Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Quinolin-8-ol; 8-hydroxyquinoline

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

CLH-O-0000001412-86-60/F

Adopted

5 June 2015

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5 June 2015

CLH-O-0000001412-86-60/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonized classification and labelling (CLH) of:

Chemical names: **Quinolin-8-ol; 8-hydroxyquinoline**

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

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

The proposal was submitted by **Spain** and received by RAC on **17 September 2014**.
In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Spain has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **26 September 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 November 2014**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Agnes Schulte**

Co-rapporteur, appointed by RAC: **Stephen Dungey**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonized classification and labelling was adopted on **5 June 2015** by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion on Quinolin-8-ol; 8-hydroxyquinoline that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal		Quinolin-8-ol; 8-hydroxyquinoline	205-71 1-1	148-24-3	Repr. 2 Acute Tox. 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H301 H318 H317 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H361d H301 H318 H317 H410		 M = 1 M = 10	
RAC opinion		Quinolin-8-ol; 8-hydroxyquinoline	205-71 1-1	148-24-3	Repr. 1B Acute Tox. 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H301 H318 H317 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H360D H301 H318 H317 H410		 M = 1 M = 1	
Resulting Annex VI entry if agreed by COM		Quinolin-8-ol; 8-hydroxyquinoline	205-71 1-1	148-24-3	Repr. 1B Acute Tox. 3 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H301 H318 H317 H400 H410	GHS05 GHS06 GHS08 GHS09 Dgr	H360D H301 H318 H317 H410		 M = 1 M = 1	

RAC general comment

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

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.

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.

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, therefore no classification is proposed.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS based their evaluation 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).

They 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 DS 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.

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 five human studies (Pevny, 1971 – three studies; Rothe, 1977; Metzner, 1987). The highest percentages of positive response to 8-hydroxyquinoline were observed in 4.7%, 8% and 6% of patients after topical application in the three studies reported 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 human 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 un-selected dermatitis patients and ≥ 2% selected dermatitis patients). Sub-categorisation was considered not to be possible due to the 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 the 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 dermatitis patients were unselected or selected. 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 should 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 but the 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)**.

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.

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.

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 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 not confirmed in either in soma cells or 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.

The evaluations of the genotoxicity data of 8-hydroxyquinoline by the DS and RAC do not differ. Taking into consideration the reliable mutagenicity data, RAC also comes to the conclusion that there is no need for classification of 8-hydroxyquinoline as an *in vivo* mutagen.

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.

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 represent 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 lessions 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 (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**	**3000 ppm**	**Control**	**1500 ppm**	**3000 ppm**
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%)

	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%)

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

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

(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 does not seem sufficient to be considered as 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 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 as unlikely to be related 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, $p \leqslant 0.05$. †† statistically significant trend, $p \leqslant 0.01$. ***** statistically significant by survival-adjusted method (pairwise comparisons), $p \leqslant 0.05$ ****** statistically significant by survival-adjusted method (pairwise comparisons), $p \leqslant 0.01$ ^{F} statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), $p \leqslant 0.05$ $^{F\,F}$ statistically significant by Fisher's exact test for pairwise comparisons (not adjusted for survival differences), $p \leqslant 0.01$

(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 in 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. 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 does not 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.

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 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.

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 the 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 do not 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 this 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 under 3.7.2.1.1 for **Repr. 1B (H360D)** are therefore fulfilled.

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 proposed 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.

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).. 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 8-hydroxyquinoline 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.

Bioaccumulation

The n-octanol/water partition coefficient (log K_{OW}) is in the range 1.26 to 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 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). Given the low effect concentrations, RAC notes that differences in bioavailability are unlikely to 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 under Supplemental information – In depth analyses by RAC in Annex 1) 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.

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} ≤ 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.

RAC therefore agrees with the DS's proposal with the exception of the chronic M-factor that.

8-hydroxyquinoline should be classified as:

Aquatic Acute 1 - H400, M=1;

Aquatic Chronic 1 - H410, M=1.

Additional references

Albert, A. and Phillips, J. N. (1956). 264. Ionization constants of heterocyclic substances. Part II. Hydroxy-derivatives of nitrogenous six-membered ring-compounds. *Journal of the Chemical Society (Resumed)*, 1294–1304. doi:10.1039/JR9560001294.

George K. Symeon GK, Michael Goliomytis M,Iosif Bizelis I, Papadomichelakis G, Pagonopoulou O, Abas Z, Deligeorgis SG, Chadio SE (2015) Effects of Gestational Maternal Undernutrition on Growth, Carcass Composition and Meat Quality of Rabbit Offspring PLoS One. 2015; 10(2): e0118259. Published online 2015 Feb 11. doi: 10.1371/journal.pone.0118259, PMCID: PMC4324905

Katritzky, A.R., Tatham, D.B. and Maran, U. (2001). Theoretical descriptors for the correlation of aquatic toxicity of environmental pollutants by quantitative structure–toxicity relationships. *Journal of Chemical Information and Computer Sciences*, 41, 1162–1176.

Matsuoka T, Mizoguchi Y, Serizawa K, Ishikura T, Mizuguchi H, Asano Y (2006) Effects of stage and degree of restricted feeding on pregnancy outcome in rabbits. J Toxicol Sciences 31:196-175.

Stoll D, Alembik Y, Dott B, Roth M-P (2008) Omphalocele and gastroschisis and associated malformations. Am J Med Gen Part A, Volume 146A:1280-1285.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and the RAC comments (excl. confidential information).