarcinogen in rats and mice. In contrast, no clear evidence of carcinogenicity could be derived from the available information on 8-hydroxyquinoline.

The target organ for the carcinogenic activity of quinoline is the liver, where it has been shown to increase the incidence of hepatocellular carcinomas and hemangioendotheliomas or hemangiosarcomas in rats and mice. No such effects were observed in the dosed rats of either sex in the available carcinogenicity study with 8-hydroxyquinoline. In the study performed with 8-hydroxyquinoline in mice, an increase in the combined incidence of hemangiomas and hemangiosarcomas was observed only in low dose female mice, which was statistically significant by the Fisher's exact test (which doesn't adjust for survival differences), but was not statistically significant by methods that adjusted for survival. Besides, no dose-response relationship was observed for this finding. Therefore this finding was not considered related to the treatment with 8-hydroxyquinoline.

Quinoline is also classified as Muta. 2, as it has shown mutagenic activity *in vitro* and *in vivo*, while 8-hydroxyquinoline showed evidence of mutagenicity *in vitro* but not *in vivo* (based on well conducted studies).

Ten of the fourteen studies available for evaluating the genotoxicity of 8-hydroxyquinoline were published in the scientific literature, and most of them were performed to compare the mode of action between quinoline and its derivatives, including 8-hydroxyquinoline. Quinoline was found to be a powerful S-phase inducer, with an optimum response between 16 and 36 h after oral dosing of 225-500 mg/kg, whereas the same doses of 8-hydroxyquinoline did not induce S-phase activity (Ashby *et al*., 1989).

4.11 Toxicity for reproduction

Table 34: Summary table of relevant reproductive toxicity studies.

Method	Main results
Two- generation Reproduction Toxicity Study in Wistar Rats. **Fascineli, M. L. (2006b)** Guidelance: OECD 416 GLP: Yes Sex/ Strain/ Species: Wistar Han rats *(Rattus norvegicus)* from BIOAGRI Laboratórios (Brazil) Purity: 99.7% No./group animals: 26/sex/dose Route of exposure: Oral (diet) Doses: 0, 1000, 3000 and 8000 ppm equivalent to mg/kg bw/day: Premating F0 0, 95-119, 274-345, 678-933 for m-f respectively. Premating F1 0, 102-122, 292-346, 769-855 for m-f respectively. Gestation F0 0, 119, 358, 918 Gestation F1 0, 127, 330, 841 Lactation F0 0, 242, 674, 2060 Lactation F1 0, 257, 710, 1767 **Study acceptable**	**F0 PARENTS** There were no remarkable clinical signs or deaths associated to the treatment. **8000 ppm** ▪ ↓ Bodyweight, bodyweight gain and food consumption during pre-mating (in both sexes), gestation and lactation periods (in females). ▪ ↓ Relative and absolute weights of prostate and ↑ relative and absolute weights of spleen in males. ↓ Relative and absolute weights of kidneys, adrenals and ovaries in females. ▪ ↓ Average terminal bodyweight (12% in males and 11.8% in females). ▪ ↑ Incidence of pigmentation in liver (11/26 in males and 8/26 in females). **3000 ppm** ▪ ↓ Bodyweight during pre-mating (females), gestation and lactation periods, ↓ bodyweight gain during pre-mating and gestation periods and ↓ food consumption during pre-mating and lactation periods in females. ▪ ↓ Absolute weight of prostate, ↑ absolute and relative weight of spleen in males. ▪ ↓ Average terminal bodyweight (5% in females). ▪ ↑ Incidence of pigmentation in liver (5/26 in females). **1000 ppm** ▪ ↓ Food consumption during pre-mating periods (non-dose dependent) for weeks 5-6 (12.4%) in females. **REPRODUCTIVE INDICES** A decrease in the number of live born pups statistically significant (average/litter: 9.5 *vs* 12.4 control) was observed at 8000 ppm. It was out of the range of the historical control values (9.9-12.4 %) collected in the Reporting Table rev. 1-1 (08.04.2010) of 8-hydroxyquinoline. At doses of 3000 ppm and 1000 ppm there was also a decrease in the number of live born pups (10.5 and 11 respectively *versus* the control value of 12.4) but they fell into the range of historical control values mentioned above. The main reproductive indices (mating, fertility, gestation and oestrous cycle) were comparable with control groups. **F1 PUPS** **8000 ppm** ▪ ↓ Bodyweight (13.4-35.1% in males and 13.2-33.2% in females) in the interval 7-21 days. ▪ Delayed sexual maturity (age in days): ↑ Preputial separation (47.7 *vs* 42.3 control) in males and ↑ vagina opening (37.6 d *vs* 33.5 control) in females. ▪ ↑ Relative weight and ↓ absolute weight of brain in both sexes. ↓ Absolute and relative weights of spleen and thymus in both sexes. ▪ ↓ Terminal body weight: 32.4% in males and 32.2% in females. **3000 ppm** ▪ ↓ Bodyweight (7.7 % in males) on day 21. ▪ ↓ Absolute weight of thymus in males and ↓ absolute weight of spleen in females. **F1 PARENTS** There were no remarkable clinical findings and 1 female died during parturition at 8000 ppm. **8000 ppm** ▪ ↓ Bodyweight and food consumption during pre-mating, gestation and lactation periods (both sexes) and ↓ bodyweight gain during pre-mating (both sexes) and gestation (females).

	▪ ↓ Absolute and relative weights of kidneys and ↓ absolute weight of brain and right adrenals in both sexes. ↓ Absolute weight of left adrenal in males. ↓ Absolute weight of seminal vesicles, epididymides and testes and ↓ absolute and relative weights of liver in males. ↓ Absolute and relative weight of ovaries in females ▪ ↓ Average terminal bodyweight (30.3% in males and 19% in females). ▪ ↑ Incidence of pigmentation in liver (8/26 in males and 5/25 in females). **3000 ppm** ▪ ↓ Bodyweight during pre-mating (both sexes), gestation and lactation periods (females), ↓ bodyweight gain during pre-mating in males and ↓ food consumption during pre-mating and gestation periods in females. ▪ ↓ Absolute weights of seminal vesicles and right adrenal in males. ↓ Absolute weight of brain in females. ▪ ↓ Average terminal bodyweight (8.7 % in males and 8.3% in females). ▪ ↑ Incidence of pigmentation in liver (2/26 in females). **1000 ppm** ▪ ↓ Bodyweight (3%) in week 10 in females and ↓ food consumption (13.3-15.8%) during pre-mating period (weeks 1-5) in females. ▪ ↓ Average terminal bodyweight (7 % in females). **<u>REPRODUCTIVE INDICES</u>** **8000 ppm** ▪ ↓ No. of complete oestrous cycle for 3 weeks (3.5 *vs* 4.3 control). ▪ ↑ Length of oestrous cycle (days), (5.3 *vs* 4.6 control). ▪ ↓ Number of live born pups (8.5 *vs* 11.2 control) out of the range of historical controls values (9.9-12.4%) mentioned above. Reproductive parameters such as mating, fertility or gestation indices were no affected. **<u>F2 PUPS</u>** **8000 ppm** ▪ ↓Bodyweight (17.8-31.7 % in males and 15.9-28.2 % in females) in the interval 7-21 days. ▪ Delay in day of apparition of incisor eruption in males (mean day of apparition: 10.3 *vs* 9.6 control) and females (mean day of apparition: 10.3 *vs* 9.7 control). ▪ Delay in the eyes opening in males (14.5 *vs* 13.8 control) and females (14.2 *vs* 13.7 control). ▪ ↑ Relative and ↓ absolute weight of brain in both sexes. ↓Absolute and relative weights of spleen and thymus in both sexes. ▪ ↓ Terminal body weight (32.4% in males and 26.4% in females). **3000 ppm** ▪ ↓ Bodyweight in males (4.0%) on day 21 and females (5.8-6.6 %) in the interval 14-21 days. ▪ Delay in the eyes opening in males (mean day of apparition: 14.1 *vs* 13.8 control) and females (mean day of apparition: 14.1 *vs* 13.7 control). ▪ ↓ Absolute weight of brain in both sexes and absolute weight of spleen in females. ▪ ↓ Terminal body weight (6.9% in males and 5.3% in females).

Prenatal Developmental Toxicity Study in Wistar Han Rats **Fascineli, M. L. (2006c)** Guidelance: OECD 414 (2001) GLP: yes Sex/ Strain/ Species: Female Wistar Han (Hannover) rats Purity: 99.68% No./group animals: 25 females dose/group Route of exposure: Oral (gavage) from day 6 to 19 of gestation Doses: 0, 100, 300 and 600 mg/kg bw/day Vehicle:(corn oil) **Study acceptable**	**MATERNAL TOXICITY** The survival was 100% in all groups. **600 mg/kg bw/day** ▪ Nervous system excitation (during 10 minutes) after dosing followed by lethargy (during 20 minutes) in all dams. ▪ ↓ Bodyweight since day 12 of administration (6.8%) until the day of sacrifice (10.7%), ↓ bodyweight gain (32.7%) and ↓ food consumption (22.5%) during days 6-20 of the gestation period. ▪ ↓ Maternal corrected bodyweight gain (95.9%). ▪ ↓Carcass weight (24.1%). **300 mg/kg bw/day** ▪ Nervous system excitation (during 10 minutes) after dosing followed by lethargy (during 20 minutes) in all dams. ▪ ↓ Bodyweight (5%) day 20, ↓ bodyweight gain (14%) during 6-20 days and ↓ food consumption (11.8%) during days 6-20 of the gestation period. ▪ ↓ The maternal corrected bodyweight gain (42.3%). ▪ ↓ Carcass weight (17.7%). **100 mg/kg bw/day** ▪ ↓ Maternal corrected bodyweight gain (10.3 %). **REPRODUCTIVE INDICES** No effect on the live and dead foetuses, resorptions, pre and post-implantation losses, *corpora lutea* or number of litters. **FOETAL TOXICITY** **600 mg/kg bw/day** ▪ ↓ Foetal bodyweight (14.6%). ▪ ↓ Mean placental weight (18.3%). ▪ ↑ Incidence of visceral variations: Enlarged nasal cavity (16 foetuses/11 litters *vs* 5/4 control) and unilateral hydronephrosis in kidney (47 foetuses/21 litters *vs* 28/13 control) ▪ ↑ Incidence of skeletal variations: Short supernumerary rib (53 foetuses/21 litters *vs* 25/16 control) and full supernumerary rib (24 foetuses/15 litters *vs* 8/7 control) ▪ ↑ Incidence of skeletal retardations: Sternebra not ossified (49 foetuses/20 litters *vs* 2/2 control) and rudimentary sternebra (44 foetuses/18 litters *vs* 17/12 control) ▪ ↓ Number of foetal ossification centres on day 20 of gestation: Anterior phalanges (0.77 *vs* 2.91 control), metacarpals (6.83 *vs* 7.89 control), caudal vertebrae (1.85 *vs* 2.63 control) and sternebrae (5.45 *vs* 5.98 control) **300 mg/kg bw/day** ▪ ↓ Foetal bodyweight (5.8%). ▪ ↓ Mean placental weight (6.1%). ▪ ↑ Incidence of visceral variations: Enlarged nasal cavity (14 foetuses/7 litters *vs* 5/4 control) ▪ ↑ Incidence of skeletal variations: Full supernumerary rib (23 foetuses/15 litters *vs* 8/7 control) ▪ ↑ Incidence of skeletal retardations: Sternebra not ossified (19 foetuses/11 litters *vs* 2/2 control) and rudimentary sternebra (50 foetuses/19 litters *vs* 17/12 control). ▪ ↓ Number of foetal ossification centres on day 20 of gestation: Anterior phalanges (1.24 *vs* 2.91 control), metacarpals (7.43 *vs* 7.89 control), caudal vertebrae (2.08 *vs* 2.63 control) and sternebra (5.82 *vs* 5.98 control) **100 mg/kg bw/day** ▪ ↓ Placental weight for males (6.5%) and females (6.8%). ▪ ↑ Incidence of skeletal retardations: Not ossified sternebra (11 foetuses/7 litters *vs* 2/2 control) and rudimentary sternebra (37 foetuses/18 litters *vs* 17/12 control). ▪ ↓ Number of foetal ossification centres on day 20 of gestation: Anterior phalanges (1.84 *vs* 2.91 control), metacarpals (7.66 *vs* 7.89 control) and caudal vertebrae (2.29 *vs* 2.63 control).

Prenatal Developmental Toxicity Study in New Zealand White Rabbits **Fascineli, M. L.** **(2006d)** Guidelance: OECD 414 (2001) GLP: Yes Sex/ Strain/ Species: Female New Zealand White rabbits Purity: 99.68% No./group animals: 25 females dose/Group Route of exposure: Oral (gavage) from day 6 to 28 of gestation Doses: 0, 5, 15 y 60 mg/kg bw/day Vehicle: (corn oil) **Study acceptable**	**MATERNAL TOXICITY** The survival was 100% in all groups. **60 mg/kg bw/day** ▪ Nervous system excitation followed by lethargy occurred after test item administration without a specific trend in the beginning or duration of the effects in 11 dams (44% affected). These findings were dose dependent and they did not occurred in control group. **15 mg/kg bw/day** ▪ Nervous system excitation followed by lethargy occurred after test item administration without a specific trend in the beginning or duration of the effects in 4 dams (16% affected). No statistically significant. These findings were dose dependent and they did not occurred in control group. **REPRODUCTIVE INDICES** **60 mg/kg bw/day** ▪ ↓ Number of live born female pups (2.90 *vs* 4.15 controls). ▪ ↑ Pre-implantation loss (32.33% *vs* 15.81% control). This finding cannot be attributed to the test substance administration since females were exposed from implantation (day 6 after mating). **FOETAL TOXICITY** **60 mg/kg bw/day** ▪ Malformations: Omphalocele (5 foetuses/4 litters *vs* 0/0 control). The incidence for omphalocele (4.3% incidence in foetuses and 23.5% in the litters) is out of the range of the historical control data of the laboratory. (0-1.8% and 0-8.3% in foetuses and litters respectively). ▪ ↑ Incidence of head (soft tissue) variations: Periorbital haemorrhage (18 foetuses/11 litters *vs* 8/7 control) and retinal fold (19 foetuses/14 litters *vs* 13/10 control). ▪ ↑ Incidence of skeletal retardations: Sternebra not ossified (38 foetuses/14 litters *vs* 16/9 control) and rudimentary sternebra (31 foetuses/12 litters *vs* 22/12 control). ▪ ↓ Number of foetal ossification centres on day 20 of gestation: Caudal vertebrae (10.83 *vs* 12.02 control) and sternebrae (5.63 *vs* 5.86 control). ▪ 2 dams (8%) which manifested clinical signs (nervous symptoms described above) aborted on days 20 and 28. No statistically significant compared with control group. **15 mg/kg bw/day** ▪ Malformations: Omphalocele (5 foetuses/3 litters *vs* 0/0 control). The incidence for omphalocele (3.9% incidence in foetuses and 16.7% in the litters) is out of the range of the mentioned above historical control data of the laboratory. ▪ ↑ Incidence of head (soft tissue) variations: Periorbital haemorrhage in eyes (20 foetuses/12 litters *vs* 8/7 control). ▪ ↑ Incidence of skeletal retardations: Not ossified sternebra (29 foetuses/15 litters *vs* 16/9 control) and rudimentary sternebra (35 foetuses/16 litters *vs* 22/12 control). ▪ ↓ Number of foetal ossification centres on day 20 of gestation: Sternebrae (5.76 *vs* 5.86 control). ▪ 1 dam aborted on day 29 (4%) in absence of maternal toxicity (no nervous clinical signs). No statistically significant compared with control group. **5 mg/kg bw/day** ▪ ↑ Incidence of skeletal retardations: Rudimentary sternebra (46 foetuses/19 litters *vs* 22/12 control). Non-dose dependent.

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The effects on fertility of 8-hydroxyquinoline have been investigated in a two-generation study in rat (*Fascineli, 2006b*; see Table 35 and Table 36).

Parental toxicity was manifested at dose of 3000 ppm and above by statistically significant reduction of food consumption, bodyweight and bodyweight gain in parents of both generations. Parental toxicity was more pronounced in females than in males. The absolute and relative weight of spleen (F0 males) was increased. A decreased was observed in the absolute weight of seminal vesicles (F1 males), prostate (F0 males), right adrenal (F1 males) and brain (F1 females). However, no histological lesions were found, except for an increased incidence of liver pigmentation that was observed in both parent generations. In addition, the average terminal bodyweight resulted to be significantly lower than control in both generations.

Additionally, at the top dose level of 8000 ppm other toxicity effects observed were, changes in the weight of some organs, such as a decrease of absolute and relative weight of adrenals (F0 females), kidneys (F0 females and F1 both sexes), ovaries (F0 and F1 females), prostate (F0 males) and liver (F1 males). The absolute weight of testis (F1 males), epididymides (F1 males), right adrenal (F1 both sexes), left adrenal (F1 males) and brain (F1 of both sexes) were also reduced.

The Parental NOAEL was 1000 ppm (95-119) mg/kg bw/day in males and females respectively) based on effects observed at 3000 ppm (decreased of food consumption, bodyweight, bodyweight gain, reduced terminal bodyweight at sacrifice and changes in the weight of some organs).

Pup toxicity was observed at dose of 3000 ppm by statistically significant reduction of the bodyweight on day 21 in male pups of the F1 and F2 litters, and in the interval 14-21 days of the lactation period in female pups of the F2 litters. From this dose level, the necropsy of F1 and F2 pups revealed a decrease of absolute weights of spleen (F1 and F2 female pups), thymus (F1 male pups) and brain (F2 pups of both sexes). A reduced terminal bodyweight and a delay in the time of eye opening were also observed in both sexes of the F2 litters at this dose level.

Additionally, at the dose of 8000 ppm reduced bodyweight was observed in both sexes of the F1 and F2 litters in the 7-21 days interval. At this dose level the absolute and relative weight of thymus and spleen was decreased (F1 and F2 pups of both sexes) and an increase of the relative weight and a decrease of the absolute weight of brain (F1 and F2 pups of both sexes) were observed. A reduced terminal bodyweight in F1 and F2 pups (both sexes) was also seen. In addition, delayed sexual maturity (preputial separation in males and vagina opening in females) in F1 was observed, in accordance with bodyweight depression and delay in the apparition of the incisor eruption observed in both sexes of the F2 litters.

The Offspring NOAEL was 1000 ppm (95-119 mg/kg bw/day in males and females respectively) based on effects observed at 3000 ppm (bodyweight decrease, changes in the weight of some organs and delay in the eyes opening).

Reproductive toxicity was manifested by a statistically significant decrease in the number of live born pups. The effect was observable at doses from 1000 ppm, in the F1 litters and at the dose of 8000 ppm in the F2 litters, although it is only out of the range of historical controls in F1 and F2 litters at 8000 ppm.

In addition, at 8000 ppm F1 females presented a statistically significant reduction of the number of oestrus cycles and an increased duration of the oestrus cycle, although it did not appear to have adverse effects in the mating or fertility indices. All other parameters, such as mating, fertility and pregnancy indices were not altered, including sperm parameters that remained similar to control values.

Besides, changes in the weight of some reproductive organs were observed, such as decreased weight of seminal vesicles (F1 males) and prostate (F0 males) at doses of 3000 ppm and above. At the top dose level of 8000 ppm the weight of ovaries (F0 and F1 females), testes (F1 males) and epididymides (F1 males) were also decreased.

The Reproductive NOAEL was 3000 ppm (274-345 mg/kg bw /day in males and females respectively) based on the alterations in the oestrus cycle, changes in reproductive organ weights and the reduced mean number of live born pups (statistically significant) observed at 8000 ppm.

Table 35: Findings in F0 adults and F1 pups in two-generation in rat (*Fascineli, 2006b*)

Parameter	Dose level (ppm)							
	Males				Females			
	0	**1000**	**3000**	**8000**	**0**	**1000**	**3000**	**8000**
PARENTS (F0)								
Pre-mating intake (mg/kg bw/day)	0	95	274	678	0	119	345	933
Bodyweight (g)								
Pre-mating (week 1)	282.27	288.92	287.19	271.77	193.23	192.54	188.46	**185.04* (-4.2%)**
Pre-mating (week 10)	396.12	396.12	402.77	**367.38* (-7.3%)**	240.04	235.65	**229.62* (-4.3%)**	**220.69* (-8.1%)**
Gestation Day 0					241.50	238.19	**229.09* (-5.1%)**	**220.19* (-8.8%)**
Gestation Day 21					359.88	361.85	**339.65* (-5.6%)**	**305.56* (-15.1%)**
Lactation Day 0					269.35	270.12	259.25	**233.12* (-13.45%)**
Lactation Day 7					293.69	291.96	**278.71* (-5.1%)**	**247.54* (-15.7%)**
Lactation Day 21					290.27	287.96	283.13	**242.65* (-16.4%)**
Bodyweight gain (g)								
Pre-mating	145.65	140.73	147.96	**107.04* (-26.3%)**	61.27	57.58	**54.15* (-11.6%)**	**44.77* (-26.9%)**
Gestation					118.38	123.65	**109.90* (-7.2%)**	**85.12* (-28.1%)**
Lactation					20.92	17.85	23.88	9.54 (54.4%)
Food consumption (g/animal/day)								
Pre-mating (week 1-2)	28.67	30.38	29.07	28.25	25.08	23.45	22.68	21.82
Pre-mating (week 5-6)	30.43	32.02	31.80	**27.01* (-11.2%)**	29.84	**26.15* (-12.4%)**	**24.84* (-16.8%)**	25.07
Pre-mating (week 7-8 males) (week 6-7 females)	31.12	33.31	31.38	**26.18* (-15.9%)**	29.59	26.55	**23.89* (-19.3%)**	**24.45* (-17.4%)**
Pre-mating (week 9-10)	29.88	31.85	29.34	**24.24* (-18.9%)**	25.95	24.79	23.08	21.94
Gestation (Day 3-6)					29.42	29.56	28.42	**26.74* (-9.1%)**
Gestation (Day 18-21)					32.09	32.59	31.49	29.06

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Lactation (Day 15-18)					99.10	93.26	**89.25* (-10%)**	88.40 (10.8%)
Lactation (Day 18-21)					111.27	112.42	104.33 (6.3%)	**93.82* (-15.7%)**
Fertility								
Mating index (%)	100	100	100	100	100	100	100	100
Fertility index (%)	100	100	92.3	100	100	100	92.3	100
Gestation index (%)					100	100	100	100
No. complete oestrous cycle					4.5	4.6	4.6	4.3
Length of oestrous cycle (days)					4.4	4.3	4.4	4.6
No. litters evaluated					26	26	24	26
No. live born								
Male pups					6.5	6.1	**5.4***	**5.3***
Female pups					5.9	4.9	5	**4.2***
Average litter					12.4	**11.0***	**10.5***	**9.5***
No. live born on postnatal day 4								
Male pups					6.5	6.1	**5.2***	**5.3***
Female pups					5.8	4.8	5	**4.2***
Average litter					12.3	**10.9***	**10.1***	**9.5***
Absolute (g) and relative (to brain) organ weights								
Prostate								
Absolute	0.44	0.38	**0.37* (-15.9%)**	**0.36* (-18.2%)**				
Relative	21.45	18.58	18.27 (-14.8%)	**18.04* (-15.9%)**				
Liver								
Absolute	12.04	12.83	**13.17* (+9.4%)**	12.80 (+6.3%)				
Relative	587.09	622.37	**650.94* (+10.9%)**	635.08 (+8.2%)				
Spleen								
Absolute	0.67	0.71	**0.77* (+14.9%)**	**0.81* (+20.9%)**				
Relative	32.59	34.23	**37.73* (+15.8%)**	**40.09* (+23.0%)**				
Right Ovary								
Absolute					0.06	0.06	0.05	**0.05* (-16.7%)**
Relative					3.00	2.96	2.76	**2.46* (-18%)**
Left Ovary								
Absolute					0.06	0.06	0.05	**0.05* (-16.7%)**
Relative					2.91	2.94	2.87	**2.47* (-15.1%)**
Right Kidney								
Absolute					0.95	0.94	0.93	**0.86* (-9.5%)**
Relative					50.66	49.59	49.58	**45.87* (-9.5%)**
Left Kidney								
Absolute					0.91	0.89	0.89	**0.83* (-8.8%)**
Relative					48.34	47.37	47.18	**44.06* (-8.9%)**

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Right Adrenal								
Absolute					0.040	0.038	0.038	**0.035* (-12.5%)**
Relative					2.13	2.02	2.0	**1.85* (-13.2%)**
Left Adrenal								
Absolute					0.042	0.041	0.040	**0.038* (-9.5%)**
Relative					2.20	2.16	2.11	**2.02* (-8.2%)**
Terminal Body weight (g) at sacrifice								
Terminal body weight	418.98	427.65	412.61	**368.86* (-12%)**	256.29	252.47	**242.96* (-5.2%)**	**226.13* (-11.8%)**
Pathology								
No. examined	26	26	26	26	26	26	26	26
Unilateral hydronephrosis	2	4	1	5	0	0	1	0
Bilateral hydronephrosis	0	1	0	0	0	0	1	1
Liver pigmentation	1	0	0	**11***	0	1	**5***	**8***
Prostatic atrophy	4	7	7	7				
Uterus pigmentation					10	12	**20***	8
LITTERS (F1)								
Bodyweight pup (g)								
Day 7	15.7	16.5	15.2	**13.6* (-13.4%)**	15.1	16.0	15.2	13.1* (-13.2%)
Day 14	30.9	30.2	28.8	**23.0* (-25.6%)**	29.8	29.4	28.8	22.4* (-24.8%)
Day 21	48.1	47.6	**44.4* (-7.7%)**	**31.2* (-35.1%)**	45.8	46.0	44.0	30.6* (-33.2%)
Physical evaluation (mean day apparition)								
Pinna unfolding	2.3	2.1	2.1	2.1	2.3	**2.1***	2.0	**2.1***
Fur	7.0	7.0	7.1	7.0	7.0	7.0	7.0	7.0
Incisor eruption	9.7	9.4	9.8	9.9	9.7	9.4	9.9	9.9
Opening of eyes	13.7	13.6	13.7	14.0	13.5	13.5	13.5	13.8
Sexual maturity (age in days) and bodyweight at sexual maturity (g)								
Preputial separation	42.3	41.8	43.6	**47.7***				
Vagina opening					33.5	33.2	33.8	**37.6***
Bodyweight	172.98	172.02	174.82	**174.24***	104.15	103.34	101.94	**89.18***
Reflexes (mean day apparition)								
Grip reflex	2.1	**2.0***	**2.1***	**2.0***	2.1	2.0	2.0	2.0
Postural reflex	2.0	**2.0***	2.1	2.0	2.0	2.0	2.1	2.0
Negative geotaxis	7.2	7.1	7.1	**7.0***	7.2	7.1	7.1	**7.0***
Absolute (g) and relative (to bodyweight) organ weights								
Brain								
Absolute	1.43	1.47	1.42	**1.33* (-7%)**	1.40	1.41	1.38	**1.29* (-7.9%)**
Relative	2.85	2.94	3.05	**3.89* (+36.5%)**	2.89	2.92	3.08	**3.96* (+37.0%)**
Thymus								
Absolute	0.23	0.22	**0.18* (-21.7%)**	**0.10* (-56.5%)**	0.22	0.22	0.20	**0.10* (-54.5%)**
Relative	0.45	0.43	0.39	**0.28* (-37.8%)**	0.44	0.44	0.44	**0.30* (-31.8%)**

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Spleen								
Absolute	0.19	0.19	0.16	**0.10* (-47.4%)**	0.19	0.18	**0.16* (-15.8%)**	**0.10* (-47.4%)**
Relative	0.38	0.37	0.35	**0.28 * (-26.3%)**	0.39	0.36	0.36	**0.30 * (-23.1%)**
Terminal bodyweight (g)								
Terminal bodyweight	51.09	50.63	47.32	**34.53* (-32.4%)**	48.96	49.11	45.30	**33.20* (-32.2%)**

**significantly different to controls (p<0.05)*

Table 36: Findings in F1 adults and F2 pups in two-generation in rat (*Fascineli,. 2006b*)

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
PARENTS (F1)								
Pre-mating intake (mg/kg bw/day)	0	102	291	769	0	122	346	855
Bodyweight (g)								
Pre-mating (week 1)	189.38	191.85	179.65	**139.54* (-26.3%)**	143.85	141.54	**135.65* (-5.7%)**	**114.12* (-20.7%)**
Pre-mating (week 10)	378.62	382.88	355.27	**272.46* (-28.4%)**	231.08	**224.27* (-3%)**	**216.27* (-6.4%)**	**187.04* (-19.1%)**
Pre-mating (week 11)	390.77	392.04	**259.73* (-33.5%)**	**278.12* (-28.8 %)**	232.62	227.62	**217.81* (-6.4%)**	**188.68* (-18.9%)**
Gestation Day 0					233.08	229.72	**217.0* (-6.9%)**	**186.0* (-20.2%)**
Gestation Day 21					347.32	347.08	**326.71* (-5.9%)**	**266.95* (-23.1%)**
Lactation Day 0					257.68	258.12	247.13	**206.05* (-20%)**
Lactation Day 14					293.12	292.35	**276.08* (-5.8%)**	**227.43* (-22.4%)**
Lactation Day 21					278.64	275.04	271.25	**229.71* (-17.6%)**
Bodyweight gain (g)								
Pre-mating	242.77	242.35	**221.58* (-8.7%)**	**174.35* (-28.2%)**	107.35	102.62	100.5	**93.96* (-12.5%)**
Gestation					114.24	117.36	109.71	**80.95* (-29.1%)**
Lactation					20.96	16.92	24.13	23.67
Food consumption (g/animal/day)								
Pre-mating (week 1-2)	24.40	25.96	23.95	**18.79* (-23%)**	22.08	**19.15* (-13.3%)**	**19.13* (-13.4%)**	**16.60* (-24.8%)**
Pre-mating (week 5-6 males) (week 4-5 females)	28.63	30.08	26.25	**18.56* (-35.2%)**	28.31	**23.85* (-15.8%)**	**21.60* (-23.8%)**	**16.69* (-41.0%)**
Pre-mating (week 9-10)	29.12	30.13	23.64	**17.76* (-39%)**	27.53	23.35	**20.97* (-23.8%)**	**14.82* (-46.2%)**
Gestation (Day 3-6)					33.93	32.17	**22.65* (-33.2%)**	**19.91* (-41.3%)**
Gestation (Day 18-21)					35.56	34.93	**26.0* (-26.9%)**	**18.25* (-48.7%)**
Lactation (Day 3-6)					53.51	55.65	50.10	**38.89* (-27.3%)**
Lactation (Day 18-21)					118.49	121.96	110.56	**89.51* (-24.5%)**
Fertility								
Mating index (%)	100	100	100	100	100	100	100	100
Fertility index (%)	96.2	100	92.3	88.0	96.2	100	92.3	88.0
Gestation index (%)					100	100	100	95.5

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
No. complete oestrous cycle					4.3	4.2	4.3	**3.5***
Length of oestrous cycle (days)					4.6	4.8	4.4	**5.3***
No. litters evaluated					25	26	24	21
No. live born								
Male pups					5.5	6.2	5.7	**4.2***
Female pups					5.6	5.5	4.6	**4.0***
Average litter					11.2	11.7	10.3	**8.5***
No. live born postnatal day 4								
Male pups					5.5	6.1	5.6	4.4
Female pups					5.5	5.5	4.5	**4.0***
Average litter					11.0	11.6	10.2	**8.4***
Absolute (g) and relative (to brain) organ weights								
Seminal vesicles								
Absolute	1.86	1.88	**1.47* (-21.0%)**	**1.50* (-19.4%)**				
Relative	92.13	92.57	**74.34* (-19.3%)**	82.29 (-10.7%)				
Right Epididymis								
Absolute	0.73	0.74	0.70	**0.65* (-11.0%)**				
Left Epididymis								
Absolute	0.72	0.72	0.71	**0.63* (-12.5%)**				
Right Testis								
Absolute	2.04	2.03	1.97	**1.84* (-9.8%)**				
Left Testis								
Absolute	2.04	2.02	1.97	**1.84* (-9.8%)**				
Liver								
Absolute	13.92	14.39	14.13	**11.24* (-19.3%)**				
Relative	689.71	709.42	709.97	**616.67* (-10.6%)**				
Right Kidney								
Absolute	1.43	1.40	1.34	**1.05* (-26.6%)**	0.98	0.93	0.92	**0.80* (-18.4%)**
Relative	70.88	69.18	67.43	**57.53* (-18.8%)**	52.05	50.40	50.07	**47.05* (-9.6%)**
Left Kidney								
Absolute	1.39	1.37	1.33	**1.04* (-25.2%)**	0.94	0.91	0.90	**0.78* (-17.0%)**
Relative	68.69	67.47	66.64	**57.24* (-16.7%)**	49.91	49.55	48.94	**45.61* (-8.6%)**
Right Adrenal								
Absolute	0.031	0.03	**0.028* (-9.7%)**	**0.025* (-19.4%)**	0.040	0.040	0.038	**0.036* (-10%)**
Left Adrenal								
Absolute	0.032	0.033	0.030	**0.026* (-18.8%)**	0.042	0.045	0.042	0.038 (-9.5%)
Brain								
Absolute	2.02	2.03	1.99	**1.86* (-7.9%)**	1.88	1.84	**1.83* (-2.7%)**	**1.73* (-8.0%)**

Parameter	Dose level (ppm)							
	Males				Females			
	0	1000	3000	8000	0	1000	3000	8000
Right Ovary								
Absolute					0.05	0.05	0.05	**0.03* (-40%)**
Relative					2.70	2.52	2.58	**1.75* (-35.2%)**
Left Ovary								
Absolute					0.05	0.05	0.05	**0.03* (-40%)**
Relative					2.54	2.57	2.51	**1.62* (-36.22%)**
Terminal bodyweight (g) at sacrifice								
Terminal bodyweight	424.01	423.76	**387.26* (-8.7%)**	**295.48* (-30.3%)**	248.23	**230.95* (-7%)**	**227.69* (-8.3%)**	**201.89* (-18.7%)**
Pathology								
No examined	26	26	26	26	26	26	26	26
Unilateral hydronephrosis	6	2	5	1	3	1	1	1
Liver pigmentation	0	0	0	8	0	0	2	5
Prostatic atrophy	5	6	9	2				
Uterus pigmentation					10	5	13	11
LITTERS (F2)								
Bodyweight pup (g)								
Day 7	16.9	16.4	16.6	**13.9* (-17.8%)**	16.4	16.0	15.9	**13.8* (-15.9%)**
Day 14	32.2	31.8	30.3	**22.3* (30.7%)**	31.7	31.1	**29.6* (-6.6%)**	**22.7* (-28.4%)**
Day 21	47.9	48.9	**46.0* (-4.0%)**	**32.7* (-31.7%)**	46.5	47.4	**43.8* (-5.8%)**	**33.4* (-28.2%)**
Physical evaluation (mean day apparition)								
Pinna unfolding	2.0	2.1	2.0	2.2	2.0	2.1	2.0	2.1
Fur	7.0	7.0	7.0	7.1	7.0	7.0	7.0	7.1
Incisor eruption	9.6	9.4	9.8	**10.3***	9.7	9.3	10.0	**10.3***
Opening of eyes	13.8	13.9	**14.1***	**14.5***	13.7	13.7	**14.1***	**14.2***
Reflexes (mean day apparition)								
Grip reflex	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Postural reflex	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Negative geotaxis	7.1	7.1	7.2	7.1	7.1	7.2	7.2	7.1
Absolute (g) and relative (to bodyweight) organ weights								
Brain								
Absolute	1.51	1.48	**1.45* (-4.0%)**	**1.38* (-8.6%)**	1.46	1.43	**1.39* (-4.8%)**	**1.37* (-6.2%)**
Relative	3.15	3.05	3.29	**4.31* (+36.8%)**	3.15	3.10	3.22	**4.10* (+30.2%)**
Thymus								
Absolute	0.21	0.22	0.19	**0.12* (-42.8%)**	0.21	0.23	0.20	**0.14* (-33.3%)**
Relative	0.43	0.46	0.42	**0.37* (-14.0%)**	0.46	0.49	0.46	**0.41* (-10.9%)**
Spleen								
Absolute	0.19	0.21	0.18	**0.10* (-47.4%)**	0.19	0.19	**0.17* (-10.5%)**	**0.11* (-42.1%)**
Relative	0.40	0.42	0.40	**0.30* (25%)**	0.41	0.42	0.38	**0.31* (-24.4%)**
Terminal bodyweight	48.12	48.88	**44.81* (-6.9%)**	**32.53* (-32.4%)**	46.33	46.49	**43.87* (-5.3%)**	**34.10* (-26.4%)**

**significantly different to controls ($p<0.05$)*

4.11.1.2 Human information

No information available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The developmental toxicity of 8-hydroxyquinoline has been investigated in rats and rabbits.

Developmental rat study (*Fascineli, 2006c*; see Table 37)

The dams showed marked maternal toxicity at doses of 300 mg/kg bw/day and above, manifested by a decrease of the bodyweight, bodyweight gain and food consumption, and clinical signs (all females suffered 10 minutes of nervous system excitation after dosing followed by 20 minutes of lethargy). At the lower dose of 100 mg/kg bw/day, dams only showed a statistically significant decrease of the maternal corrected bodyweight gain (10.3%). The LOAEL for maternal toxicity was 100 mg/kg bw/day based on decrease of the maternal corrected bodyweight gain at this dose level.

Foetal toxicity was observed at dose of 100 mg/kg bw/day and above. The lowest dose of 100 mg/kg bw/day produced a statistically significant reduction in the number of ossification centres and an increase in skeletal retardations (not ossified and rudimentary sternebrae). The placenta weight was markedly depressed from this dose level. At 300 mg/kg bw/day and above, the mean foetal bodyweight and the number of ossification centres for sternebra were decreased. From this dose level there was a statistically significant increase in visceral variations (nasal cavity enlargement) and skeletal variations (full supernumerary ribs). At 600 mg/kg bw/day, 8-hidroxyquinoline also produced a statistically significant increase in the incidence of visceral variation (kidney hydronephrosis) and a statistically significant increase in skeletal variations (short supernumerary ribs).

The LOAEL for developmental toxicity was 100 mg/kg bw/day based on a statistically significant reduction in the number of ossification centres on day 20 of gestation, an increase in skeletal retardations (not ossified and rudimentary sternebrae) and reduced placental weight at this dose level.

Table 37: Findings in the developmental rat study (*Fascineli, 2006c*)

Parameter	Dose level (mg/kg bw/day)			
	0	**100**	**300**	**600**
MATERNAL TOXICITY				
Effects				
Nervous system excitation 10 min after dosing followed by 20 min of lethargy No. dams affected/examined	0/25	0/25	**25***/25 **(100%)**	**25***/25 **(100%)**
Bodyweight (g)				
Day 12	238.35	234.76	229.87	**222.18*** **(-6.8%)**
Day 20	310.39	303.4	**294.74*** **(-5.0%)**	**277.05*** **(-10.7%)**
Bodyweight gain (g)				
Day 6-20	109.64	106.92	**94.09** **(-14.2%)**	**73.72*** **(-32.7%)**
Food consumption (g/kg bw/day)				
Day 6-20	112.96	107.97	**99.58*** **(-11.8%)**	**87.55*** **(-22.5%)**

Parameter	Dose level (mg/kg bw/day)			
	0	**100**	**300**	**600**
Corrected maternal body weight gain (g)[1]				
Corrected maternal bw gain (% decrease)	35.19	**31.57*** **(-10.3%)**	**20.29*** **(-42.3%)**	**1.45*** **(-95.9%)**
Carcass (% decrease)	284.24	239.57	**233.81*** **(-17.7%)**	**215.72*** **(-24.1%)**
Necropsy				
Hydronephrosis right kidney No. dams affected/ examined	0/25	0/25	1/25 (4%)	0/25
REPRODUCTIVES INDICES				
Corpora lutea/dams	11.87±2.42	12.88±2.30	12.61±1.44	**14.77±2.09***
No. of litters	10.57±2.63	11.28±2.79	11.04±1.77	**12.36±1.92***
No. live born pups /litter	10.26±2.47	11.04±2.76	10.78±1.78	**12.18±1.87***
FOETAL TOXICITY				
Mean placental weight [g]	0.508	0.482	**0.477*** **(-6.1%)**	**0.415*** **(-18.3%)**
Male	0.521	**0.487*** **(-6.5%)**	**0.491*** **(-5.8%)**	**0.424*** **(-18.6%)**
Females	0.509	**0.474*** **(-6.8%)**	**0.462*** **(-9.2%)**	**0.405*** **(-20.4%)**
Mean foetal body weight [g]	3.996	3.879	**3.765*** **(-5.8%)**	**3.414*** **(-14.6%)**
Male	4.089	3.971	**3.850*** **(-5.8%)**	**3.496*** **(-14.5%)**
Females	3.914	3.781	**3.673*** **(-6.2%)**	**3.343*** **(-14.6%)**
No. of foetuses examined/ litters examined				
Visceral variations	112/23	131/25	116/23	127/22
Skeletal variations Skeletal retardations Ossification centres	124/23	145/25	132/23	141/22
No. of foetuses/ litters affected				
Visceral variations				
Nasal cavity enlarged	5/4 (4.5%/17.3%)	10/5 (7.6%/20.0%)	**14***/7 (**12.1%**/30.4)	**16*/11*** **(12.6%/50.0%)**
Unilateral hydronephrosis kidney	28/13 (25.0%/56.5%)	24/14 (18.3%/56.0%)	34/19 (29.3%/82.6%)	**47*/21*** **(37.0%/95.5%)**
Skeletal variations				
Short supernumerary rib	25/16 (20.2%/69.6%)	38/18 (26.2%/72.0%)	25/14 (18.9%/60.9%)	**53*/21*** **(37.6%/95.5%)**
Full supernumerary rib	8/7 (6.5%/30.4%)	6/5 (4.1%/20.0%)	**23*/15*** (**17.4%/65.2**)	**24*/15*** **(17.0%/68.2%)**
Skeletal retardations				
Total retardations	23/15 (18.5%/65.2%)	**47***/20 (**32.4%**/80.0%)	**75*/21*** **(56.8%/91.3%)**	**88*/22*** **(62.4%/100%)**
Sternebra not ossified	2/2 (1.6%/8.7%)	**11***/7 (**7.6%**/28.0%)	**19*/11*** (**14.4%/47.8**%)	**49*/20*** **(34.8%/90.9%)**
Sternebra rudimentary	17/12 (13.7%/52.2%)	**37***/18 (**25.5%**/72.0%)	**50*/19*** **(37.9%/82.6**%)	**44*/18*** **(31.2%/81.8%)**
Number of foetal ossification centres on day 20 of gestation				
Anterior phalanges	2.91	**1.84***	**1.24***	**0.77***
Metacarpals	7.89	**7.66***	**7.43***	**6.83***
Caudal vertebrae	2.63	**2.29***	**2.08***	**1.85***
Sternebrae	5.98	5.92	**5.82***	**5.45***

** Significantly different from the control values, $p < 0.05$*

[1]*Corrected maternal body weight gain is the carcass body weight minus the body weight on day 6*

Developmental rabbit study (*Fascineli, 2006d;* see Table 38 and Table 39).

In this study some females (16% and 44% at the doses of 15 and 60 mg/kg bw/day, respectively) suffered signs of nervous system excitation followed by lethargy occurred after test item administration without a specific trend in the beginning or duration of the effects. In addition, 1 dam on day 29 at 15 mg/kg bw/day and 2 more dams on days 20 and 28 respectively at 60 mg/kg bw/day aborted. Dam which aborted at 15 mg/kg bw/day didn't manifest any clinical signs, whereas both dams which aborted at the top dose level showed maternal toxicity (nervous symptoms). There is not available information in the study about aborted foetus. The administration of 8-hydroxyquinoline did not alter food consumption, bodyweight and bodyweight gain of female rabbits. The necropsy of the dams revealed no macroscopic or histopathological findings.

The NOAEL for maternal toxicity was 5 mg/kg bw/day based on clinical signs observed at 15 mg/kg bw/day.

Foetal toxicity was observed at dose of 15 mg/kg bw/day and above. A statistically significant increase in the incidence of an external malformation called omphalocele (abdominal wall defect) was observed, very rare in this laboratory historical control data (incidence of 0.8% [0-1.8%] for foetuses and 4% [0-8.3%] for litters). At doses of 15 and 60 mg/kg bw/day, 5 foetuses of 3 litters and 5 foetuses of 4 litters were affected, respectively. The incidence for omphalocele was 3.9% in foetuses and 16.7% in litters at 15 mg/kg bw/day, and 4.3% in foetuses and 23.5% in litters at 60 mg/kg bw/day. At both doses, the occurrence was out of the range of the historical control data and the mechanism of action was not clarified. Besides, omphalocele occurred in absence of maternal toxicity at 15 mg/kg bw/day and in presence of slight maternal toxicity at the top dose level as it can be observed in the following table:

Table 38: Individual foetal omphalocele data versus individual maternal toxicity data

Dose: 15 mg/kg bw/day			**Dose: 60 mg/kg bw/day**		
Dam number	**Fetus number**	**Clinical signs (nervous symptoms)**	**Dam number**	**Fetus number**	**Clinical signs (nervous symptoms)**
63	2	N	83	2	Y
65	1	N	83	8	Y
65	8	N	86	5	Y
74	2	N	93	4	Y
74	8	N	96	2	N

Other adverse effects in foetal development, such as soft tissue variation (periorbital haemorrhage), skeletal retardations (not ossified sternebrae and rudimentary sternebrae), reduction in the number of ossification centres in sternebrae and one abortion were observed from 15 mg/kg bw/day. At the highest dose of 60 mg/kg bw/day, there was also an increase in the foetal incidence of retina fold apparition, reduction in the number of live born pups only statistically significant in females, reduced number of foetal ossification centres in caudal vertebrae and two abortions in presence of slight maternal toxicity.

The NOAEL for developmental toxicity was 5 mg/kg bw/day based on a statistically significant increase in the incidence of foetuses with omphalocele (external malformation), periorbital haemorrhage (soft tissue variation), skeletal retardations (not ossified and rudimentary sternebrae), reduced number of ossification centres in sternebrae and one abortion observed at 15 mg/kg bw/day.

Table 39: Findings in the developmental rabbit study (*Fascineli, 2006d*)

Parameter	Dose level (mg/kg bw/day)			
	0	**5**	**15**	**60**
MATERNAL TOXICITY				
Effects				
Signs of nervous system excitation followed by lethargy occurred after test item administration without a specific trend in the beginning or duration of the effects No. dams affected/examined	0/25	0/25	4 /25 (16%)	**11***/25 **(44%)**
REPRODUCTIVE INDICES				
No. live born pups/litter	7.3±2	7.50±2.15	6.35±3.34	5.80±3.32
Mean males	3.15	3.58	3.30	2.90
Mean females	4.15	3.92	3.05	**2.90***
% pre-implantation loss	15.81±19.74	15.04±15.42	26.98±26.21	**32.33±22.66***
FOETAL TOXICITY				
Abortion No. dams affected/examined	0/25	0/25	1/25 (4%)	2/25 (8%)
No. of foetuse examined/litters examined				
External malformations	146/19	180/24	127/18	116/17
Visceral variations	146/19	180/24	127/18	116/17
Skeletal retardations	146/19	180/24	127/18	116/17
Foetal ossification centres	146/19	180/24	127/18	116/17
No. of foetuses/ litters affected				
External malformations				
Omphalocele	0/0	0/0	**5***/3 (**3.9%**/16.7%)	**5*/4*** **(4.3%/23.5%)**
Visceral variations				
Periorbital hemorrhage (eyes)	8/7 (11.4%/36/8%)	12/9 (14.3%/37.5%)	**20***/12 (**32.3%**/66.7%)	**18***/11 (**34.0%**/64.7%)
Retinal fold	13/10 (18.6%/52.6%)	19/16 (22.6%/66.7%)	20/14 (32.3%/77.8%)	**19***/14 (**35.8%**/82.4%)
Skeletal retardations (No. of foetuses /litters affected)				
Total retardations	51/19 (34.9%/100%)	**85***/23 (**47.2%**/95.8%)	**74***/18 **(58.3%**/100%)	**76***/16 **(65.5%**/94.1%)
Sternebra not ossified	16/9 (11.0%/47.4%)	27/17 (15.0%/70.8%)	**29*/15*** **(22.8%/83.3%)**	**38*/14*** **(32.8%/82.4%)**
Sternebra rudimentary	22/12 (15.1%/63.2%)	**46***/19 (**25.6%**/79.2%)	**35***/16 (**27.6%**/88.9%)	**31***/12 (**26.7%**/70.6%)
Number of foetal ossification centres on day 20 of gestation				
Caudal vertebrae	12.02	11.67	11.63	**10.83***
Sternebrae	5.86	5.85	**5.76***	**5.63***

** Significantly different from the control values, p < 0.05*

4.11.2.1 Human information

No information available

4.11.3 Other relevant information

No data available.

4.11.4 Summary and discussion of reproductive toxicity

Fertility

In the 2-generation study in rats, 8-hydroxyquinoline at the highest dose of 8000 ppm caused a statistically significant reduction in the number of oestrus cycles and an increase in the duration of the oestrus cycle in F1 generation. Besides, changes in the weight of some reproductive organs were observed, such as decreased weight of seminal vesicles (F1 males) and prostate (F0 males) at doses of 3000 ppm and above. At the top dose level of 8000 ppm the weight of ovaries (F0 and F1 females), testes (F1 males) and epididymides (F1 males) were also decreased. However none of these findings had impact in the fertility indices and can be attributed to maternal toxicity clearly manifested in a reduction in body weights. Besides, a statistically significant decrease in the number of live born pups was manifested at dose of 8000 ppm in F1/F2 litters although it was only slightly out of the range of the historical controls values and in presence of maternal toxicity

Development

8-hydroxyquinoline resulted to be teratogenic in the rabbit (*Fascineli, 2006d*) based on the increased incidence of an external malformation (omphalocele) observed from the dose of 15 mg/kg bw/day. This is a rare malformation with an incidence out of the range of the historical control data, with a mechanism of action not clarified and at dose of 15 mg/kg bw/day it occurred in absence of maternal toxicity. At the top dose level, 4 out of those 5 foetuses which suffered omphalocele did it in presence of clinical signs (nervous symptoms). However, a direct consequence of the 8-hydroxyquinoline action cannot be ruled out taking into consideration data at 15 mg/kg bw/day.

Other adverse effects in rabbit, observed from 15 mg/kg bw/day, were soft tissue variation (periorbital hemorrhage), skeletal retardations (not ossified sternebrae and rudimentary sternebrae) and reduced number of sternebrae ossification centres. One dam aborted on day 29 at this dose level. This dam didn't manifested clinical signs. Besides, at the highest dose of 60 mg/kg bw/day, there was an increase in the foetal incidence of retina fold apparition, reduction in the number of caudal vertebrae ossification centres and two dams with clinical signs aborted. At dose of 60 mg/kg bw/day the number of live born female pups was also reduced. However, this effect can be due to the statistically significant increase of the pre-implantation losses at this dose level.

Maternal toxicity in rabbits was manifested at 15 mg /kg bw/day (16% of the dams) and at 60 mg/kg bw/day (44% of the dams) by nervous system excitation followed by lethargy after test item administration without a specific trend in the beginning or duration of the effects.

In the developmental rat study (*Fascineli, 2006c)* at dose of 100 mg/kg bw/day and above, a decrease in the placental weight, a statistically significant reduction in the number of ossification centres and an increase in skeletal retardations (not ossified and rudimentary sternebrae) were observed. From the dose of 300 mg/kg bw/day onward, there was also a decrease in the mean foetal weight, a statistically significant increase in the visceral variations (nasal cavity enlargement), reduction in the number of sternebra ossification centres and increase of skeletal variations (full supernumerary ribs). In addition, at the dose of 600 mg/kg bw/day 8-hidroxyquinoline produced a statistically significant increase in the incidence of visceral variations (kidney hydronephrosis) and skeletal variations (short supernumerary ribs). These variations were seen in the presence of maternal toxicity, manifested by a decrease of the bodyweight, bodyweight gain, food consumption and nervous symptoms from the dose of 300 mg/kg bw/day onward, and a decrease of the maternal corrected bodyweight at dose of 100 mg/kg bw/day and above.

Other effects indicating developmental toxicity were observed in offspring of a 2-generation study in rat. Toxicity was manifested by a statistically significant decrease in the number of live born pups at the dose of 8000 ppm in F1/F2 litters slightly out of the range of historical controls. In addition, a delayed sexual maturity (preputial separation in males and vagina opening in females) in F1 pups at 8000 ppm, delay on the time apparition of the incisor eruption at 8000 ppm in F2 pups and a delay in the time of eye opening from the dose of 3000 ppm in F2 pups were observed. These findings occurred in presence of maternal toxicity manifested by decrease of food consumption, bodyweight and bodyweight gain, reduced terminal bodyweight at sacrifice and changes in the weight of some organs from the dose of 3000 ppm.

4.11.5 Comparison with criteria

According to the classification criteria in section 3.7.2., Annex I CLP: "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or 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 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) or from animal data (Category 1B).*

The classification of a substance in this Category 1A is largely based on evidence from humans.

The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

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, or on development, and where 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".

Comparison with classification criteria for reproductive toxicity (sexual function and fertility)

According to CLP Regulation (section 3.7.1.3 of Annex I), *any effect of substances that has the potential to interfere with sexual function and fertility has to be regarded for a classification for reproductive toxicity. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.*

Effects on fertility seen in the 2-generation study in rats at the top dose level (8000 ppm) were:

- Reduction in the number and increase in the duration of the oestrus cycles in F1 generation.
- Changes of the weight of some reproductive organs (prostate, seminal vesicles, epididymides, ovaries and testes).
- Decrease in the number of live born pups at the top dose level in F1/F2 generations, only slightly out of the range of historical controls.

However, these data don't warrant classification for fertility for the following reasons:

- Oestrus cycle changes were only observed in F1 generation.
- Changes in the weight of some reproductive organs (prostate, seminal vesicles, right epididymis and left ovary) were not accompanied of histopathological effects.
- All other fertility parameters, such as mating, fertility and pregnancy indices, were not altered by the administration of 8-hydroxyquinoline, including sperm parameters. Therefore, 8-hydroxyquinoline hasn't the capacity to interfere with reproduction.
- At this dose level (8000 ppm) there were clear signs of maternal toxicity manifested by significant decreases of bodyweight, bodyweight gain, food consumption and changes in the weight of organs. Therefore, these fertility effects are likely to be a secondary non-specific consequence of general toxicity and not a direct consequence of administration of 8-hydroxyquinoline.

Overall, the results show that 8-hydroxyquinoline does not affect fertility or reproductive performance. No effects providing sufficient evidence to cause a strong suspicion of impaired fertility were observed in the absence of marked parental toxicity.

The MSCA concludes that based on data available, comparing these data with the relevant CLP classification criteria, there is no sufficient and convincing evidence for classifying 8-hydroxyquinoline for its effects on fertility.

Comparison with classification criteria for reproductive toxicity (development)

8-hydroxyquinoline is considered teratogenic mainly based on the occurrence of an external malformation (omphalocele) in the rabbit developmetal study, in some individual cases in absence of maternal toxicity. This is a rare malformation with an incidence out of the range of historical control values and with a mechanism of action not clarified. Besides, in rabbit there were also several cases of soft tissue variations (periorbital haemorrhage and retinal fold), skeletal retardations (not ossified and rudimentary sternebrae), reduction in the number of ossification centres and abortions. These effects were observed in the presence of slight maternal toxicity manifested by nervous system excitation followed by lethargy occurred after administration of the test compound.

In developmental and 2-generation rat studies, findings related to developmental toxicity were also seen in presence of maternal toxicity and can be considered as a consequence of it. However, they can be regarded as a supporting evidence of developmental toxicity.

In section 3.7.2.4.2 of Annex I to CLP Regulation it is stated that "*developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations embryo/foetal lethality, significant post-natal functional deficiencies*".

Besides in section 3.7.2.4.3 of Annex I to CLP Regulation it is stated that "*Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects"*.

According to the classification criteria (3.7.2.4.3, Annex I CLP), MSCA is of opinion that the adverse effects in rabbits could not completely be attributed to maternal toxicity. 8-hydroxyquinoline did not cause severe disturbance or general health conditions of treated dams and the level of maternal toxicity was not sufficiently severe to explain the effects observed. Besides, all cases of omphalocele in rabbit at the mid dose level occurred in absence of maternal toxicity. Therefore, it is no reasonable to assume that development toxicity is produced solely as a secondary consequence of maternal toxicity and so to discount the developmental changes.

However, even if a casual relationship were established between developmental and maternal toxicity and the effects on the offspring could be proved to be secondary to maternal toxicity, they are still relevant for developmental classification, considering the severity of some effects observed in the developmental study in rabbit (omphalocele malformation). Therefore, the available data evaluated shows that there is reasonable evidence that 8-hydroxyquinoline can impair foetal development.

As no evidence from humans is available a classification into category 1A is not considered. The incidence of omphalocele in rabbit at the mid dose level in absence of maternal toxicity raises a discussion on what category, 1B or 2, is more suitable for classification. After a detailed review of all available data, the MSCA is of the opinion that category 2 is more appropriate since this adverse effect was not observed in rat studies and the other adverse effects were seen at dose levels where maternal toxicity also occurred.

Therefore, taking into account the severe effects observed in the rabbit study and the supporting data in rat studies, the MSCA considers that 8-hydroxyquinoline should be classified according to CLP as **Repr. Cat. 2, H361d "Suspected of damaging the unborn child".**

4.11.6 Conclusions on classification and labelling

Fertility

CLP: A classification is not required

Development

CLP: Repr. 2 (H361d: *Suspected of damaging the unborn child*)

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal
In the CLH report, the effects were summarised as follows:
Fertility
In the 2-generation study in rats, 8-hydroxyquinoline at the highest dose of 8000 ppm caused a statistically significant reduction in the number of oestrus cycles and an

increase in the duration of the oestrus cycle in the F1 generation. In addition, changes in the weight of some reproductive organs were observed, such as decreased weight of seminal vesicles (F1 males) and the prostate (F0 males) at doses of 3000 ppm and above. At the top dose level of 8000 ppm the weight of ovaries (F0 and F1 females), testes (F1 males) and epididymides (F1 males) were also decreased. However, none of these findings had an impact on the fertility indices and can be attributed to maternal toxicity, which was clearly manifested as a reduction in body weights. Besides, a statistically significant decrease in the number of live born pups was manifested at 8000 ppm in F1/F2 litters, but it was only slightly outside the range of the historical control values and occurred in the presence of maternal toxicity.

Developmental toxicity

Two developmental toxicity studies with 8-hydroxyquinoline were documented.

The DS concluded that 8-hydroxyquinoline is teratogenic in the rabbit (Fascineli, 2006d) based on the increased incidence of an external malformation (omphalocele) observed at doses $\geq$ 15 mg/kg bw/d. This is a rare malformation with an incidence outside the range of the historical control data, with a mechanism of action not clarified and at 15 mg/kg bw/d it occurred in the absence of maternal toxicity. At the highest dose, in 4 out of 5 foetuses in which omphalocele was observed, it occurred in the presence of clinical signs (nervous symptoms). However, a direct consequence of the 8-hydroxyquinoline exposure cannot be ruled out, taking into consideration the data at 15 mg/kg bw/d.

Other adverse effects in the rabbit, observed from 15 mg/kg bw/d, were soft tissue variations (periorbital haemorrhage), skeletal retardations (not ossified sternebrae and rudimentary sternebrae) and reduced number of sternebrae ossification centres. One dam aborted on day 29 at this dose level. This dam didn't manifest clinical signs. In addition, at the highest dose of 60 mg/kg bw/d, there was an increase in the foetal incidence of retina fold apparition, reduction in the number of caudal vertebrae ossification centres and two dams with clinical signs aborted. At 60 mg/kg bw/d the number of live born female pups was also reduced. However, this effect can be due to the statistically significant increase in the pre-implantation losses seen at this dose level.

Maternal toxicity in rabbits was manifested at 15 mg /kg bw/d (16% of the dams) and at 60 mg/kg bw/d (44% of the dams) by nervous system excitation followed by lethargy after test item administration without a specific trend in the beginning or duration of the effects. However, when individual data for offspring is correlated with their parents, the teratogenic effects were observed in all animals without maternal toxicity.

In the developmental rat study (Fascineli, 2006c) at doses of 100 mg/kg bw/d and above, a decrease in the placental weight, a statistically significant reduction in the number of ossification centres and an increase in skeletal retardations (not ossified and rudimentary sternebrae) were observed. From the dose of 300 mg/kg bw/d onward, there was also a decrease in the mean foetal weight, a statistically significant increase in the visceral variations (nasal cavity enlargement), reduction in the number of sternebra ossification centres and increase of skeletal variations (full supernumerary ribs). In addition, at the dose of 600 mg/kg bw/d 8-hydroxyquinoline produced a statistically significant increase in the incidence of visceral variations (kidney hydronephrosis) and skeletal variations (short supernumerary ribs). These variations were seen in the presence of maternal toxicity, manifested by a decrease of the body weight, body weight gain, food consumption and nervous symptoms from 300 mg/kg bw/d onward, and a decrease of the maternal corrected body weight at doses of 100 mg/kg bw/d and above.

In conclusion, the DS is of opinion that the adverse effects in rabbits could not completely be attributed to maternal toxicity. 8-Hydroxyquinoline did not cause severe disturbances in the general health conditions of treated dams and the level of maternal toxicity was not sufficiently severe to explain the effects observed. Besides, all cases of omphalocele in rabbit at the mid dose level occurred in absence of maternal toxicity. Therefore, it is not reasonable to assume that development toxicity is produced solely as a secondary consequence of maternal toxicity and to dismiss the developmental changes. However, even if a causal relationship were established between developmental and

maternal toxicity and the effects on the offspring could be proven to be secondary to maternal toxicity, they are still relevant for developmental classification, considering the severity of some effects observed in the developmental study in rabbit (the omphalocele malformation). Therefore, the available data evaluated showed that there is reasonable evidence that 8-hydroxyquinoline can impair foetal development.

As no evidence from humans was available, classification as Repr. 1A is not considered. The incidence of omphalocele in rabbit at the mid-dose, in the absence of maternal toxicity, raises the issue of whether Repr. 1B or Repr. 2 is the more suitable classification. After a detailed review of all available data, the DS original opinion was that Repr. 2 is more appropriate since this adverse effect was not observed in rat studies and the other adverse effects were seen at dose levels where maternal toxicity also occurred.

After public consultation, the DS took the arguments from one MSCA into account and re-assessed the data. The revised position of the DS was to support classification as Repr. 1B – H360D (May damage the unborn child).

Comments received during public consultation

Fertility
Two MSCAs agreed with no classification for fertility and lactation.

Developmental toxicity
One MSCA mentioned that the low live birth rate in the 2-generation study (significant, dose related and outside historical control incidence, both generations) may be considered a developmental effect supporting the classification as Category 1B. In their response the DS referred to the general toxicity observed at 3000 and 8000 ppm in this study (for details, see above) and to the fact that these dose levels are above the LD_{50} obtained in rats.
This MSCA disagreed with the proposed reproductive toxicity classification (Cat. 2) as proposed by the DS because the teratogenic effects in rabbits (increase in omphalocele, a rare malformation) at 15 mg/kg bw/d were seen in the presence of maternal toxicity at this dose level (16% of dams showed nervous system symptoms including excitation followed by lethargy at 15 mg/kg bw/d). Other developmental anomalies were also reported in a developmental and 2-generation rat study in the presence of maternal toxicity. In their opinion, classification as Repr. 1B should be considered because the teratogenic effects at 15 mg/kg bw/d were observed in specific animals in which the maternal toxicity was absent.
The general agreement of another MSCA is interpreted as agreement with the proposed (original) classification as Repr. 2 (H361d).
One Industry organisation disagreed with the proposed classification. They considered that there was no justification for the (originally) proposed classification for developmental toxicity, as the findings in the rabbit developmental study (Fascineli, 2006) were considered as not relevant to humans.

Assessment and comparison with the classification criteria

Fertility
In order to conclude on whether a classification is warranted or not, the comparison with the criteria as proposed by the DS was considered and additional information and arguments were added by RAC:
Effects on fertility seen in the 2-generation study in rats at the top dose level (8000 ppm) were:

- Significant reduction in the number of complete oestrus cycles (3.5 vs. 4.3 in control females) and increase in the duration of the oestrus cycles (5.3 d vs. 4.6 d in control females) in the F1 generation (8000 ppm = 855 mg/kg bw/d).
- Changes in the weight of some reproductive organs (prostate, seminal vesicles, epididymides, ovaries and testes).

- Decrease in the number of live born pups at the high dose level in F1/F2 generations, only slightly outside the range of historical controls.

However, these data don't warrant classification for fertility for the following reasons:

- Oestrus cycle changes were only observed in the F1 generation.

- Changes in the weight of some reproductive organs (prostate, seminal vesicles, right epididymis and left ovary) were not accompanied by histopathological effects.
  Decreases in absolute/relative seminal weights were also seen at 3000 ppm (291 mg/kg bw/d), but these were not clearly related to the dose. For example, relative weights of seminal vesicle were -19.3% at 3000 ppm and -10.7% at 8000 ppm in F1 males. Significant reductions in testis and epididymis weight (absolute and relative) corresponded to lower food consumption and body weight in F1 males during the premating treatment. Dose-dependent lower prostate weight was observed in F0 males at 3000 and 8000 ppm, while body weight and food consumption was lower than in controls at 8000 ppm. No data were reported on testis weight in F0 males.

- No other fertility parameters, such as mating, fertility and pregnancy indices, were altered by the administration of 8-hydroxyquinoline, including sperm parameters. Therefore, 8-hydroxyquinoline wasn't considered to interfere with reproduction.

- At this dose level (8000 ppm) there were clear signs of maternal toxicity in F1 females manifested by significant decreases of body weight (-20.7% -19.1%, -18.9% at premating week 1, 10, 11 in F1 female parents, respectively), body weight gain, food consumption (-24.8%, -41%, -46% at premating weeks 1-2, 4-5, 9-10, respectively) and changes in organ weights. A dose-related reduction in food consumption was observed in all three F1 female dose groups during the premating period. At 8000 ppm food consumption remained reduced during gestation (-41.3% on GD 3-6 and -48.7% on GD 18-21) and lactation (-27.3% on LD 3-6) and -24.5% on LD 18-21). The same is true for the body weight and the lower increase in body weight could be interpreted as being related to the low food consumption.
- Oestrus cycle changes were not observed in the F0 females. Body weight and food consumption were also lowered in F0 females, but were less severe.
- The DS indicated that pup viability was reduced at 8000 ppm in F1/F2, however data show that a dose-related lower pup viability compared to the control levels was seen on day 0 and day 4 in F1 pups in all three dose groups (12.4%, 11.0%, 10.5%, 9.5% for control, 1000, 3000, 8000 ppmrespectively, at day 0 in F1). Pup survival was only affected at the high dose in the F2 generation (11.2% in controls vs. 8.5% at 8000 ppm). It was stated that the values were slightly outside the controls, but no data were given on the laboratory's historical controls for the rat strain in the report. Irrespective of the lack of historical control data, the dose-relationship of the reductions strongly supports that the effect was treatment related. Lower pup survival could be linked to significantly lower body weight and food consumption in dams for the 3000 and 8000 ppm groups in the F0/F1 generation and for the 8000 ppm groups in the F1/F2 generation. The only inconclusive observation is that pup viability was significantly lower in F1 compared to the control level, but was not accompanied by an effect on the body weight in F0 females at 1000 ppm.
- The pup growth of survivors was significantly lower from day 7 to day 21 at 8000 ppm in F1 males and females compared to control values. This could be related to the general health conditions (due to lower food consumption and body weight

gain) and/or lactation, but no clear evidence for lactational effects can be drawn from these observations.

- Therefore, these fertility effects are likely to be a secondary non-specific consequence of general toxicity and not a direct consequence of administration of 8-hydroxyquinoline.

RAC shares the view of the DS that the results show that 8-hydroxyquinoline does not affect fertility or reproductive performance. No effects providing sufficient evidence to cause a strong suspicion of impaired fertility were observed in the absence of marked parental toxicity in the available 2-generation study.

RAC notes that no concerns for fertility-related abnormalities were raised by the repeated dose toxicity studies.

The lower pup survival in treated F1 and F2 pups which was not linked to maternal toxicity in the low dose F1 group, should be considered for developmental toxicity.

RAC concludes, in agreement with the DS proposal, that no classification is warranted for fertility.

Developmental toxicity

RAC agrees with the DS that 8-hydroxyquinoline is teratogenic and toxic to the developing rabbits.

The treatment related effects in the rabbit study (Fascineli, 2006d) are relevant for the conclusion. The most critical effect (see Table 39 CLH report) that warrants classification as Repr. 1B (H360D) was:

- Omphalocele in 5 foetuses in 3 litters (3.9%/16.7%) at 15 mg/kg bw/d, and in 5 foetuses in 4 litters (4.3%/23.5%) at 60 mg/kg bw/d (vs. none in controls and low dose animals)
    - The omphalocele occurred in the absence of maternal toxicity at 15 mg/kg bw/d (no clinical signs in any of the 3 females, while CNS symptoms were seen in 3/4 females at 60 mg/kg/d).
    - Omphalocele is very rare in historical control data from this laboratory (incidence of 0.8% [0-1.8%] for foetuses and 4% [0-8.3%] for litters).
    - The administration of 8-hydroxyquinoline did not alter food consumption, body weight or body weight gain of female rabbits up to 60 mg/kg bw/d.
    - The observations support the conclusion that it is unlikely that the omphalocele was secondary to maternal toxicity. There are no data on the underlying mode of action.
    - Even if omphalocele occurs in the same animals that suffer from transient CNS symptoms, a link between these symptoms and the malformation appears unlikely and has not been demonstrated by mechanism of action (MoA) considerations.
    - The DS indicated that the 8-hydroxyquinoline MoA for teratogenicity could be chelation of relevant micronutrients such as metal ions. Several publications have noted that chelators can induce developmental toxicity in humans (Domingo, 1998; NRS, 2000; Keen, 2003). The developing organism seems to be more susceptible to this MoA and the long-term consequences are more severe than in the adult. The mother might recover while the offspring could be permanently affected; this appears to be worsened in cases of offspring from mothers with suboptimal nutritional status (see the RCOM).

- Omphalocele is a known malformation of the abdominal wall in children which may occur in the presence of malformations of other organs (Stoll *et al*., 2008). Incidences of 1:2000 or 1:5000 are reported (with tendency to increase), with unknown aetiology.

RAC considers other treatment-related effects to be of lower significance for the classification:

- Abortion in 1/25 dams at 15 mg/kg bw/d and in 2/25 dams at 60 mg/kg bw/d (vs 0 in controls and 5 mg/kg bw/d)
  - Nervous system excitation followed by lethargy (without mortalities) was observed in 4/25 pregnant rabbits at 15 mg/kg bw/d and in 11/25 at 60 mg/kg bw/d. No clinical signs were observed in the rabbit which aborted at 15 mg/kg bw/d, whereas both dams which aborted at the top dose showed maternal toxicity (nervous symptoms).
  - Abortion at 15 mg/kg bw/d did not appear to be linked to maternal (CNS) toxicity.
  - Although the incidence of abortions increased with dose, RAC considered that the abortions could be coincidental, as the overall incidences were low and single cases of abortion may occur spontaneously in this species. Abortions have been observed in studies on effects of undernutrition of the dams (Matsuoka *et al.*, 2006, Symeon *et al.*, 2015).
- Increased incidences of visceral variations:
  - Periorbital hemorrhage (eyes) (head soft tissue variation) 20 foetuses/12 litters (32.3%/66.7%) at 15 mg/kg bw/d and 18 foetuses/11 litters (34.0%/64.7%) at 60 mg/kg bw/d vs. 11.4%/36.8% in controls.
  - Retinal fold 19 foetuses/14 litters at 60 mg/kg bw/d (35.8%/82.4%) vs. 18.6%/52.6% in controls.
- Skeletal retardations
  - Unossified sternebrae increased in a dose-related manner in all dose groups in the rat and in the rabbit. While the incidence in the control group was rather low (1.6% of fetuses in 8.7% of litters), increased incidences were observed (7.6%/28%, 14.4%/47.8%, 34.8%/90.9%) at 100, 300 and 600 mg/kg bw/d, respectively, in the rat study. No maternal toxicity other than -10% lower corrected body weight gain was observed at the low dose. The same trend was seen for rudimentary sternebrae.
  - Both effects (unossified and rudimentary sternebrae) were increased in all dose groups of the rabbit study, also without being accompanied by any clinical symptoms at the low dose.
- Reduced pup viability:
  - Live born pups/litter 5.8 at 60 mg/kg bw/d vs. 7.3 in control rabbits.
  - The same effect was observed in pups from F0 females of all dose groups in the 2-generation study on rats (Fascineli, 2006b) without any evidence of maternal toxicity at the low dose of 1000 ppm (119 mg/kg bw/d in F0 females) and in F1 females at 8000 ppm. In contrast to these findings, increased pup survival occurred at the high dose of the developmental study in the rat (Fascineli, 2006c).

RAC agrees with the DS that the main effects can not be attributed to the maternal

toxicity.

Based on the observed teratogenic effects and developmental toxicity in rabbits and in accordance with the criteria for Category 1B, the omphalocele is the effect of highest concern that occurred (also) in foetuses at doses without maternal (CNS) toxicity. Moreover the clinical CNS symptoms in rabbits were not assumed to be linked to these effects. Dose-related high increases in incidences of unossified/rudimentary sternebrae in both rats and rabbits are supportive findings.

The CLP criteria 3.7.2.1.1 for Repr. 1B (H360D) are therefore fulfilled.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No neurotoxicity studies have been submitted. Neurotoxic adverse effects observed in other studies are summarized in the following table. Additionally it has been taken into account neurotoxicity data from the EMEA document (EMEA/MRL/464/98-FINAL).

Table 40: Summary table of relevant neurotoxic effects observed in toxicity studies

Method	Main Neurotoxic Effects	Remarks
Acute oral study (Dickhaus and Heisler, 1981a) *(see section 4.2)* The study is pre-guideline GLP: No Study acceptable supported by short term toxicity data	All animals at all dosage groups showed ataxia, gasping breathing and disturbed coordination within 1 hour after administration. Sedation (at all dose levels) and coma (at 756 and 1200 mg/kg bw) were noted after that. The surviving rats also displayed increased nervousness.	Wistar rats Doses of 600, 756, 953 and 1200 mg/kg bw Administration by rigid bulb headed cannula. Vehicle: 1% Tylose and Tween Purity: Not specified
Acute oral study (Dickhaus and Heisler , 1981b) *(see section 4.2)* The study is pre-guideline GLP: No Study acceptable as additional information	Up to 24 hours the animals displayed dose-related reduced activity, a decrease in respiratory rate, spasm and diminished reflex response. During the rest of the follow-up observation period, the surviving mice displayed sedation and reduced reactions.	CFI mice Doses of 120, 151, 190 and 240 mg/kg bw Administration by a rigid stomach tube Vehicle: 1% Tylose and Tween Purity: Not specified
Mammalian Micronucleus Test of Murine Peripheral Blood Cells (Hofman-Hünther, 2008) *(see section 4.9)* OECD 474 GLP: Yes Study acceptable	In the pre-test experiment 3 female mice and 3 male mice received a single dose of 35 mg/kg bw i.p. and showed toxic symptoms as reduction of spontaneous activity, prone position, palpecral closure, increased breathing rate and constricted opisthosome but survived 72 h after the treatment. All animals of the main experiment treated with 35 mg/kg bw showed toxic effects as reduction of spontaneous activity, palpebral closure and staggered walk.	NMRI mice Main doses of 7, 17.5 and 35 mg/kg bw Intra peritoneal Vehicle: Cottonseed oil Purity: 99.7%.
***In vivo* Mammalian**	At 300 mg/kg bw slightly reduced motility,	NMRI mice

Spermatogonial Chromosome Aberration Test (August, 2007) *(see section 4.9)* OECD 483 GLP: Yes Study acceptable	reduced muscle tone, slight ataxia and slight dypsnea were noted in 7/7 animals of the high dose group (24 hours sampling time). Slightly reduced motility, reduced muscle tone and slight ataxia were noted in 5/7 animals at 300 mg/kg bw (48 hours sampling time) immediately to 6 hours after administration.	Main doses of 75, 150 and 300 mg/kg bw Oral gavage Vehicle: 0.8% hydroxypropylmethylcellulose gel Purity: 99.8%
Teratology study (Fascineli, 2006c) *(see section 4.11)* OECD 414 GLP: Yes Study acceptable	All dams at 300 and 600 mg/kg bw/d suffered 10 minutes of nervous system excitation after dosing followed by lethargy for 20 minutes.	Wistar Han rats Doses of 0, 100, 300 and 600 mg/kg bw/d Oral gavage Vehicle: Corn oil Purity: 99.68%.
Teratology study (Fascineli, 2006d) *(see section 4.11)* OECD 414 GLP: Yes Study acceptable	Transient nervous excitation followed by lethargy was observed in 16% of the dams at 15 mg/kg bw/d and in 44% of the dams at 60 mg/kg bw.	New Zealand rabbits Doses of 0, 5, 15 and 60 mg/kg bw/d Oral gavage Vehicle: Corn oil Purity: 99.68%.

4.12.1.2 Immunotoxicity

No data

4.12.1.3 Specific investigations: other Studies

No data

4.12.1.4 Human information

No data

4.12.2 Summary and discussion

Neurotoxicity

There are no neurotoxicity studies available for the test compound.

Neurotoxic effects have been observed in other toxicity studies supplied for the inclusion of 8-hydroxyquinoline in the Annex I of Council Directive 91/414/EEC (currently repealed by Regulation (EC) No 1107/2009) as referred in Table 40. During the EFSA peer review, it was reported that "Transient dose related neurotoxic effects occurred in the developmental studies in both rats and rabbits. It was noted that these effects occurred after bolus administration, whereas after oral administration (relevant for consumers) there are no neurotoxic effects reported". EFSA, in its conclusion, regarded these effects related to the administration of the test substance by gavage. However, the MSCA deems that these effects observed in developmental studies and also in some acute toxicity and genotoxicity studies are not related to gavage administration. A detailed review of the studies in which neurotoxicity effects were observed has revealed that they only occurred at high dose levels, e.g.: in Fascineli teratology study (2006c) neurotoxic effects were observed at 300 and 600 mg/kg bw/d but neither at 100 mg/kg bw/d nor in controls. If the method of administration had been the cause of these effects, there would have been seen at all dose levels. Moreover, they were also observed after

different methods of administration, as it has seen in one genotoxicity study (Hofman-Hünther, 2008; see Table 40) in which intra peritoneal administration was used. Therefore, the MSCA cannot rule out that these effects are a direct consequence of neurotoxicity damage.

Besides, neurotoxic data about 8-hydroxyquinoline derivatives such as hydroxyquinoline halogenates have been compiled in several documents.

EMEA has reported that "Partly irreversible effects, the so-called subacute myelo-optic neuropathy, have been attributed to halogenated hydroxyquinoline derivatives after short-termed high dose or long-termed low dose oral administration to diarrhoic human patients, beginning at oral doses of higher 10 mg/kg bw and above". In 2011, EFSA concluded that "sufficient margin of safety was provided with the agreed reference values when considering any potential neurotoxic effect as reported by the EMEA from human data after exposure to halogenated hydroxyquinoline derivatives". The MSCA regards reasonable to suppose that these effects could be covered with the agreed reference values established for 8-hydroxyquinoline.

In Baumgartner, G. *et al*, 1979, it was mentioned a neurological disturbance consisted of an acute reversible encephalopathy with isolated optic atrophy as the most common manifestation, usually related to the ingestion of a high dose of clioquinol (halogenated hydroxyquinoline) over a short period.

In MSCA opinion, more information about neurotoxicity of the test substance should be needed to draw a conclusion. No information or adequate epidemiological data are available to assess the neurotoxic properties of 8-hydroxyquinoline.

4.12.3 Comparison with criteria

Neurotoxicity

8-hydroxyquinoline does not meet the criteria for classification according to CLP Regulation.

4.12.4 Conclusions on classification and labelling

Available information is not sufficient to classify the test compound regarding its neurotoxicity.

CLP: Not classified based on available data

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

8-hydroquinoline was found to be not ready biodegradable according to the OECD 301 D (EC Method C.4-E. Part VI) Closed Bottle Test.

In soil laboratory incubations under aerobic conditions in the dark (8-hydroxyquinoline sulfate was dosed) 8-hydroxyquinoline salts exhibit very low to moderate persistence forming no metabolites. It is noted however that, due to serious interferences of the soil matrix in several samples or when the recovered radioactivity in the extracts was small, no identifications were carried out. Mineralization to carbon dioxide accounted for about 10% AR after 120 days (study end). The formation of un-extractable residues (not extracted using acidified methanol or alkaline water) were a significant sink, accounting for 26-82% applied radioactivity (AR) already just after (about three hours) the application. After 120 days these figures were between 61-79% AR. 8-hydroxyquinoline is immobile in soil (8-hydroxyquinoline sulfate was investigated). There was an indication that the adsorption was pH and clay dependent, but statistically significant correlations could not be established. The study on photolysis in soil indicated that 8-hydroxyquinoline (8-hydroxyquinoline sulfate was investigated) is stable towards photolytic degradation in this compartment.

8-hydroxyquinoline was stable to hydrolysis. Aqueous photolysis was not investigated and was considered as not necessary due to the absorption maximum beneath 290 nm. In laboratory incubations in aerobic natural sediment water systems the majority of 8-hydroxyquinoline partitioned to sediment very quickly (maximum occurrence has been reached within one day). That was followed by a slow degradation (estimated biphasic whole system DT_{50} 230 days) with formation of no major metabolites. Mineralization to carbon dioxide accounted for 4.3-10.4% AR at the end of the study, while residues not extracted from the sediment represented 11.9-40.9% AR after 60-100 days.

Table 41: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD 301 D. EC Method C.4-E. Part VI Ready biodegradability	Not ready biodegradable	Closed bottle test	Dengler, D. (2005) Report-no. 20051323-01/AACB
EU Method C.7. Abiotic Degradation: Hydrolysis as a Function of pH	Stable to hydrolysis at pH 4, 7 and 9. Degradation < 10% after 120 h	-	García, A. (2004) Report-no. INF-F/2004/19-A
OECD Guideline draft. Photo-transformation of Chemicals on Soil Surfaces)	Stable to photolysis in soil. No significant degradation after 28 d of irradiation (> 83% 8-HQ unchanged at study termination)	-	Hennecke, D. (2004) Report-no. GAB-004/7-06
OECD 307 Aerobic transformation in soil	DT_{50} < 1 d (n=5) DT_{90} = 0.3 – 37.7 d (n=5) No metabolites > 10% Mineralization up to 12%	Route and rate of degradation in soil	Hennecke, D. (2004). Report-no. GAB-004/7-15

OECD 308 Aerobic transformation in water/sediment systems	DT_{50} system = 99 – 266 d (n=2) DT_{90} system = 886 – 328 d (n=2) DT_{50} water < 1 d (n=2; represents dissipation) Distribution: Max. in water 98.9% after 0 d. max. sed. 86.1 % after 1 d No metabolites > 10% Mineralization up to 10.38%	Route and rate of degradation in soil	Prata, A.P, (2009) Report No. 3154.220.001.08

5.1.1 Stability

The stability of 8-hydroxyquinoline and 8-hydroxyquinoline sulfate to hydrolysis at pH 4, 7 and 9 was tested on a preliminary study at 50ºC (García, A. 2004; report no. INF-F/2004/19-A). The study was conducted according to the EEC C.7 guideline, "abiotic degradation: hydrolysis as a function of pH". The concentrations of test item were quantified via HPLC and external standards.

The results indicate that 8-HQ was stable to hydrolysis at pH 4 and 7 and 50ºC after 5 days (< 10% degradation). At pH 9 it was also stable in the absence of oxygen (< 10% degradation). 8-HQ sulfate was stable to hydrolysis at pH 4 and 50ºC (< 10% degradation). At pH 7 and 9 precipitation occurred.

Hence, it is concluded that 8-hydroxyquinolines is stable in water and does not undergo hydrolysis at pH 4, 7 and 9 (at 50ºC).

Regarding the direct photo-degradation in water, Due to the absorption maximum beneath 290 nm, determination of the photochemical transformation or theoretical lifetime in aqueous systems is not required.

The photo-degradation of 8-hydroxyquinoline (as 8-hydroxyquinoline sulfate) in a loamy sand soil (LUFA 2.2) during 28 days was investigated (corresponding to 95.8 days of summer sunlight at 50°N) according to the OECD Guideline draft (Phototransformation of Chemicals on Soil Surfaces) and SETAC Guideline “Procedures for assessing the environmental fate and ecotoxicity of pesticides” (Hennecke, D., 2004; Report no. GAB-004/7-06).

The study was conducted at 20ºC with air dried soil. A Heraeus Suntest apparatus equipped with appropriate cut-off filters which ensured a minimum wavelength of the irradiation of 290 nm and a maximum of 800 nm. Extraction was conducted either methanol with 2% of concentrated sulphuric acid (x3). The radioactivity was measured by LSC and quantified and characterized with HPLC. Volatiles were also trapped.

The total recovery for the irradiated and the dark control group was always about 93 ± 2% of the applied radioactivity. Non-extractable residues were low reaching maximum proportions of 6.1 and 6.8% in the irradiated and non-irradiated soils, respectively. No significant degradation of 8-hydroxyquinoline sulfate in the irradiated samples was observed compared to the samples kept in darkness. The radioactivity extracted from the dark control samples and the respective irradiated samples decreased only slightly, ranging in average from 92.8% at the beginning to 86.2 and 84.6% at the end of the irradiation period, respectively.

The majority of extractable radioactivity could be attributed to unchanged 8-hydroxyquinoline sulfate (maximal 2.3% of the ITR was not identified). Due to the slow dissipation of 8-hydroxyquinoline sulfate, no valid determination of the DT_{50} value for photolytical degradation on soil surfaces was possible.

No metabolite was found in any sample. Non-extractable residues were nearly constant with time in the dark controls, being mostly between 2.4 and 2.8% of applied radioactivity and reaching 6.8% of applied radioactivity after an irradiation time of 28 days. The non-extractable residues of the irradiated samples slightly increased from 3.4% at day 1 to 6.1% of applied radioactivity at day 28 after start of irradiation.

According to these results it can be considered that 8-hydroxyquinoline does not undergo photo-degradation in soil.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No results based on estimations are provided. Please refer to experimental data presented below.

5.1.2.2 Biodegradation

The biodegradability of 8-hydroxyquinoline was investigated in an aerobic aquatic medium at a concentration of 2 mg/L (Dengler, D., 2005; Report-no. 20051323-01/AACB) according to the OECD 301 D (EC Method C.4-E. Part VI: Closed Bottle Test). The solution was inoculated with a small number of micro-organisms from a mixed population and maintained in closed bottles in the dark at a constant temperature of 20 ± 2°C. The degradation was determined by oxygen measurements over a 28 day period. A control with inoculum, but without test item was run in parallel for the determination of oxygen blanks. A reference compound (Na-benzoate) was tested in the same way to check the activity of the inoculum, along with a toxicity control with Na-benzoate and 8-Hydroxyquinoline.

The tests (inoculum blank, 8-hydroxyquinoline, reference and toxicity test) were performed in BOD flasks with ground-in-glass stoppers in 3-fold test assays for each measurement date. Due to the low solubility in water, the application of the test item was performed by means of an inorganic solvent (acetone).

Oxygen concentration was determined after 7, 14, 21 and 28 days with a WTW Microprocessor Oximeter OXI 340.

For each date of measurement the BOD (biochemical oxygen demand) was calculated by subtracting the oxygen concentration (mg O_2/L) of the mean initial inoculum blank from that of the other study groups. Then, mean measured values according to the controls were subtracted. This corrected depletion was divided by the concentration (mg/L) of the test item, to obtain the specific BOD. Percentage biodegradation was calculated by dividing the BOD by the specific oxygen demand.

The calculated oxygen demands were the following:

ThOD8-Hydroxyquinoiline:	2.53 mg O_2/mg test item
ThODNa-Benzoate:	1.67 mg O_2/mg reference item
ThODTox control:	2.10 mg O_2/mg substance mixture

The results of the degradation are presented below in Table 42.

Table 42: Degradation (%) of 8-Hydroxyquinoline, Na-Benzoate and Toxicity control

Time [d]	Degradation [%]		
	8-Hydroxyquinoline	Na-Benzoate	Toxicity control
7	1.2	64.4	20.0
14	-1.1	78.7	20.9
21	0.3	85.9	19.1
28	6.6	88.0	20.1

Biodegradation of 8-Hydroxyquinoline at the end of a 28-d period was measured to be 6.6% of the Theoretical Oxygen Demand (ThOD) for this molecule. Since the criterion for ready biodegradability of > 60% removal of the ThOD within a 28-period was not matched, 8-hydroxyquinoline cannot be considered as readily biodegradable.

In addition, degradation of the toxicity control was < 25% after 14 days. Therefore, toxic effects of 8-Hydroxyquinoline cannot be excluded. This is not unusual, as the test item acts as a fungicide and bactericide agent.

5.1.2.3 Simulation Tests

The route and rate of degradation of 8-hydroxyquinoline (as 8-hydroxyquinoline sulfate) in 4 soils incubated at 10 and 20°C and 40% MWHC was investigated for a period of up to 120 days under aerobic laboratory conditions in the dark (Hennecke, 2004; Report-no. GAB-004/7-15) according to the OECD 307. Incubation in sterilized soils was also conducted. Following application to soil surfaces, incubation vessels were sampled on days 0 (immediately after treatment), 1, 3, 7, 14, 28, 50, 80 and 120 and soil and volatile samples analyzed and characterized. Samples were analyzed by LSC and HPLC.

Individual recoveries ranged from 75% to 100% of AR. Non-extractable residues increased during the study reaching a maximum of 79% AR after 120 days. Mineralization was significant, since up to 12% AR was assigned to $^{14}CO_2$ (after 120 days).No organic volatiles were detected throughout the experiment (120 days), since they did not exceed 1 % of the applied radioactivity

8-HQ sulfate quickly dissipates from soil and no metabolites were formed. The rapid dissipation of 8-hydrxyquinoline sulfate to bound residues is reflected in its biphasic behaviour in soil. The best fit was obtained with FOMC and DFOP kinetics. The DT_{50} values at 20 ºC and 40% MWHC were < 1 d in all cases, and DT_{90} varied between 7.6 h and 34.3 d.

Table 43: DT_{50} values calculated by FOMC kinetics

Soil code	Texture class	a	b	Co [%]	DT_{50} [d]	DT_{90} [d]	R^2	SRM SE	Error Level Chi^2 test
LUFA 2.2	Loamy sand	0.32688± 0.06032	0.097792± 0.067591	97.583± 5.8076	0.7	112.0	0.9723	14.87	12.53
LUFA 10 ºC	Loamy sand	0.32818± 0.060416	0.098413± 0.067003	97.827± 5.7939	0.7	109.6	0.9723	18.71	**15.77**
Marisfeld soil	Silty clay loam	0.22194± 0.046838	$9.921E^{-06}$± 0.001415	100± 1.5165	0.0002	0.3	0.9980	8.65	7.30
Soest soil	Silt loam A	0.40207± 0.021722	0.0035039± 0.00096259	99.986± 0.79938	0.0161	1.1	0.9995	4.27	3.60
Ebbinghof	Silt loam B	0.31401± 0.045995	0.037134± 0.022024	99.236± 5.275	0.3005	56.8	0.9769	15.39	12.98

Table 44: DT_{50} values calculated by RMS with DFOP kinetics (calculated by the RMS during the EU evaluation)

Soil code	Texture class	K 1 [d^{-1}]	K 2 [d^{-1}]	g	DT_{50} [d]	DT_{90} [d]	R^2	Error Level Chi^2 test
LUFA 2.2 20 ºC	Loamy sand	6.98625	0.043544	0.555126	0.3	34.3	0.983207	9.7
Optimised error		0.0093719	1.74736	0.0365788	-	-	-	-
P-value (t-test)		0.000	0.49 (*)					
Ebbinghof	Silt loam B	9.30714	0.0534548	0.61977	0.2	25	0.98907	8.9
Optimised error		1.69701	0.0111423	0.0302131	-	-	-	-
t-test		0.000	0.001		-	-	-	-

(*)Not significantly different from zero at P=0.05

Table 45: DT_{50} and DT_{90} values calculated by the EFSA to be considered for regulatory purposes (provided during the EU review; EFSA Journal 2011;9(1):1964)

Soil code	Texture class	pH	T^a and moisture	DT_{50} [d]	DT_{90} [d]	Error Level Chi^2 test	Kinetics
LUFA 2.2	Loamy sand	5.8	20 ºC, 40% MWHC	0.3	34	9.7	DFOP
Marisfeld soil	Silty clay loam	6.0	20 ºC, 40% MWHC	0.016	1.1	3.6	FOMC
Soest soil	Silt loam	6.4	20 ºC, 40% MWHC	0.0002	0.3	7.3	FOMC
Ebbinghof	Silt loam	5.6	20 ºC, 40% MWHC	0.2 d	25	8.9	DFOP
LUFA 2.2 (10 ºC)	Loamy sand	5.8	10 ºC, 40% MWHC	0.9 d	37.7	11.7	DFOP

The route and rate of degradation of 8-hydroxyquinoline (as 8-hydroxyquinoline sulfate) in two water sediment systems (fine and coarse, identified as CHA and IRA) at 20°C was investigated for a period of up to 100 days under aerobic laboratory conditions in the dark (Prata, A.P, 2009; Report No. 3154.220.001.08) according to the OECD 308. The samples taken from natural areas were first acclimated under aerobic conditions in the dark prior to treatment until equilibrium was reached.

Duplicate samples were taken for analysis at specified intervals up to 100 days after application. Organic volatiles and carbon dioxide were also trapped. Radioactivity in the water was quantified by LSC, while the characterization was conducted through HPLC with true standards. The sediment was extracted with methanol and chloroform.

Each sampling during the test was done in duplicate. The mean recoveries from the water/sediment system were in the range of 91.32% to 101.94% for the CHA system and from 101.19% to 108.90% for the IRA system.

The obtained results indicate that there is a very rapid partitioning to sediment. The radioactivity in water decreases from 80.5 and 98.9% AR to 4.4 and 22.8% AR 1 day after application respectively for the system CHA and IRA. The bond residues increased steadily through the incubation, with higher amounts observed on the system IRA (11.89 and 40.89% after 100 and 60 days respectively for CHA and IRA). Volatilization was not significant in any of the systems while the mineralization represented 4.31% AR in CHA and 10.38% AR in IRA.

Regarding the degradation products, numerous metabolites were found although none of them above 10% AR. The fraction that was not identified represented a maximum of 11.03% AR in CHA and 6.90% AR in IRA.

Regarding the kinetics The $DT_{50}$and DT_{90} of 8-Hydroxyquinoline sulfate in the CHA and IRA systems were 266 and 99 days and 886 and 328 days, respectively (SFO).

Table 46: Calculated half-lives for 8-hydroxyquinoline in two water sediment systems

System	Parameters of the exponential model C_0 [a]	k [b]	R^2	DT_{50}	DT_{90}
	%	$days^{-1}$		days	days
CHA (whole system)	94.66	0.0026	0.867	266	886
IRA (whole system)	93.23	0.007	0.864	99	328
CHA (sediment)	86.52	0.0031	0.999	222	720
IRA (sediment)	66.93	0.0044	0.988	158	523
CHA (water phase)	80.44	5.72	0.975	0.12 [b]	0.402
IRA (water phase)	98.08	4.34	0.874	0.16 [b]	0.530

[a]Initial concentration
[b]Represent dissipation to sediment

Table 47: DT_{50} and DT_{90} values calculated by the EFSA (provided during the EU review; EFSA Journal 2011;9(1):1964)

System	pH water	pH system	DT_{50}/DT_{90} whole system (Chi^2)	DT_{50}/DT_{90} whole system (Chi^2)	DT_{50}/DT_{90} whole system (Chi^2)	Kinetics
CHA	4.30 – 6.18	4.67 – 6.13	229.55/862.51 (5.1)	< 1d *	No reliable value estimated	HS
IRA	5.21 – 6.02	5.62 – 6.09	No reliable value estimated	< 1d *	No reliable value estimated	-

*Represents dissipation to sediment

5.1.3 Summary and discussion of degradation

The results obtained in the ready biodegradability test indicate that 8-hydroquinoline is not ready biodegradable, it is mentioned that it could be due to toxic effect to bacteria since the 8-HQ is used as bactericide and fungicide, however the toxicity test on soil organisms showed no toxicity effects on the bacteria nitrogen metabolism (Kölzer, U., 2003). Nevertheless the occurrence of biodegradation was demonstrated in the soil metabolism studies conducted in five soils under sterile and non-sterile conditions but not in the water/sediment studies. In soil, 8-hydroxyquinoline shows very low persistence with DT_{50} values below 1 day and DT_{90} values below 35 days. In the water/sediment studies, 8-hydroxyquinoline quickly dissipates from water with DT_{50} values below 1 day. The disappearance from the whole system is longer with half-lives of 99 and 266 days for the tested systems.

Regarding the abiotic degradation, 8-hydroxyquinoline is stable to hydrolysis in water and to photolysis in soil. Direct photo-degradation in water is not expected due to the low absorption < 290 nm.

According to the data summarized above 8-hydroxiquinoline is not ready biodegradable, hydrolytically stable and the DT_{50}'s in the water/sediment system vary from 99 to 266 days for the whole system and from 158 to 222 in sediment, therefore it is considered as not rapid degradable.

5.2 Environmental distribution

Soil is expected to be relevant compartment for 8-HQ since it is highly immobile and of low volatility.

5.2.1 Adsorption/Desorption

The adsorption/desorption properties of radio-labeled 8-HQ (as 8-HQ sulfate) were investigated in five soils according to OECD 106 (Hennecke, D., 2004; Report-no: GAB 004/7-13). The analytical measurements were performed by HPLC. All tests were performed at 20°C in an air-conditioned room in the dark. Based on experimental data a soil to solution ratio of 1/50 (w/v) for all tests was chosen. An initial concentration of 50 mg/L, was used for determination of adsorption or desorption kinetics. Adsorption as well as desorption equilibrium was achieved after 24 hours which was used as agitation times for further testing. 8-HQ sulfate was stable under the applied test conditions following tests on the stability of the test item.

To derive adsorption isotherms application solution was added to the soils and the suspensions were shaken during the equilibration time using a soil/solution ratio as defined above. The concentration levels based on aqueous solutions were fixed at 1 mg/L, 2.5 mg/L, 5 mg/L, 10 mg/L and 20 mg/L. After equilibration time and subsequent sedimentation, the supernatant was decanted and analysed for the test substance by HPLC.

Prior to desorption experiments an adsorption test was performed as described above with an agitation time to reach adsorption equilibrium. Then both phases were separated by centrifugation and the aqueous phase was removed as complete as possible. The volume of solution removed was determined and replaced with an equal volume of 0.01 M $CaCl_2$ without test item. After the agitation time the phases were separated by centrifugation and the concentration of the test item was determined analytically in the supernatant. For the determination of desorption isotherms all soils and five concentrations (1 mg/L, 2.5 mg/L, 5 mg/L, 10 mg/L and 20 mg/L) were used.

The Freundlich adsorption coefficients (K_F^{ads}) ranged between 286 and 2965 mL/g. Normalization to the organic carbon content of the soils resulted in K_{FOC}^{ads} values from 12796 to 111459 mL/g. A strong adsorption to organic matter as well as to the clay fraction was observed. The Freundlich desorption coefficients (K_F^{des}) ranged from 441 to 6761. Adsorption is almost irreversible, only 3 to 8% was desorbed. Hence, 8-HQ is highly immobile in soil.

5.2.2 Volatilisation

8-Hydroxyquinoline sulfate has negligible volatility (6.7×10^{-5} hPa at 20°C) and its DT_{50} in air is very short (0.647 hours according to Atkinson). Also considering that the recommended application via drip irrigation in greenhouses reduces evaporation of irrigation water to a minimum, it can be concluded that residues of the active substance in air will be negligible. Therefore, a study with 8-Hydroxyquinoline sulfate investigating the rate and route of degradation in air is not required.

5.2.3 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

There is no indication for a bioaccumulation potential of 8-Hydroxyquinoline (log P_{OW} < 3.0).

5.3.1.2 Measured bioaccumulation data

Since the octanol-water partition coefficient of 8-Hydroxyquinoline was determined as log P_{OW} < 3.0, there is no indication for a bioaccumulation potential of 8-Hydroxyquinoline. Therefore a study with 8-Hydroxyquinoline investigating the aquatic bioaccumulation is not required.

5.3.2 Summary and discussion of aquatic bioaccumulation

8-Hydroxyquinoline is not considered to have potential for bioaccumulation, since the log P_{OW} is below 3.0.

5.4 Aquatic toxicity

For clarifications, all references on 8-HQS concentrations have not been included on the tables due to all analytical measurements in the experiments are for 8-HQ instead of 8-HQS. So the toxicity endpoints are expressed on 8-HQ measured and Beltanol-L nominal or calculated (from the actual content on the measured 8-HQ) concentrations.

The typical Beltanol-L formulation composition is approximately a 50/50 (w/w) of 8-HQS and water, for more clarifications on the composition of the Beltanol-L and the endpoint calculations, please read in depth the explanations provided in every test section below.

Table 48: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
OECD 203	LC_{50} (96 h) = 6.56 mg/L Beltanol-L (n) NOEC (96 h) = 2.56 mg/L Beltanol-L(n) LC_{50} (96 h) = 2 mg/L 8-HQ (m) NOEC (96 h) = 0.78 mg/L 8-HQ (m)	-	Stäbler, D. (2004), Document No: 20031207/01-AAOm
OECD 204 OECD 215	LC_{50} (28 d) > 0.024 mg/L Beltanol-L (c) NOEC (28 d) = 0.024 mg/L Beltanol-L (c) LC_{50} (28 d) > 0.01 mg/L 8-HQ (m) NOEC (28 d) = 0.01 mg/L 8-HQ (m)	-	Gonsior, G. (2011), Document No: S11-02694
OECD 202	EC_{50} (48 h) = 10.9mg/L Beltanol-L (n) NOEC (48 h) = 7.61mg/L Beltanol-L (n) EC_{50} (48 h) = 3.67 mg/L 8-HQ (m) NOEC (48 h) = 2.32 mg/L 8-HQ (m)	-	Stäbler, D. (2004), Document No: 20031207/01-AADm
OECD 211	$NOEC_{rep}$ (21 d) = 0.09 mg/L Beltanol-L (c) $NOEC_{rep}$ (21 d) = 0.039 mg/L 8-HQ (m)	-	Weber K. (2012), Document No: S11-02695

OECD 201	E_yC_{50} (72 h) = 1.33 mg/L Beltanol-L (c) E_rC_{50} (72 h) = 1.74 mg/L Beltanol-L (c) E_yC_{10} (72 h) = 0.52 mg/L Beltanol-L (c) E_rC_{10} (72 h) = 0.66 mg/L Beltanol-L (c) E_yC_{50} (72 h) = 0.54 mg/L 8-HQ (m) E_rC_{50} (72 h) = 0.71 mg/L 8-HQ (m) E_yC_{10} (72 h) = 0.21 mg/L 8-HQ (m) E_rC_{10} (72 h) = 0.27 mg/L 8-HQ (m)		Falk, S. (2011), Document No: S11-02696

[n]Nominal concentration
[m]Measured
[c]Calculated from the 8-HQ content.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of 8-Hydroxyquinoline to fish is summarised in Table 49.

Table 49: Acute toxicity of 8-Hydroxyquinoline (8-HQ) to fish

Guideline / Test method	Species	Exposure		Results		Reference
		Design	Duration (h), tested substance	Endpoint	Value (mg/L)	
OECD 203	*Oncorhynchus mykiss*	semi-static	96, Beltanol-L 8-HQ	LC_{50} LC_{50}	6.56 (n) 2 (m)	Stäbler, D. (2004), Document No: 20031207/01-AAOm

Stäbler D. (2004a). Report No. 20031207/01-AAOm. Acute toxicity testing of Beltanol-l (batch: 208613, purity: nominal: 500 g/L of 8-Hydroxyquinoline sulphate (8-HQS), analysed: 504.2 g/L of 8-HQS) in rainbow trout (*O. mykiss*) (teleostei, salmonidae). The study was conducted following OECD 203 guideline and under GLP.

Deviations

Dissolved oxygen saturation was above 60 % saturation during holding. The guideline requires this to be above 80 % during this period. Test water conductivity was 518 µS/cm instead of the recommended ≤ 10 µS/cm. Tank loading was approximately 2.0 g fish/L instead of the recommended maximum of 1.0 g fish/L. These deviations are not considered to have affected the outcome of the study.

The acute toxicity of 8-Hydroxyquinoline (8-HQ) formulated as Beltanol-L to Rainbow trout (*O. mykiss*) was investigated under semi-static test conditions for 96 hours. Aquaria with 10 L volume of test solution were used to test each of six test concentrations and one blank control with 10 fish in each treatment group. The nominal test concentrations were 1.0, 1.6, 2.56, 4.1, 6.55 and 10.5 mg formulation/L and were based on the results of a range finding test.

Fish of 4 to 6 cm body length were acclimatised for more than 20 days in dechlorinated and deionised water at conditions in the range of 15 to 17 °C, pH of 6.5 to 8.5, total hardness (as $CaCO_3$) of 140 to 268 mg/L, dissolved oxygen content above 60 % and 12 to 16 hours light per day. Granular rearing food to approx. 2 % of the fish body weight was fed daily during acclimatisation until 24 hours prior to the test start. During the study no feed was provided. There was continuous aeration during the study and test media were renewed daily.

The test media for the treatment groups 2.56, 4.1 and 10.5 mg formulation/L were analysed initially and following 24, 48 and 72 hours from fresh and aged media, and at the end of the study after 96 hours from aged media for 8-Hydroxyquinoline concentration with HPLC-UV. Fish were observed for mortality and abnormal behaviour 3, 6, 24, 48, 72 and 96 hours after introduction into the test media and all fish were weighed and measured at the end of the study.

Analytical concentrations

Mean 8-Hydroxyquinoline (8-HQ) concentrations were found to be between 92.8 and 105 % of nominal values throughout the study. Nominal concentrations were therefore used for all subsequent result calculations. The content of 8-HQ in the formulation Beltanol-L was 30.5% (w/w) as given by sponsor. The nominal content of the active substance 8-Hydroxyquinoline sulphate (8-HQS)in the formulation Beltanol-L was 50.4% (w/v).

Test conditions in the test media ranged from 16.2 to 17.3 °C, oxygen concentration of > 60 % saturation, pH from 7.89 to 8.44, hardness of 10°dH (as $CacO_3$) under 16 hours light per day. Average body weight of the fish across all treatment groups was 2.07 g.

Biological observations:

Mortality only occurred in the two highest test concentrations of 10.5 and 6.55 mg formulation/L after 48 and 72 hours, respectively. No sub-lethal effects or abnormal behaviour were observed up to 2.56 mg formulation/L. Three fish in the 4.1 mg formulation/L group displayed difficulties maintaining equilibrium at 96 hours. Other effects were observed in the two higher test concentration groups after 48 hours, these are summarised below in Table 50.

Table 50: Mortality and abnormal behaviour observed in Rainbow trout exposed to various concentrations of 8-Hydroxyquinoline formulated as Beltanol-L.

		Time (hours)					
Formulation concentration (mg/L)	**Observed abnormalities and mortality**	**3**	**6**	**24**	**48**	**72**	**96**
0	None						
1.0	None						
1.6	None						
2.56	None						
4.1	b						3/10
6.55	a b c d				4/10	3/10 5/10 1/10	 5/10 5/10
10.5	a b c d				9/10 1/10	 1/10 9/10	 10/10 10/10

[a]unusual behaviour (reduced activity and or orientation to bottom or surface of the test vessels)
[b]difficulties with maintenance of equilibrium
[c]fish upside down with loss of equilibrium, showing only movement of gills as a sign of life
[d]dead

Conclusion

Based on nominal values, the LC_{50} (96 h) of Beltanol-L to Rainbow trout was determined to be 6.56 mg formulation/L with 95 % confidence limits of 5.63 to 7.65 mg/L. The NOEC (96

h) was 2.56 mg formulation/L. Expressed in terms of 8-HQ, the LC_{50} and NOEC are 2 and 0.78 mg/L, respectively. Expressed in terms of 8-HQS, the LC_{50} and NOEC are 2.68 and 1.05 mg/L, respectively.

5.4.1.2 Long-term toxicity to fish

The long-term toxicity of 8-HQ to fish is summarised in Table 51.

Table 51: Long-term toxicity of 8-HQ to fish

Guideline / Test method	Species	Exposure		Results		Reference
		Design	Duration (d), tested substance	Endpoint	Value (µg/L)	
OECD 204 OECD 215	*Oncorhynchus mykiss*	semi-static	28, Beltanol-L 8-HQ	LC_{50} NOEC LC50 NOEC	> 30 (n) 30 (n) > 10.01 (TWA) 10.01 (TWA)	Gonsior, G. (2011), Document No: S11-02694

Autor: GONSIOR, G. (2012)

Report: Beltanol-L - 28-Day Toxicity Test in Rainbow Trout *(O. rnykiss)* (Teleostei, Salmonidae).

Report No.: S11-02694.

Guidelines: OECD 204 (1984)

OECD 215 (2000)

Deviations: none

GLP: Yes (certified laboratory).

The aim of the study was the assessment of the lethal and sublethal effects (development of body weight and length) , including the evaluation of the no observable effect concentration (NOEC) of Beltanol-L in fish , over an observation period of 28 days within the framework of laws for registration according to OECD Guideline 204 and 215.

Material and methods:

Beltanol-L, Batch no: 11060502; content of active substance (analysed): 49.85% (w/v) 8-HQS. Test species: *O. mykiss*, size between 4 and 6 cm. Ten organisms per test concentration were used. The duration of the test was 28 days. The test item was evaluated in a semi-static test with renewal of test media three times a week (Monday, Wednesday and Friday). The nominal test concentrations were 30.0, 12.0, 4.80, 1.92, 0.768 and 0 µg/L Beltanol-L. Test media were prepared by dilution of the test item in test water (= stock solutions), and application of defined volumes of the stock solutions to the test vessels. The fish were observed daily. Records were made on mortality and visible abnormalities, if observed. Analytical determinations of test item content in test solutions were conducted. Endpoints reported are the LC_0, LC_{50}, LC_{l00} and the NOEC (No Observed Effect Concentration). Temperature, pH-value and % oxygen saturation of the test solutions were measured three times a week at each test medium renewal, prior to and after renewal of the test medium. Hardness of the test water was measured at the start.

Findings:

Table 52: NOEC and LCx-values after 28 days of exposure to the test item.

	8-HQ (TWA) [µg/L]	Beltanol-L* [µg/L]
NOEC	10.01	23.6
LC0	≥ 10.01	≥ 23.6
LC50	> 10.01	> 23.6
LC100	> 10.01	> 23.6

*** Based on a 42.49% of 8-HQ content .**

The total hardness (as $CaCO_3$) of the test medium was determined to be 11 - 13°dH; the mean pH-value of the untreated control was determined to be 8.24 ± 0.10, the overall mean temperature was measured to be 16.1 ± 0.3°C and the overall mean oxygen saturation was determined to be 93 ± 5 %. The test item had no influence on the pH-value of the test solutions.

Analytical determinations were done on Beltanol-L nominal concentrations of 30.0, 12.0, 4.80 µg/L and control. Samples were taken at 0h (initial value) and after each renewal of test medium from fresh and at 2, 5, 14, 23 and 26 days from aged test medium.

The mean measured 8-HQ in the fresh test solutions was 97% of the nominal concentration of 8-HQ, however the measured concentrations of 8-HQ in the aged test solutions ranged from 57% to 62% (based on geomeans) of fresh medium concentration. Due to the arithmetic mean of losses is greater than the 40%, the TWA's (Time Weighted Average) have been calculated for every concentration measured. Therefore the Beltanol-L endpoints have been expressed as calculated from TWA values multiplied by geomean of the % of the measured 8-HQ in the fresh medium (in this case 42.49%).

1.92 µg/L concentration was excluded from calculations due to infection of the fish at this concentration level only; furthermore the concentration of 0.768 µg/L also was excluded by the lab, no explanation has been reported, we suppose that it is not technically possible to detect it nor to quantify it. These had no effects on the outcome of the study.

Table 53: Losses calculations for the 8-HQ nominal concentration of 0.00198 mg/L corresponding to a nominal Beltanol-L concentration of 0.0048 mg/L.

Day	**Fresh 8-HQ (measured)**	**Day**	**Aged 8-HQ (measured)**	**% respect to fresh medium**	
0	0.00227				
2	0.00237	2	0.00103	45.37	
12	0.00188	5	0.0016	67.51	
21	0.00204	14	0.00112	59.57	
23	0.002	23	0.00124	60.78	
		26	0.00105	52.50	
					% losses
			Geomean	**56.63**	**43.37**

Table 54: Losses calculations for the 8-HQ nominal concentration of 0.00494 mg/L corresponding to a nominal Beltanol-L concentration of 0.012 mg/L.

Day	**Fresh 8-HQ (measured)**	**Day**	**Aged 8-HQ (measured)**	**% respect to fresh medium**	
0	0.00539				
2	0.00506	2	0.00275	51.02	

12	0.00495	5	0.00357	70.55	
21	0.00545	14	0.00312	63.03	
23	0.00570	23	0.00365	66.97	
		26	0.00336	58.95	
					% losses
			Geomean	**61.72**	**38.28**

Table 55: Losses calculations for the 8-HQ nominal concentration of 0.0124 mg/L corresponding to a nominal Beltanol-L concentration of 0.03 mg/L.

Day	**Fresh 8-HQ (measured)**	**Day**	**Aged 8-HQ (measured)**	**% respect to fresh medium**	
0	0.0135				
2	0.014	2	0.00795	58.89	
12	0.0135	5	0.0097	69.29	
21	0.012	14	0.00989	73.26	
23	0.0138	23	0.00743	61.92	
		26	0.00594	43.04	
					% losses
			Geomean	**60.29**	**39.71**

The arithmetic mean of the losses is 40.45% with respect to the initial concentration in the fresh medium.

As above mentioned TWA's have been calculated due to the losses on the concentrations with respect to the fresh medium. A first order kinetic for the losses has been supposed hence the natural logarithm correction has been applied to know the actual exposure concentrations in the test (Table 56, 58 and 59).

Table 56: TWA calculations for the 8-HQ nominal concentration of 0.00198 mg/L, corresponding to a nominal Beltanol-L concentration of 0.0048 mg/L.

Days	**[8-HQ] in fresh medium (µg/L)**	**[8-HQ] in aged medium (µg/L)***	**Days**	**Ln of fresh medium**	**Ln of aged medium**			
0	2.27			0.82				
2	2.37	1.03	2	0.86	0.03	3.14		
5	2.11	1.60	3	0.75	0.47	5.88		
7	2.04	**1.19**	2	0.71	0.18	3.22		
9	1.71	**1.16**	2	0.54	0.14	3.11		
12	1.88	**0.97**	3	0.63	-0.03	3.91		
14	1.92	1.12	2	0.65	0.11	2.93		
16	2.08	**1.09**	2	0.73	0.08	2.93		
19	2.11	**1.18**	3	0.75	0.16	4.76		
21	2.04	**1.19**	2	0.71	0.18	3.22		
23	2	1.24	2	0.69	0.22	3.21		
26	2.02	1.05	3	0.70	0.05	4.42		
28		**1.14**	2		0.13	3.08		
geomean	**2.04**	∑	**28**		∑	**43.82**	**TWA**	**1.57**

*** Bold numbers on aged medium column have been calculated based on a geomean of 56.63% (see Table 53).**

Table 57: TWA calculations for the 8-HQ nominal concentration of 0.00494 mg/L, corresponding to a nominal Beltanol-L concentration of 0.012 mg/L.

Days	[8-HQ] in fresh medium (µg/L)	[8-HQ] in aged medium (µg/L)*	Days	Ln of fresh medium	Ln of aged medium			
0	5.39			1.68				
2	5.06	2.75	2	1.62	1.01	7.85		
5	5.06	3.57	3	1.62	1.27	12.82		
7	5.01	**3.12**	2	1.61	1.14	8.03		
9	4.23	**3.09**	2	1.44	1.13	7.95		
12	4.95	**2.61**	3	1.60	0.96	10.07		
14	4.75	3.12	2	1.56	1.14	7.93		
16	4.89	**2.93**	2	1.59	1.08	7.54		
19	5.37	**3.02**	3	1.68	1.10	11.64		
21	5.45	**3.31**	2	1.70	1.20	8.52		
23	5.7	3.65	2	1.74	1.29	8.98		
26	5.43	3.36	3	1.69	1.21	13.28		
28		**3.35**	2		1.21	8.61		
geomean	**5.09**	∑	**28**		∑	**113.2**	**TWA**	**4.04**

*** Bold numbers on aged medium column have been calculated based on a geomean of 61.72% (see Table 54).**

Table 58: TWA calculations for the 8-HQ nominal concentration of 0.0124 mg/L, corresponding to a nominal Beltanol-L concentration of 0.03 mg/L.

Days	[8-HQ] in fresh medium (µg/L)	[8-HQ] in aged medium (µg/L)*	Days	Ln of fresh medium	Ln of aged medium			
0	13.5			2.60				
2	14	7.95	2	2.64	2.07	20.96		
5	14.2	9.70	3	2.65	2.27	35.16		
7	12.5	**8.56**	2	2.53	2.15	22.29		
9	10.7	**7.54**	2	2.37	2.02	19.62		
12	13.5	**6.45**	3	2.60	1.86	25.19		
14	13.2	9.89	2	2.58	2.29	23.20		
16	11.8	**7.96**	2	2.47	2.07	20.72		
19	12.4	**7.11**	3	2.52	1.96	27.78		
21	12	**7.48**	2	2.48	2.01	19.46		
23	13.8	7.43	2	2.62	2.01	19.07		
26	12.1	5.94	3	2.49	1.78	27.97		
28		**7.30**	2		1.99	18.99		
geomean	**12.77**	∑	**28**		∑	**280.41**	**TWA**	**10.01**

*** Bold numbers on aged medium column have been calculated based on a geomean of 60.29% (see Table 55).**

NOEC and LOEC calculations

The body weight and length of fish was determined at the beginning and the end of the test. No significant differences were detected at start of the test. NOEC as function of length and weight development was analysed (p = 0.05). No statistically significant influence of the test item on the fish growth and length could be detected (Table 59).

Table 59: Mean of size and body weight of fish at the start and end (day 28) of the test.

Beltanol-L (nominal) (µg/L)	Mean body weight (g)		Mean fish lenght (mm)	
	Start of the test (t = 0 d)	End of the test (t = 28 d)	Start of the test (t = 0 d)	End of the test (t = 28 d)
Control	1.13	2.68	49	63
0.768	1.15	2.67	49	63
1.92	1.16	- [1)]	50	- [1)]
4.8	1.18	2.89	50	65
12	1.13	2.62	50	63
30	1.18	2.65	49	63

[1)] **No data, since concentration was not used for biological data evaluation; fish were killed two days after test start because an infection on the fish was detected.**

No effects on fish weight and length were observed. Therefore the NOEC was determined to be 30 µg/L Beltanol-L (nominal).

Conclusion

All endpoints are based on the TWA calculations, so according to the results of the test, the LC_0 (28 d) was determined to be ≥ 10.01 µg/L, the LC_{100} (28 days) was > 10.01 µg/L and the LC_{50} (28 days) was calculated to be > 10.0 µg/L of 8-HQ. No visible abnormalities and no effects on the development of body weight and length were observed at 10 µg/L. Therefore the NOEC was determined to be 10.01 µg/L of 8-HQ that correspond to a Beltanol-L calculated concentration of 23.6 µg/L.

Table 60: NOEC and LCx-values after 28 days of exposure to the test item.

	8-HQ (TWA) [µg/L]	Beltanol-L* [µg/L]
NOEC	10.01	23.6
LC0	≥ 10.01	≥ 23.6
LC50	> 10.01	> 23.6
LC100	> 10.01	> 23.6

*** Based on a 42.49% of 8-HQ content .**

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of 8-HQ to invertebrates is summarised in Table 61.

Table 61: Acute toxicity of 8-HQ to invertebrates

Guideline / Test method	Species	Exposure		Results		Reference
		Design	Duration (h), tested substance	Endpoint	Value (mg/L)	
OECD 202	*Daphnia magna*	semi-static	48, Beltanol-L (n) 8-HQ (m)	EC_{50} NOEC EC_{50} NOEC	10.9 7.61 3.67 2.32	Stäbler, D. (2004), Document No: 20031207/01-AADm

Stäbler D. (2004b): Assessment of toxic effects of Beltanol-L (batch: 208613, purity: nominal: 500 g/L of 8-HQS (measured: 504.2 g/L) on *D. magna* using the 48 h acute immobilisation test. Report No. 20031207/01-AADm. The assay was conducted following the OECD 202 guideline and under GLP.

Deviations

The test substance was analysed from three test concentrations including the highest, but not the lowest as recommended in the guideline. This is not considered to have affected the outcome of the study.

The acute toxicity of 8-HQ formulated as Beltanol-L in *D. magna* was investigated under semi-static conditions for 48 hours. Following a range finding test, Daphnids were exposed in 100 mL glass beakers holding 50 mL test medium to nine concentrations of the formulation, ranging from 1.0 to 25.7 mg formulation/L, one blank control and two concentrations of a reference item, potassium-dichromate, at 0.9 and 1.9 mg/L. Each treatment group consisted of four replicates each containing five Daphnids.

The acute toxicity of 8-HQ formulated as Beltanol-L in *D. magna* was investigated under semi-static conditions for 48 hours. Following a range finding test, Daphnids were exposed in 100 mL glass beakers holding 50 mL test medium to nine concentrations of the formulation, ranging from 1.0 to 25.7 mg formulation/L, one blank control and two concentrations of a reference item, potassium-dichromate, at 0.9 and 1.9 mg/L. Each treatment group consisted of four replicates each containing five Daphnids.

Daphnids of between 6 and 24 hours of age were taken from the laboratory's stock culture and bred in a RUMED chamber at 20 ± 2 °C in dechlorinated and deionised water with 16 hours of light per day. Water used in the test media was composed from dechlorinated drinking water and deionised water and had a hardness of 213.6 mg/L as $CaCO_3$. The photoperiod during the study was 16 hours light per day. The test water was gently aerated for 30 minutes prior to use. No aeration or feeding took place during the actual test.

Oxygen, temperature and pH measurements were made at test start, after 24 hours from aged and fresh test media and after 48 hours. Daphnids were observed for immobilisation 24 and 48 hours after introduction into the test media. Test media from the 5.07, 7.61 and 25.7 mg formulation/L treatment groups were analysed initially, following 24 hours from aged and fresh test media, and after 48 hours for 8-Hydroxyquinoline sulphate concentration.

Findings

The mean 8-Hydroxyquinoline concentrations in the analysed treatment groups were all around 110 % of nominal concentrations, results were thus based on nominal concentrations. The content of 8-HQ in the formulation Beltanol-L was 30.5% (w/w) as given by sponsor. The nominal content of the active substance 8-HQS in the formulation Beltanol-L was 50.4% (w/w).

The test media temperature ranged from 19.6 to 20.7 °C, pH from 8.27 to 8.52 and oxygen levels were greater than 97 % of saturation value during the whole study. Daphnia immobilisation in the toxic reference item treatment groups after 48 hours exposure was 5 % and 95 % in the 0.9 and 1.9 mg potassium-dichromate/L groups, respectively. Therefore the Daphnids were demonstrated to be suitable for the determination of toxicological effects of 8-Hydroxyquinoline formulated as Beltanol-L.

Immobilisation results are displayed in Table 62. No mortality or effect was observed at concentrations up to 7.61 mg formulation/L.

Table 62: Cumulative acute immobilisation* (%) observed in *D. magna* exposed to 8-Hydroxyquinoline formulated as Beltanol-L.

	% Daphnia immobilization	
Mean actual concentration (mg formulation/L)	**24h**	**48 h**
Blank control	0	0
1.0	0	0
1.5	0	0
2.25	0	0
3.38	0	0
5.07	0	0
7.61	0	0
11.4	15	60
17.1	60	100
25.7	100	100

*** mean of four replicates, five Daphnids per replicate**

Conclusion

Based on nominal data, the EC_{50} (48 h) of formulated Beltanol-L to *D. magna* was calculated to be 10.9 mg formulation/L, with 95 % confidence limits of 10.0 to 12.0 mg formulation/L. The NOEC (48 h) was 7.61 mg formulation/L. Expressed in terms of 8-HQ, the EC_{50} and NOEC as calculated by RMS are 3.67 and 2.32 mg/L, respectively. Expressed in terms of 8-HQS, the EC_{50} and NOEC are 5.5 and 3.11 mg/L, respectively.

5.4.2.2 Long-term toxicity to aquatic invertebrates

The long-term toxicity of 8-HQ to invertebrates is summarised in Table 63.

Table 63: Long-term toxicity of 8-HQ to invertebrates

Guideline / Test method	Species	Exposure		Results		Reference
		Design	Duration (d), tested substance	Endpoint	Value (mg/L)	
OECD 211	*Daphnia magna*	semi-static	21, Beltanol-L (calculated) 21, 8-HQ (measured)	NOEC (reproduction) NOEC (reproduction)	0.09 0.039	Weber, K. (2012), Document No: S11-02695

Autor: WEBER K, (2012)

Report: Beltanol-L - Assessment of Toxic Effects on *D. magna* Using the 21 Day Reproduction Test.

Report No.: S11-02695.

Guidelines: OECD 211

Deviations: none

GLP: Yes (certified laboratory).

The aim of the study was the assessment of the long-term effect of the test item on *D. magna* in a 21 day reproduction toxicity test and the determination of the NOEC and the EC_{50} for mortality and reproduction according to OECD 211 (2008).

Material and methods:

Beltanol-L, Batch number: 11060502; content of active substance (analysed): 49.85 % (w/v) 8-HQS. Test species: *D. magna* Strauss, Clone V, age between 6 and 24 hours. 10 Daphnids per test item concentration and the untreated control were exposed to the test solutions for 21 days. The results were evaluated in a semistatic test with concentrations of 0, 0.125, 0.25, 0.5, 1 and 2 mg/L with renewal of test solutions every Monday, Wednesday and Friday. Assessments on immobilisation and other effects were performed each day. Offspring were counted and removed daily after appearance of first brood. Test item concentrations were verified by analysis at each renewal of the fresh test solutions and once a week of aged test solutions. Analytical determinations were performed at 0.125, 0.5 and 2 mg/L and the control. Temperature, pH-value and oxygen concentration of the fresh and aged test solutions was measured at each renewal of the test solutions. Hardness of the test water was measured in the fresh solutions on the days of test solution renewal. Endpoints reported are EC_{50}, NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) for reproduction and mortality of adult Daphnis.

Findings:

Mortality of Adults

At the control and up to 0.500 mg/L no adult Daphnis died during the test. At 1 mg/L a mortality rate of 10 % was observed on day 10 and 20 % on day 13. At 2 mg/L 10 % mortality was observed on day 5. Mortalities were all within the validity criteria for the control.

Reproduction

On the basis of the sum of alive offspring per adult at the end of the test, the number of offspring decreased significantly with increasing concentration levels between 0.25 mg/L and 1 mg/L. At 2 mg/L no significant inhibition of reproduction was observed.

The NOEC (21 d) for inhibition of reproduction was determined to be 0.039 mg/L and 0.09 mg/L for 8-HQ and Beltanol-L (calculated) respectively. The EC_{50} for mortality of adults (21d) was determined to be >0.73 mg/L and > 1.7 mg/L of 8-HQ and Beltanol-L (calculated) respectively. The NOEC (21 d) for mortality of adults was observed at 0.73 mg/L and 1.7 mg/L of 8-HQ and Beltanol-L (calculated) respectively.

The first offspring at the control and all concentration levels was observed on day 11.

Table 64: ECx- values of daphnia exposed to Beltanol-L

	8-HQ (TWA) [mg/L]	Beltanol-L* [mg/L]
EC_{50} (mortality of adults)	> 0.73	> 1.7
NOEC(mortality of adults)	0.73	1.7
NOEC (reproduction)	0.039	0.09
LOEC (reproduction)**	**0.103****	**0.25****

*** Based on a 42.94% of 8-HQ content .**
**** Nominal concentrations, because these concentrations were not measured by the lab.**

The total hardness (as $CaCO_3$) of the test water at the day of test solution preparation was between 11 and 12°dH. The mean pH-value of the untreated control was determined to be 8.23 ± 0.47. The mean temperature was measured to be 20.4 ± 0.3°C and the mean oxygen

saturation was determined to be 104 ± 16 %. The test item had no influence on the pH-value of the test solutions.

Analytical Determinations

The chemical analysis of samples was done in accordance with SANCO/3029/99 rev.4.

The mean content of 8-HQ in the fresh test solutions was 104% of the nominal concentration of 8-HQ, however the measured concentrations of 8-HQ in the aged test solutions ranged from 56.1% to 74.4% (based on geomeans) of fresh medium concentration. Due to these losses the TWA's (Time Weighted Average) have been calculated for every concentration measured. Therefore the Beltanol-L endpoints have been expressed as calculated from TWA values multiplied by geomean of the % of the measured 8-HQ in the fresh medium (in this case 42.94%).

Only 3 tested concentrations were measured, 0.25mg/L and 1 mg/L concentrations were excluded from calculations by the lab, no explanation has been reported. Although, these exclusions had no effects on the outcome of the study.

Table 65: Losses calculations for the 8-HQ nominal concentration of 0.0515 mg/L corresponding to a nominal Beltanol-L concentration of 0.125 mg/L.

Day	Fresh 8-HQ (measured)	Day	Aged 8-HQ (measured)	% respect to fresh medium	
0	0.0525				
2	0.049				
5	0.0543	5	0.0452	92.24	
7	0.0498				
9	0.0516				
12	0.0551				
14	0.0268	14	0.0359	65.15	
16	0.0514				
19	0.0614	19	0.0147	28.6	
21		21	0.0353	57.49	
					% losses
			Geomean	**56.07**	**43.93**

Table 66: Losses calculations for the 8-HQ nominal concentration of 0.206 mg/L corresponding to a nominal Beltanol-L concentration of 0.5 mg/L.

Day	Fresh 8-HQ (measured)	Day	Aged 8-HQ (measured)	% respect to fresh medium	
0	0.2				
2	0.21				
5	0.202	5	0.181	86.19	
7	0.213				
9	0.206				
12	0.202				
14	0.106	14	0.155	76.73	
16	0.213				
19	0.243	19	0.102	47.89	
21		21	0.145	59.67	
					% losses
			Geomean	**65.93**	**34.067**

Table 67: Losses calculations for the 8-HQ nominal concentration of 0.824 mg/L corresponding to a nominal Beltanol-L concentration of 2 mg/L.

Day	Fresh 8-HQ (measured)	Day	Aged 8-HQ (measured)	% respect to fresh medium	
0	0.844				
2	0.899				
5	0.863	5	0.768	85.43	
7	0.883				
9	0.856				
12	0.877				
14	0.436	14	0.698	79.59	
16	0.841				
19	1.03	19	0.516	61.36	
21		21	0.755	73.3	
					% losses
			Geomean	**74.36**	**25.64**

The arithmetic mean of the losses is 34.55% with respect to the initial concentration in the fresh medium.

As above mentioned TWA's have been calculated due to the losses on the concentrations with respect to the fresh medium. A first order kinetic for the losses has been supposed hence the natural logarithm correction has been applied to know the actual exposure concentrations in the test (Table 68, 70 and 71).

Table 68: TWA calculations for for the 8-HQ nominal concentration of 0.0515 mg/L corresponding to a nominal Beltanol-L concentration of 0.125 mg/L.

Days	[8-HQ] in fresh medium (µg/L)	[8-HQ] in aged medium (µg/L)*	Days	Ln of fresh medium	Ln of aged medium			
0	52.5			3.96				
2	49	**29.44**	2	3.89	3.38	79.73		
5	54.3	45.20	3	3.99	3.81	141.22		
7	49.8	**30.45**	2	3.91	3.42	82.46		
9	51.6	**27.92**	2	3.94	3.33	75.62		
12	55.1	**28.93**	3	4.01	3.36	117.54		
14	26.8	35.90	2	3.29	3.58	89.63		
16	51.4	15.03	2	3.94	2.71	40.70		
19	61.4	**14.70**	3	4.12	2.69	87.95		
21		**35.30**	2		3.56	94.30		
geomean	**53.02**	∑	**21**		∑	**809.16**	**TWA**	**38.53**

*** Bold numbers on aged medium column have been calculated based on a geomean of 56.07% (see Table 65)**

Table 69: TWA calculations for the 8-HQ nominal concentration of 0.206 mg/L corresponding to a nominal Beltanol-L concentration of 0.5 mg/L.

Days	[8-HQ] in fresh medium (µg/L)	[8-HQ] in aged medium (µg/L)*	Days	Ln of fresh medium	Ln of aged medium			
0	200			5.30				
2	210	**131.86**	2	5.35	4.88	327.14		
5	202	181	3	5.31	5.20	585.42		
7	213	**133.18**	2	5.36	4.89	330.41		
9	206	**140.43**	2	5.33	4.94	348.41		
12	202	**135.82**	3	5.31	4.91	505.44		
14	106	155	2	4.66	5.04	354.93		
16	213	**69.89**	2	5.36	4.25	173.39		
19	243	102	3	5.49	4.62	452.25		
21		145	2		4.98	379.60		
geomean	**211**	∑	**21**		∑	**3456.99**	**TWA**	**164.62**

*** Bold numbers on aged medium column have been calculated based on a geomean of 65.93% (see Table 66).**

Table 70: TWA calculations for the 8-HQ nominal concentration of 0.824 mg/L corresponding to a nominal Beltanol-L concentration of 2 mg/L.

Days	[8-HQ] in fresh medium (µg/L)	[8-HQ] in aged medium (µg/L)*	Days	Ln of fresh medium	Ln of aged medium			
0	844			6.74				
2	899	**627.60**	2	6.80	6.44	1460.93		
5	863	768.00	3	6.76	6.64	2495.34		
7	883	**641.73**	2	6.78	6.46	1493.82		
9	856	**656.60**	2	6.75	6.49	1528.44		
12	877	**636.52**	3	6.78	6.46	2222.55		
14	436	698.00	2	6.08	6.55	1568.20		
16	841	**324.21**	2	6.73	5.78	754.70		
19	1030	516.00	3	6.94	6.25	1995.97		
21		755.00	2		6.63	1770.79		
geomean	**885**	∑	**21**		∑	**15290.73**	**TWA**	**728.13**

*** Bold numbers on aged medium column have been calculated based on a geomean of 74.36% (see Table 67).**

Calculation of the NOEC and LOEC values for mortality

The EC_{50}, LOEC and NOEC for mortality were determined to be > 1.7 mg/L, > 1.7 mg/L and 1.7 mg/L respectively for calculated Beltanol-L concentrations. For 8-HQ measured concentrations the EC_{50} and NOEC were >0.73 and 0.73 respectively (see Table 73 below)

Table 71: Mortality rates of adult animals in percentage.

	Nominal concentrations of Beltanol-L (mg/L)					
Day	Control	0.125	0.25	0.5	1	2
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	10	0	0	0	0	0
5	10	0	0	0	0	10
6	10	0	0	0	0	10
7	10	0	0	0	0	10

8	10	0	0	0	0	10
9	10	0	0	0	0	10
10	10	0	0	0	10	10
11	10	0	0	0	10	10
12	10	0	0	0	10	10
13	10	0	0	0	20	10
14	10	0	0	0	20	10
15	10	0	0	0	20	10
16	10	0	0	0	20	10
17	10	0	0	0	20	10
18	10	0	0	0	20	10
19	10	0	0	0	20	10
20	10	0	0	0	20	10
21	10	0	0	0	20	10

Calculation of the NOEC and LOEC values for reproduction

In Table 72 the mean number of alive offspring per a1ive adult and replicate over the period from day 0 to day 21 are presented.

Table 72: Number of alive offspring (0d - 21d) per alive adult and replicate, reduction of reproduction (%).

Beltanol-L concentration tested (mg/L)		**Mean number of alive offspring per adult**	**Inhibition (%)**
Nominal	**Calculated***		
Control	Control	99.6	-
0.125	**0.09**	71.8	27.9
0.25	**-**	55.2	44.6
0.5	**0.38**	54.2	45.6
1	**-**	53.3	46.5
2	**1.7**	67.7	32.0

*** Based on an actual geomean content of a 42.92% of 8-HQ.**

Comparison of the test item quotients (number of all alive offspring per number of alive adults per replicate) to the control quotients showed statistically significant decrease of reproduction at 0.25, 0.5 and 1mg/L Beltanol-L. No effects were observed at 1.7 mg/L Beltanol-L. However, due to the fact that significant differences were determined at 0.25, 0.5 and 1 mg/L Beltanol-L the LOEC and NOEC for reproductions were set at 0.25 mg/L and 0.09 mg/L (see Table 73 below) respectively for nominal and calculated Beltanol-L concentrations. For 8-HQ measured concentration the NOEC was 0.039 mg/L (see Table 73 below).

The EC_{50} for reproduction was not determinable due to data structure.

Conclusion:

The NOEC (21 d) for inhibition of reproduction was determined to be 0.039 mg/L and 0.09 mg/L for 8-HQ and Beltanol-L (calculated) respectively. The EC_{50} for mortality of adults (21d) was determined to be >0.73 mg/L and > 1.7 mg/L of 8-HQ and Beltanol-L (calculated) respectively. The NOEC (21 d) for mortality of adults was observed at 0.73 mg/L and 1.7 mg/L of 8-HQ and Beltanol-L (calculated) respectively.

Table 73: EC_{50}, LOEC and NOEC calculations for mortality and reproduction

	8-HQ (TWA) [mg/L]	Beltanol-L* [mg/L]
EC_{50} (mortality of adults)	> 0.73	> 1.7
NOEC (mortality of adults)	0.73	1.7
NOEC (reproduction)	0.039	0.09
LOEC (reproduction)**	**0.103****	**0.25****

*** Based on a 42.49% of 8-HQ content.**
**** Nominal concentrations, because these concentrations were not measured by the lab.**

5.4.3 Algae and aquatic plants

The toxicity of 8-HQ to algae and aquatic plants is summarised in Table 74.

Table 74: Acute and long-term toxicity of 8-HQ to algae and aquatic plants

Guideline / Test method	Species	Exposure		Results		Reference
		Design	Duration, tested substance	Endpoint	Value (mg/L)	
OECD 201	*Desmodesmus subspicatus*	static	72h, Beltanol-L	E_bC_{50} E_rC_{50} NOEC	0.60 1.42 0.35	Dengler, D. (2004), Document No: 20031207/01-AADs
OECD 201	*Desmodesmus subspicatus*	static	72h, Beltanol-L* 72h, 8-HQ	E_yC_{50} E_rC_{50} E_rC_{10} E_yC_{10} E_yC_{50} E_rC_{50} E_rC_{10} E_yC_{10}	1.33 1.74 0.66 0.52 0.71 0.54 0.27 0.21	Falk, S. (2011), Document No: S11-02696

*Based on an 8-HQ content of 40.81%

The study from Dengler, D (2004) was not validated due to some irregularities on pH and the possible subestimation of the endpoints, for these reasons it was repeated therefore the study from Falk, S. (2011), can be regarded as the key study for the acute aquatic toxicity of 8-Hydroxyquinoline and hence for classification and labelling. Therefore the study is presented in more detail below:

Toxicity of 8-HQ to *D. subspicatus*

Author: Falk, S. (2011)

Report: Testing of toxic effects of Beltanol-L on the single cell green alga *D. subspicatus.*

Report No.: S11-02696

Guidelines: OECD 201

Deviations: Minor

GLP/GEP: Yes

Validity: Acceptable

The aims of this study is to determine the effects of 8-HQ on the single cell green algae *D. subspicatus* within the framework of laws for registration according to OECD Guideline 201 was assessed.

Material and methods:

Beltanol-L, Batch number: 11060502 (content of, 8-HQS (8-Hydroxyquinoline sulphate), a.i. 49.85% (w/v), actual content of 8-HQ of 40.81%); Test system: *D. subspicatus.* Initial concentration of 0.5 x 10^4 cells/ml. in each test vessel, were exposed in a static test system for 3 days. No range-finding test was performed. The main test was performed with the Beltano-L nominal concentrations 0.278, 0.833, 2.50, 7.50 and 22.5 mg/L. Six replicates were performed for the control and three for each test item concentration. The test was performed in 500 mL Erlenmeyer flasks under continuous illumination at 5000 - 6000 lux at cell culture level.

The final volume in each test vessel was approx. 167 mL. After 1, 2 and 3 days, the cell growth was determined by fluorescence detection, The mean value of the cell concentration was plotted versus time to produce growth curves for each concentration. Endpoints reported are the EC_x values for growth rate (E_rC_{50}) and yield (E_yC_{50}) calculated by graphic approach. EC_{50} and EC_{10} were measured after 72 h. The temperature was recorded after 0, 24, 48 and 72 hours and the pH values of the test solutions were measured after O and 72 h. Analytical samples were taken at 0 hours (initial value) from fresh test solution and after 24, 48 and 72 hours from aged test solution. All test concentrations and control were analysed at t = 0 and t = 72 hours to verify test concentrations.

Findings:

Table 75: EC_x for growth rate and biomass inhibition.

Endpoint	**8-HQ (mg/L)**	**Beltanol-L (c) (mg/L)**
E_vC_{50} (72 h)	0.54	1.33
E_rC_{50} (72 h)	0.71	1.74
E_rC_{10} (72 h)	0.27	0.66
E_vC_{10} (72 h)	0.21	0.52

*** Based on a 40.81% of 8-HQ content .**

Analytical Determinations

The actual concentration of 8-HQ were the 103% of the nominal concentrations, however the measured concentrations of 8-HQ at the end of test was 77.3 % (based on geomeans between fresh and aged medium) of the initial concentrations in the medium; therefore the toxicological endpoints were evaluated using actual calculated concentrations based on geomeans.

Table 76: 8-HQ actual calculated concentrations.

Beltanol-L (nominal) (mg/L)	8-HQ (mg/L) fresh medium	8-HQ (mg/L) aged medium	% respect to the fresh medium		8-HQ (mg/L) geomean	% of 8-HQ in fresh medium respect to Beltanol-L
0.278	0.115	0.075	65.22		0.093	41.37
0.833	0.343	0.211	61.52		0.269	41.18
2.5	1.06	0.795	75		0.918	42.4
7.5	3.03	2.62	86.47		2.818	40.4
22.5	8.73	9.27	106.19		8.996	38.8
				% of losses		
		geomean	**77.32**	**22.68**	**geomean**	**40.81**

Calculations of endpoints for growth rate and biomass inhibition

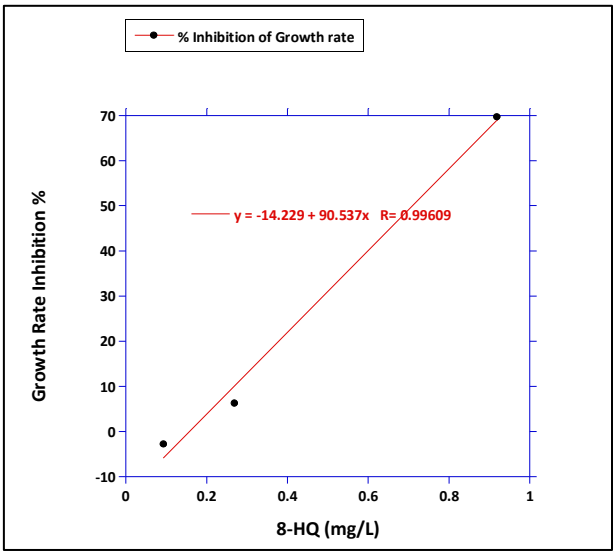
In the Table 77 are shown the percentage of inhibition for the different concentration tested in the main test.

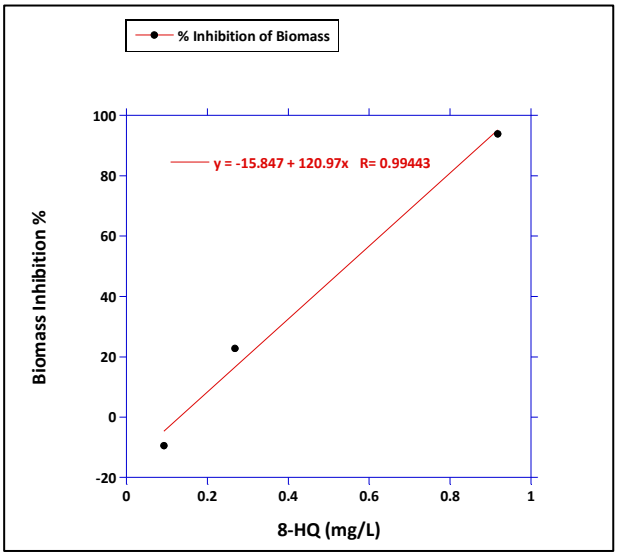
Table 77: Percetage of inhibition of growth rate and yield in the main test.

Beltanol-L (nominal) (mg/L)	8-HQ (mg/L) geomean	Growth rate inhibition %			Yield inhibition %		
		0-1d	0-2d	0-3d	0-1d	0-2d	0-3d
Control	Control	0.0	0.0	0.0	0.0	0.0	0.0
0.278	0.093	-4.8	-1.6	-2.8	-9.7	-3.6	-9.4
0.833	0.269	4.5	0.5	6.3	4.6	0.7	22.8
2.5	0.918	-0.9	49.8	69.7	-2.9	76	93.9
7.5	2.818	5.6	195.2	n.c.	9.7	106.7	106.9
22.5	8.996	37.8	157.1	n.c.	78.9	113.6	110.2

n.c. not calculate due to negative cell numbers

The endpoints were calculated by graphical approach instead of a probit analysis using the 8-HQ geomean measured concentration values versus the % of inhibition, below you can see the plots and the equation curve fits for growth rate and biomass inhibition (left and right plot respectively). To calculate the endpoints only the linear part of the curve was used. For this reason E_xC_{10} was calculated instead of the $NOEC_x$.





Conclusion:

The E_yC_{50} after 72 h was 1.33 mg/L and the E_rC_{50} (72 h) was 1.74 mg/L for Beltanol-L (calculated from an 8-HQ content of 40.81%). Expressed in terms of measured 8-Hydroxyquinoline, the E_yC_{50} and E_rC_{50} were 0.54 and 0.71 mg/L, respectively.

The E_yC_{10} and the E_rC_{10} (72 h) were 0.52 and 0.66 mg/L of Beltanol-L respectively (calculated from an 8-HQ content of 40.81%), corresponding to and E_rC_{10} and E_yC_{10} of 0.27 and 0.21 mg/L of measured 8-Hydroxyquinoline respectively.

Table 78: EC_x for growth rate and biomass inhibition.

Endpoint	8-HQ (mg/L)	Beltanol-L (c) (mg/L)
E_yC_{50} (72 h)	0.54	1.33
E_rC_{50} (72 h)	0.71	1.74
E_rC_{10} (72 h)	0.27	0.66
E_yC_{10} (72 h)	0.21	0.52

*** Based on a 40.81% of 8-HQ content .**

5.4.4 Other aquatic organisms (including sediment)

No information available.

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

8-Hydroxyquinoline is assessed as very toxic to aquatic life with long lasting effects, based on the acute toxicity data to algae and estimation of degradation for the substance.

8-Hydroxyquinoline was found to be not readily biodegradable according to the OECD 301 D (EC Method C.4-E. Part VI) Closed Bottle Test.

8-Hydroxyquinoline has a log K_{OW} of 2.395 (20ºC, pH = 6.8) and is not considered to have potential for bioaccumulation. No BCF study was conducted, since it is required if log P_{OW} >3.

8-Hydroxyquinoline shows the lowest acute toxicity to algae 72h E_rC_{50} = 0.71 mg/L.

Conclusion of environmental classification according to Regulation (EC) No 1272/2008

In aquatic toxicity studies, the lowest acute relevant value for 8-Hydroxyquinoline was obtained for algae. 72 h E_rC_{50} = 0.71 mg/L and a fish 28 days NOEC of 0.01 mg/L.

8-Hydroxyquinoline was found to be not rapidly biodegradable (for details please refer to Point 5.1).

Classification according to Regulation (EC) No 1272/2008 is required.

Acute Category 1, M factor of 1.

Chronic Category 1, M factor of 10.

GHS Pictogram

Signal Word
Warning

Hazard Statement
H410: Very toxic to aquatic life with long lasting effects.

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

8-Hydroxyquinoline does fulfil the criteria for classification.

Classification: Acute Category 1 with an M factor of 1 and Chronic Category 1 with an M factor of 10, according to Regulation (EC) No 1272/2008 is required.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed the environmental hazard classification Aquatic Acute 1 - H400 with an M-factor of 1 based on acute aquatic toxicity to the alga *Desmodesmus subspicatus* (72 h E_rC_{50} = 0.71 mg/L), and Aquatic Chronic 1 - H410 with an M-factor of 10, based on chronic aquatic toxicity to the fish *Oncorhynchus mykiss* (28 d NOEC = 0.01 mg/L) combined with a lack of rapid degradation.

Comments received during public consultation

One MSCA agreed with the classification but suggested that "as no valid chronic data are available for algae, a chronic classification should be considered based on the lowest NOEC as well as on the lowest LC_{50}". This was based on a misunderstanding, as a valid E_rC_{10} value is available for algae.

Another MSCA asked for better justification of the use of data for the 'Beltanol-L' formulation (an approximate 50% w/w solution of the sulphate salt) to fulfil the aquatic ecotoxicity endpoints. The DS re-iterated the statement from the first paragraph of section 5.4 of the CLH report that the toxicity endpoints were based on the measured concentrations of 8-hydroxyquinoline in the tests but did not provide a justification for conducting the ecotoxicity tests with the formulation rather than the active substance.

In addition, the same MSCA asked for a case to be made to justify the use of the 28 days juvenile fish growth test as a chronic endpoint, suggesting that the surrogate approach to chronic fish classification could also be used as a confirmatory check. The DS replied that a chronic NOEC/EC_{10} will be at least equal to that for subchronic effects, and so did not modify the environmental classification proposal.

RAC's view on both of these elements is included below.

Supplemental information - In depth analyses by RAC

QSAR predictions

ECOSAR v1.11 classifies the substance in the class "Phenols" and produces the following predictions:

Fish acute toxicity:	LC_{50} (96h) = 45 mg/L
Daphnia acute toxicity:	LC_{50} (48h) = 11 mg/L
Algae acute toxicity:	EC_{50} (96h) = 56 mg/L

Daphnia chronic toxicity: NOEC = 1.6 mg/L

Algae chronic toxicity: NOEC = 18 mg/L

Fish chronic toxicity: NOEC = 3 mg/L

The predictions for acute aquatic toxicity are considered to be within the domain of the models. The models for chronic aquatic toxicity do have a smaller training set and it should be considered that these predictions do have a higher uncertainty.

TOPKAT produces the following predictions:

Fish acute toxicity: LC_{50} = 0.04 mg/L (However some structural features of the target were not found in the training set. Therefore this prediction is not in the optimal prediction space.)

Daphnia acute toxicity: EC_{50} = 5.7 mg/L (This prediction is in the optimal prediction space.)

Danish QSAR database (Multicase models) produces the following predictions:

Fish acute toxicity: LC_{50} = 18 mg/L

Daphnia acute toxicity: LC_{50} = 7 mg/L

Algae acute toxicity: EC_{50} = 122 mg/L

The algae predicition is within the domain of the model while some uncertainties are flagged for the fish and *Daphnia* predictions as they are not considerd fully in the domain of the models.

Additional key elements

RAC notes that there is a registration for this substance under REACH, but only as an intermediate with no (eco)toxicity data.

8-Hydroxyquinoline is a monoprotic bidentate chelating agent containing two functional groups that can ionise. In the CLH report a measured pKa of 4.88 and 9.45, respectively, at 25 °C were cited. Furthermore, there is no discussion of the influence of ionisation on the properties of the substance, but in the reporting table attached to the CLH report the DS states that "between pH 5.88 and pH 8.45 ... approximately 90% of the substance is in the unionised form". In neutral solution, the hydroxyl group is in the protonated (i.e. unionised) form (pKa≈9-10) and the nitrogen atom is also not protonated (pKa≈5) (i.e. unionised) (Albert & Phillips, 1956). However, an excited-state zwitterionic isomer exists in which a proton is transferred from the hydroxyl group (giving an oxygen anion) to the nitrogen atom (giving a protonated nitrogen cation). Some of the available studies used a sulphate salt in which the substance will be ionised. This introduces some uncertainty as to whether the results of studies using the sulphate salt reflect the properties of the parent substance in terms of its bioavailability (ionised compounds are usually less bioavailable than neutral forms, and so are potentially less toxic).

The DAR makes it clear that the tests have been conducted using the form of the substance sold to the end-user. However, this does not necessarily apply to all potential uses of the substance. The active substance approved and included in Annex I of Regulation (EU) No. 993/2011 is 8-hydroxyquinoline and not the sulphate salt. The DS did not provid any explanation about the (eco)toxicological equivalence of the salt and parent substance, but in the DAR it is stated that they are dissociated in water as different ionic species depending on pH, which implies that there could be a difference.

Assessment and comparison with the classification criteria

Degradation

8-Hydroxyquinoline is hydrolytically stable after 5 days at 50 °C at pH 4 and 7, and also pH 9 in the absence of oxygen (< 10% degradation). In the CLH report it is not explain what happens when oxygen is present, but given the low hydrolysis rates at other environmentally relevant pHs, this is not important. Aqueous photolysis was not investigated since the UV absorption maximum is below 290 nm (a 28 d photodegradation study in soil also indicated no significant degradation). The substance was not readily biodegradable according to an OECD 301D Closed Bottle Test, achieving 6.6% removal after 28 days. However, degradation in the toxicity control was below 25% after 14 days so toxic effects cannot be excluded (N.B. the substance acts as a fungicide and bactericide).

Simulation tests in two aerobic water-sediment systems at 20 °C using radio-labelled substance as the sulphate salt indicated the formation of numerous metabolites (though none of them above 10% of the applied radioactivity), with a first order degradation DT_{50} value for the whole system of 99 – 266 days, and relatively little mineralisation over 100 days (4.3 – 10.4% of applied radioactivity). Based on the lack of hydrolysis and whole system degradation half-lives exceeding 16 days in aquatic simulation studies with limited mineralisation, RAC agrees with the DS's proposal that 8-hydroxyquinoline does not meet the criteria for being rapidly degradable in the environment. (Aerobic soil simulation studies indicated that the substance rapidly forms bound residues (DT_{50} < 1 day), but this appears to be less relevant for the aquatic compartment. In any case, mineralisation to carbon dioxide was low, accounting for about 10% of applied radioactivity after 120 days).

Bioaccumulation

The n-octanol/water partition coefficient (log K_{OW}) is in the range 1.26 – 1.95 at 22 °C and pH 4.1 – 9.1. Since the log K_{OW} is below 4, the substance does not meet the bioaccumulation criteria of the CLP Regulation.

Aquatic Toxicity

The substance is used as a fungicide and bactericide. The lowest reliable ecotoxicity results reported in the CLH report were as follows (the key data are highlighted in bold):

Trophic level	Species	Short-term result	Long-term result
Fish	Rainbow Trout *Oncorhynchus mykiss*	96 h LC_{50} = 2 mg/L[a]	**28 d NOEC ≥ 0.01 mg/L**[b]
Aquatic invertebrates	*Daphnia magna*	48 h EC_{50} = 3.67 mg/L	21 d NOEC = 0.039 mg/L[c]
Aquatic algae and plants	*Desmodesmus subspicatus*	**72 h E_rC_{50} = 0.71 mg/L**[c]	72 h E_rC_{10} = 0.27 mg/L[d]

Note: a Additional data identified by RAC in the open literature includes two additional results indicating the same or lesser toxicity, but also an LC_{50} of 0.015 mg/L for Guppy *Poecillia reticulata* (duration/test guideline not specified) (Katritzky *et al*., 2001). It has not been possible to validate this result.

b This study was based on OECD TG 204 & 215, and the end points of body weight and length. The results are based on time-weighted average concentrations, due to losses of around 40% in test concentrations over 28 days. The reported value is ≥ 0.01001 mg/L.

c The results are based on time-weighted average concentrations, due to losses of up to 44% in test concentrations over one week.

d The results are based on geometric mean concentrations using measurements made at the beginning and end of the test (after which there had been around a 23% loss in test concentration).

The tests were performed using an aqueous formulation ('Beltanol-L') of the sulphate salt of the substance, with the results provided in terms of measured concentrations of the parent substance. Apart from the concentration of the salt and parent compound, no further composition details are provided in the CLH report itself. In the reporting table attached to the CLH report the DS stated that: "The formulation is a solution of [the substance] and sulfuric acid in water". The water solubility of this salt is not stated in the CLH dossier, but EFSA (2011) indicated that it is 773 g/L (at 20 °C), which is at least 300 times higher than the parent compound (0.7 – 2.4 g/L at 20 ºC and environmentally relevant pH). In the CLH dossier no justification was provided for the use of the salt for testing the properties of the parent substance, and the DS did not provide any additional information in their response to public comments. RAC notes that the bioavailability of the parent molecule might be lower when it is present as the sulphate salt (due to differences in ionisation) i.e. the non-ionised form might possibly be more toxic. No information seems to be available on this issue in the CLH report. Given the low effect concentrations, RAC notes that differences in bioavailability (if they exist) will not affect the environmental classification, but could influence the M-factors. Estimates of acute and chronic aquatic toxicity for the neutral molecule using quantitative structure-activity relationships (QSARs, see further RAC analysis below) provided some reassurance that the neutral molecule is not more toxic than suggested by the available test data.

In addition, EFSA (2011) indicated that at the water solubility limit of the sulphate salt, the pH of the solution is 1.57. Aqueous solutions might therefore be acidic, although the test substance had no influence on the pH-value of the test solutions in the fish and *Daphnia* tests. No information was provided about the influence on pH for the algal data in the CLH dossier, except that one earlier study (Dengler, 2004) was not validated due to "some irregularities on pH and the possible subestimation of the endpoints". EFSA (2011) mentioned that a steep increase in pH was observed in algal studies, which could have led to a potential underestimation of algal toxicity, and this appeared to relate to the Dengler (2004) study. In the Falk (2011) study report (provided by the DS) it was indicated that acidity increased with test concentration, but that the pH increased in a somewhat random manner during the course of the study, as indicated in the table below.

Initial test concentration, mg/L (nominal)	**pH**	
	t = 0	**t = 3 d**
Control	6.63	7.56
0.278	6.57	7.89
0.833	6.52	8.13
2.50	6.47	7.33
7.50	6.44	7.44
22.5	6.28	7.37

The DS commented that the pH at the start (6.28 – 6.63) and the end (7.33 – 8.13) of this study was below that of the Dengler (2004) study (7.9 – 8.3 at the start, 8.3 – 10.9 at the end). The Falk (2011) study is therefore more reflective of neutral pH conditions, whereas the Dengler (2004) study reflects more alkaline conditions.

If an outdoor use is applied for at MS level, a new study with algae may be required, which could affect the classification in future.

As highlighted in the public consultation, the study used to fulfil the long-term fish toxicity end point is a fish juvenile growth test. No significant effects were observed at the highest test concentration. Although this method is considered to be of insufficient duration to examine all the sensitive points in the fish life-cycle, it provides a shorter and less expensive option to an early life stage test for substances with log K_{OW} < 5 (such as 8-hydroxyquinoline). In the REACH Guidance on Information Requirements and Chemical Safety Assessment (Chapter R.7b: Endpoint specific guidance, Version 2.0) it is indicated that this test can be accepted on a case-by-case basis if there are well founded justifications suggesting that growth inhibition is the most relevant effect in fish for the

assessed substance. No such justification was offered in the CLH report or in the DS reply to the public comments. RAC does not think that the statement from the DS that the chronic NOEC/EC_{10} will be at least equal to that for subchronic effects is useful, since the result is a "greater than or equal to" value. It is therefore not known if effects might occur at lower concentrations for other life stages. In view of this uncertainty, RAC therefore considers that it is appropriate to also consider the surrogate approach for chronic classification for the fish trophic group, as a supporting line of evidence.

Classification according to CLP

Acute aquatic hazard: Reliable acute aquatic toxicity data are available for the three trophic levels fish, aquatic invertebrates and algae. The lowest reliable short-term aquatic toxicity result is a 72 h E_rC_{50} of 0.71 mg/L for the green alga *D. subspicatus*. This concentration is below the threshold value of 1 mg/L, so 8-hydroxyquinoline is classifiable as Aquatic Acute 1 - H400. As $0.1 < E_rC_{50} \leq 1$ mg/L, the acute M-factor is 1, as proposed by the DS.

Long-term aquatic hazard: Reliable long-term aquatic toxicity data are available for aquatic invertebrates and algae. As discussed above, the long-term fish toxicity study is reliable, but potentially does not cover sensitive life stages. The lowest long-term aquatic toxicity result is a 28 d NOEC of ≥ 0.01001 mg/L for the fish *O. mykiss*. 8-hydroxyquinoline is not rapidly degradable, and as this concentration is below the threshold value of 0.1 mg/L, the substance is classifiable as Aquatic Chronic 1 - H410. As the NOEC exceeds 0.01 mg/L (albeit only just), the chronic M-factor is 1 (not 10, as proposed by the DS).
RAC notes that the NOEC is based on the highest concentration tested (i.e. the true NOEC could be higher). If the surrogate approach were used, i.e. the long-term hazard classification for fish were based on the acute fish toxicity data (96-h LC_{50} of 2 mg/L), the resulting classification would be Aquatic Chronic 2. The next most sensitive value (for *Daphnia*) is a 21-d NOEC of 0.039 mg/L, which leads to classification as Aquatic Chronic 1 - H410, with an M-factor of 1. The result based on the available chronic aquatic toxicity data is more conservative, so is selected.
In summary, 8-hydroxyquinoline should be classified as:
Aquatic Acute 1 - H400, M=1;
Aquatic Chronic 1 - H410, M=1.

RAC therefore agrees with the DS's proposal with the exception of the chronic M-factor.

Supplemental information - In depth analyses by RAC

QSAR predictions

ECOSAR v1.11 classifies the substance in the class "Phenols" and produces the following predictions:

Fish acute toxicity:	LC_{50} (96h) = 45 mg/L
Daphnia acute toxicity:	LC_{50} (48h) = 11 mg/L
Algae acute toxicity:	EC_{50} (96h) = 56 mg/L
Daphnia chronic toxicity:	NOEC = 1.6 mg/L
Algae chronic toxicity:	NOEC = 18 mg/L
Fish chronic toxicity:	NOEC = 3 mg/L

The predictions for acute aquatic toxicity are considered to be within the domain of the models. The models for chronic aquatic toxicity do have a smaller training set and it should be considered that these predictions do have a higher uncertainty.

TOPKAT produces the following predictions:

Fish acute toxicity: LC_{50} = 0.04 mg/L (However some structural features of the target were not found in the training set. Therefore this prediction is not in the optimal prediction space.)

Daphnia acute toxicity: EC_{50} = 5.7 mg/L (This prediction is in the optimal prediction space.)

Danish QSAR database (Multicase models) produces the following predictions:

Fish acute toxicity: LC_{50} = 18 mg/L

Daphnia acute toxicity: LC_{50} = 7 mg/L

Algae acute toxicity: EC_{50} = 122 mg/L

The algae predicition is within the domain of the model while some uncertainties are flagged for the fish and *Daphnia* predictions as they are not considerd fully in the domain of the models.

6 OTHER INFORMATION

No other data available for consideration in determining the classification of 8-hydroxyquinoline.

7 REFERENCES

- Spain, 2010a, Draft Assessment Report (DAR) on the active substance 8-hydroxyquinoline prepared by the rapporteur Member State Spain in the framework of Directive 91/414/EEC. July 2009. Updated May 2010.
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7.1 Physico-chemical properties

Author(s)	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**
Gil, A.G.	2004a	DETERMINATION OF SOLUBILITY IN WATER AND ORGANIC SOLVENTS OF 8-HQ AND 8-HQS. DETERMINATION OF THE PARTITION COEFFICIENT N-OCTANOL/WATER Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2003/27-A GLP: yes Published: no
Gil, A.G.	2004b	DETERMINATION OF THE HYDROLYSIS RATE OF 8-HQ AND 8-HQS AS A FUNCTION OF PH Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2004/19-A GLP: yes Published: no

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
Gil, A.G.	2010	EFECTO DEL PH EN LA SOLUBILIDAD DE 8- HIDROXIQUINOLEINA EN AGUA. EFECTO DEL PH EN EL COEFICIENTE DE PARTICION POW DE 8-HIDROXIQUINOLEINA (EFFECT OF PH ON WATER SOLUBILITY OF 8-HYDROXYQUINOLINE. EFFECT OF PH ON PARTITION COEFFICIENT POW OF 8- HYDROXYQUINOLINE) Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no.: F/2010/06 GLP: yes Published: no
Gomez, A.G.	2004a	DETERMINATION OF PHYSICAL STATE, COLOUR, ODOUR AND RELATIVE DENSITY IN 8-HQ AND 8-HQS Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2003/25-A GLP: yes Published: no
Gomez, A.G.	2004b	DETERMINATION OF 8-HQ AND 8-HQS DISOCIATION IN WATER. DETERMINATION OF IR, NMR AND MS SPECTRA OF 8-HQ AND 8-HQS Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2004/20-A GLP: yes Published: no
Gomez, A.G.	2010	AMENDMENT TO THE FINAL REPORT INF-F/2004/20-A: DETERMINATION OF THE DISSOCIATION CONSTANT OF 8-HQ AND 8-HQS IN WATER. DETERMINATION OF IR, RMN, MS SPECTRA OF 8-HQ AND 8-HQS Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no.: INF-F/2004/20-A GLP: yes Published: no
Gonzalez, M.B.	2004a	DETERMINATION OF THE MELTING AND THE BOILING POINTS OF 8-HQ. CALCULATION OF THE HENRY'S LAW CONSTANT Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2004/18-A GLP: yes Published: no
Gonzalez, M.B.	2004b	DETERMINATION OF THE MELTING POINT, THE BOILING POINT, THE FLAMMABILITY AND SELF-IGNITION POINT OF 8-HQS Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2004/24-A GLP: yes Published: no
Gonzalez, M.B.	2004c	DETERMINATION OF THE FLAMMABILITY AND SELF-IGNITION POINT OF 8-HQ Laboratorio BPL de Probelte, Spain Probelte S.A. Report-no. INF-F/2003/28-A GLP: yes Published: no

Author(s)	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**
Smeykal, H.	2003	8-HYDROXYQUINOLINE - VAPOUR PRESSURE Sicherheitstechnik Siemens Axiva GmbH & Co. KG, Frankfurt Probelte S.A. Report-no. 20030854.01 GLP: yes Published: no
Smeykal, H.	2004	8-HYDROXYQUINOLINE - EXPLOSIVE PROPERTIES Siemens AG, Prozess-Sicherheit, Frankfurt am Main, Germany Probelte S.A. Report-no. 20040820.01 GLP: yes Published: no
Tiemann, J.	2004	8-HYDROXYQUINOLINE OXIDIZING PROPERTIES OF THE ACTIVE SUBSTANCE GAB Consulting GmbH, Lamstedt, Germany Probelte S.A. Report-no. 115123-A2-0215-01 GLP/GEP: no Published: no
Walter, D.	2004	SURFACE TENSION OF 8-HYDROXYQUINOLINE GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Probelte S.A. Report-no. 20041384/01-PCST GLP: yes Published: no

7.2 Toxicology and metabolism

Author(s)	**Year**	**Title** **Testing Facility** **Owner / Source (where different from owner)** **Report No** **GLP or GEP status (where relevant)** **Published or not**
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August, M.	2007a	IN VIVO MAMMALIAN SPERMATOGONIAL CHROMOSOME ABERRATION TEST OF 8-HYDROXYQUINOLINE IN NMRI MOUSE BY ORAL ADMINISTRATION LPT Lab. of Pharmacology and Toxicology KG, Hamburg, Germany Probelte S.A. Report-no. 19589/05 GLP: yes Published: no

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Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
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Bulnes Goicochea, C.	2004	DOSE RANGE-FINDING STUDY FOR REPEATED DOSE 90-DAY ORAL TOXICITY STUDY IN RATS (RATTUS NORVEGICUS) FOR 8-HYDROXYQUINOLINE BioAgri Laboratórios, NL-2582 AB Den Haag Probelte S.A. Report-no. PS - 3154.307.017.04 GLP: yes Published: no
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ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
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ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
Fascineli, M.L.	2006d	PRENATAL DEVELOPMENTAL TOXICITY STUDY IN NEW ZEALAND WHITE RABBITS FOR 8-HYDROXYQUINOLINE BIOAGRI Laboratórios, Planaltina/DF - 73301-970 - Brazil Probelte S.A. Report-no. RF-3154.315.009.04 GLP: yes Published: no
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Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
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Metzner, H.H.	1987	KONTAKTSENSIBILISIERUNGEN DURCH TOSYLCHLORAMIDNATRIUM (CHLORAMIN) UND HYDROXYCHINOLIN (SULFACHIN) not applicable Dermatol Monatsschrift, 173, 674-677 Report-no. not applicable GLP/GEP: no Published: yes
Neurath, G.	2007	SUMMARY OF MAMMALIAN TOXICITY AND OVERALL EVALUATION GAB Consulting GmbH, Lamstedt, Germany Probelte S.A. Report-no. 115123-A2-0510-01 rev. 1 GLP/GEP: no Published: no
Neurath, G.	2010	8-HYDROXYQUINOLINE EVALUATION OF THE THYROID EFFECT OBSERVED IN THE 90-DAY STUDY IN BEAGLE DOGS GAB Consulting GmbH, Lamstedt, Germany Probelte S.A. Report-no. 123115-A2-050302-01 GLP/GEP: not applicable Published: no

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
NTP National Toxicology Program	1985	TOXICOLOGY AND CARCINOGENESIS STUDIES OF 8-HYDROXYQUINOLINE (CAS NO. 148-24-3) IN F344/N RATS AND B6C3F1 MICE (FEED STUDIES) National Institute of Health, USA US NTIS PB 85-213361, Springfield, VA., 1-170 Report-no. NTP TR 276 GLP/GEP: no Published: yes
Pevny, I., Schellenberg, J.	1971	SENSIBILISIERUNGEN UND GRUPPENSENSIBILISIERUNGEN DURCH CHINOLINDERIVATE not applicable Der Hautarzt, 22, 13-18 Report-no. not applicable GLP/GEP: no Published: yes
Rothe, A.	1978	HYDROXYCHINOLIN - EIN SCHWACHES KONTAKTALLERGEN not applicable Medicamentum, 19, 366-367 Report-no. not applicable GLP/GEP: no Published: yes
Stelter, D.	2008a	ACUTE DERMAL IRRITATION/CORROSION WITH 8-HYDROXYQUINOLINE BSL Bioservice. Scientific Laboratories. GmbH, Germany Probelte, S.A. Project no. 082383 GLP/GEP: Yes Published: no
Stelter, D.	2008b	ACUTE EYE IRRITATION/CORROSION WITH 8-HYDROXYQUINOLINE BSL Bioservice. Scientific Laboratories. GmbH, Germany Probelte, S.A. Project no. 082382 GLP/GEP: Yes Published: no
Zeiger, E., Anderson, B., Haworth, S. et al	1988	SALMONELLA MUTAGENICITY TESTS: IV RESULTS FROM THE TESTING OF 300 CHEMICALS not applicable Environmental and Molecular Mutagenesis, 11, Suppl. 12, 1-158 Report-no. not applicable GLP/GEP: no Published: yes

7.3 Environment

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
Adelberger, I.	2003	BELTANOL-L: TOXICITY TO THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) IN THE LABORATORY (RATE RESPONSE TEST) ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-NLTp GLP: yes Published: no
Dengler, D.	2004	TESTING OF TOXIC EFFECTS OF BELTANOL-L ON THE SINGLE CELL GREEN ALGA DESMODESMUS SUBSPICATUS (FORMERLY SCENEDESMUS SUBSPICATUS) ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-AADs GLP: yes Published: no
Dengler, D.	2005	ASSESSMENT OF THE READY BIODEGRADABILITY OF THE 8-HYDROXYQUINOLINE WITH THE CLOSED BOTTLE TEST. Testing laboratory: GAB Biotechnologie GmbH & GAB Analytik GmbH, Germany. Report no.: 20051323/01-AACB. Owner company: Probelte S. A.
Falk, S.	2011	BELTANOL-L – TESTING OF TOXIC EFFECTS ON THE SINGLE CELL GREEN ALGA DESMODESMUS SUBSPICATUS. Testing laboratory: Eurofins Agroscience Services EcoChem GmbH, Germany. Report no.: S11-02696. Owner company: Probelte S. A.
Gonsior, G.	2012	BELTANOL-L - 28- DAY TOXICITY TEST IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE). Testing laboratory: Eurofins Agroscience Services EcoChem GmbH, Germany. Report no.: S11-02694. Owner company: Probelte S. A.
Hennecke, D.	2004a	SOIL PHOTOLYSIS OF 8-HYDROXYQUINOLINE SULFATE. Testing laboratory: Fraunhofer Institut, Germany. Report no.: GAB-004/7-06. Owner company: Probelte S. A.
Hennecke, D.	2004b	AEROBIC TRANSFORMATION OF 8-HYDROXYQUINOLINE SULFATE IN SOIL. Testing laboratory: Fraunhofer Institut, Germany. R Report no.: GAB004/7-15. Owner company: Probelte S. A.
Hennecke, D.	2004c	DETERMINATION OF ADSORPTION/ DESORPTION OF 8-HYDROXYQUINOLINE SULFATE. Testing laboratory: Fraunhofer Institut, Germany. Report no.: GAB-004/7-13. Owner company: Probelte S. A.
Kling, A.	2001	ASSESSMENT OF SIDE EFFECTS OF BELTANOL-L TO THE HONEY BEE, APIS MELLIFERA L. IN THE LABORATORY ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20011181/01-BLEU GLP: yes Published: no

ANNEX 1 – BACKGROUND DOCUMENT TO THE RAC OPINION ON QUINOLIN-8-OL; 8-HYDROXYQUINOLINE

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not
Kölzer, U.	2003	ASSESSMENT OF THE SIDE EFFECTS OF BELTANOL-L ON THE ACTIVITY OF THE SOIL MICROFLORA ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-ABMF GLP: yes Published: no
Leuschner, J.	2003	ACUTE TOXICITY STUDY OF 8-HYDROXYQUINOLINE BY ORAL ADMINISTRATION BY GAVAGE TO BIRDS (JAPANESE QUAIL). Testing laboratory: LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany. Report no.: 16984/1/03. Owner company: Probelte S. A.
Leuschner, J.	2004	ACUTE TOXICITY STUDY OF 8-HYDROXYQUINOLINE BY ORAL ADMINISTRATION VIA THE DIET TO BIRDS (JAPANESE QUAIL) (LIMIT TEST). Testing laboratory: LPT Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany. Owner company: Probelte S. A.
Prata, A.P.	2009	AEROBIC TRANSFORMATION OF 14C-8-HYDROXYQUINOLINE SULFATE IN TWO TYPES OF AQUATIC SEDIMENT SYSTEMS. Testing laboratory: BIOAGRI Laboratórios Ltda. Report no.: 3154.220.001.08. Owner company: Probelte S. A.
Stäbler, D.	2003	ACUTE TOXICITY OF BELTANOL-L ON EARTHWORMS, EISENIA FETIDA USING AN ARTIFICIAL SOIL TEST ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-NLEf GLP: yes Published: no
Stäbler, D.	2004a	ACUTE TOXICITY TESTING OF BELTANOL-L IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE) ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-AAOm GLP: yes Published: no
Stäbler, D.	2004b	ASSESSMENT OF TOXIC EFFECTS OF BELTANOL-L ON DAPHNIA MAGNA USING THE 48 H ACUTE IMMOBILISATION TEST ArGe GAB Biotech/IFU, Niefern-Öschelbronn, Germany Probelte S.A. Report-no. 20031207/01-AADm GLP: yes Published: no
Warmers, C.	2004	TOXICITY TO THE APHID PARASITOID, APHIDIUS RHOPALOSIPHI (HYMENOPTERA, BRACONIDAE) DE STEFANI PEREZ IN THE LABORATORY (DOSE RESPONSE). Testing laboratory: GAB Biotechnologie & IFU Umweltanalytik, Niefern-Öschelbronn, Germany. Report no.: 20031207/01-NLAp. Owner company: Probelte S. A.
Weber, K.	2012	BELTANOL-L - ASSESSMENT OF TOXIC EFFECTS ON *DAPHNIA MAGNA* USING THE 21 DAY REPRODUCTION TEST. Testing laboratory: Eurofins Agroscience Services EcoChem GmbH, Germany. Report no.: S11-02695. Owner company: Probelte S. A.

8 ANNEXES

None.