Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

quinoclamine (ISO);
2-amino-3-chloro-1,4-naphthoquinone

EC Number: 220-529-2
CAS Number: 2797-51-5

CLH-O-0000006853-67-01/F

Adopted

17 September 2020

17 September 2020

CLH-O-0000006853-67-01/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 harmonised classification and labelling (CLH) of:

Chemical name: **quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone**

EC Number: **220-529-2**

CAS Number: **2797-51-5**

The proposal was submitted by **Sweden** and received by RAC on **15 May 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **17 June 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **16 August 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

[Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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 harmonised classification and labelling was adopted on **17 September 2020** by **consensus**.

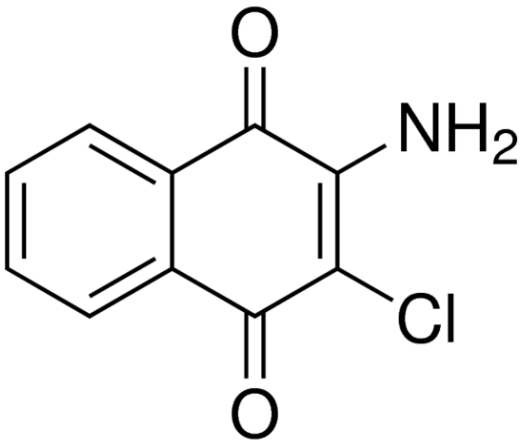
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone	220-529-2	2797-51-5	Carc. 2 Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1A STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361d H302 H319 H317 H373 (blood system, kidney) H400 H410	GHS07 GHS08 GHS09 Wng	H351 H361d H302 H319 H317 H373(blood system, kidney) H410		oral: ATE = 500 mg/kg bw M=10 M=10	
RAC opinion	TBD	quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone	220-529-2	2797-51-5	Carc. 2 Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1A STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361d H302 H319 H317 H373 (blood system, kidney) H400 H410	GHS07 GHS08 GHS09 Wng	H351 H361d H302 H319 H317 H373 (blood system, kidney) H410		oral: ATE = 500 mg/kg bw M=10 M=10	
Resulting Annex VI entry if agreed by COM	TBD	quinoclamine (ISO); 2-amino-3-chloro-1,4-naphthoquinone	220-529-2	2797-51-5	Carc. 2 Repr. 2 Acute Tox. 4 Eye Irrit. 2 Skin Sens. 1A STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H361d H302 H319 H317 H373 (blood system, kidney) H400 H410	GHS07 GHS08 GHS09 Wng	H351 H361d H302 H319 H317 H373 (blood system, kidney) H410		oral: ATE = 500 mg/kg bw M=10 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

RAC notes that the application for renewal of quinoclamine under EU Reg. 1107/2009 was withdrawn by the applicant shortly after submission of the RAR to EFSA in May 2018. Therefore, no recent EFSA peer review has taken place for this substance. Quinoclamine was previously discussed in expert meetings organised by EFSA in March 2007 (EFSA PRAPeR 19) and by experts of the TC C&L (May 2007).



Quinoclamine is a quinone herbicide/algaecide and is very similar to the quinone fungicide dichlone (2,3-dichloro-1,4-naphthoquinone), which is a relevant impurity in quinoclamine technical material. Quinoclamine inhibits photosynthesis by interfering with electron transfer at two target sites: i) inhibiting the D1–protein complex of Photosystem II similar to other herbicides such as triazines or ureas, and ii) binding at the electron-donating side of the Photosystem I.

Quinoclamine has no existing harmonised classification. The representative uses of quinoclamine were against algae and mosses in lawns/turf, ornamentals (outdoors) and nursery stock plants (out- and indoor). Currently (April 2020), it is not registered for use in the EU.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification of quinoclamine for physical hazards. Since the melting point is > 40 °C, the flash point was not considered applicable. According to the DS, the active substance is not flammable, auto flammable nor explosive and has no oxidising properties as determined in accordance with some tests recommended in the data requirements (Reg No 283/2013). However, the DS also stated that there was a lack of data for several physical hazard endpoints which prevented classification in a conclusive manner.

- Quinoclamine was not considered a flammable substance. This was based on a negative screening test in EEC A.10 (*Bates, 2000*), which is valid for CLP.
- Quinoclamine was not considered an explosive substance (*Bates, 2000*). The explosive properties were investigated by calculation of the oxygen balance and comparing bond groupings present in the molecule. Although the oxygen balance was

-161.83 (higher than the CLP criteria for non-explosiveness: -200), quinoclamine does not contain any chemical groups associated with explosive properties as given in section 2.1.4.3(a) of the CLP Regulation. Differential scanning calorimetry (DSC), showed no exothermic processes between 20°C and 600°C with enthalpies near the trigger value for explosion: 500 J/g.

- No information on self-reactivity for quinoclamine was provided (i.e. for substances that undergo a strongly exothermic decomposition even without the participation of oxygen (air)).
- No data on self-ignition for quinoclamine was provided. However, quinoclamine has been handled in air within all studies available in the CLH dossier and there were no reports of self-ignition.
- Quinoclamine was tested for oxidising properties using EEC Method A.17. The test was negative. However, this test is not sufficient to determine oxidising potential under CLP. The DS described how the CLP decision logic could not be followed in this case and that information is insufficient to rule out any oxidising potential. No classification was proposed due to lack of data.
- Quinoclamine is not an organic peroxide.
- There is no data indicating quinoclamine is corrosive to metals. The DS did not propose classification.

Quinoclamine is an orange solid (technical grade, 99.0 % purity). A melting point of 200-202°C was determined for the technical grade substance. The boiling point is 348-350°C. The vapour pressure was determined to be 7 x 10^{-6} Pa at 25°C and 3 x 10^{-6} Pa at 20°C (extrapolated values from measurements at higher temperatures). Quinoclamine has a calculated pKa of 0.93 (deprotonation of the amino group), since no dissociation occurred between pH 2-11. Solubility in water is poor at 20.7 mg/l ± 1.0 mg/l at pH 4 (citrate buffer), 19.8 mg/l ± 0.4 mg/l at pH 8.5 (unadjusted distilled water) and 20.7 mg/l ± 0.7 mg/l at pH 9 (borax buffer). In organic solvents the substance is soluble in acetone (12.2-12.8 g/L) but poorly soluble in several other solvents tested (1,2-dichloroethane, ethyl acetate, n-heptane, methanol and p-xylene, all <10g/L). The log P_{ow} value of the active substance was determined to be 1.58 at 30°C and pH 11.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

The DS did not propose classification for physical hazards. RAC has summarised the available information and conclusions regarding all physical hazards (table below) and agrees with the DS. RAC does not propose classification for physical hazards.

Table: *Summary of physical hazard data and classification conclusions*

Hazard Class	Chapter in CLP criteria Guidance	Comments	Conclusion
Explosives	2.1	Conforms to exclusion criteria. There are no functional groups associated with explosive properties. A calculated oxygen balance greater than -200 was presented but not required in light of no structural alerts for explosive properties. The exothermic decomposition	**No classification, conclusive.**

		energy was shown to be < 500 J/g at > 500°C (303 J/g at 530°C).	
Flammable gases	2.2	Not applicable.	**No classification** (Not applicable)**.**
Flammable aerosols	2.3	Not applicable.	**No classification** (Not applicable)**.**
Oxidising gases	2.4	Not applicable.	**No classification** (Not applicable)**.**
Gases under pressure	2.5	Not applicable.	**No classification** (Not applicable)**.**
Flammable liquids	2.6	Not applicable.	**No classification** (Not applicable)**.**
Flammable solids	2.7	Negative screening test in EEC A.10	**No classification, conclusive.**
Self-reactive substance/mixture	2.8	There are no chemical groups associated with explosive or self-reactive properties as shown in tables A6.1 or A6.3 in appendix 6 of the UN RTDG, Manual of Tests and Criteria. In addition, the exothermic decomposition energy was previously shown to be < 300 J/g (240 J/g) at an onset temperature of 370°C (and slightly above 300 J/g but only at > 500°C).	**No classification, conclusive.**
Pyrophoric liquids	2.9	Not applicable.	**No classification** (Not applicable)**.**
Pyrophoric solids	2.10	No test data. Experience in handling is sufficient for waiving a test (CLP, Annex I, 2.10.4.1).	**No classification. Conclusive.**
Self-heating substance/mixture	2.11	No data on self-ignition.	**No classification due to lack of data.**
Water-reactive - emits flammable gases	2.12	No test data. No reports of violent reaction and emission of gas on contact with water from handling experience. The molecule does not contain metals or metalloids (CLP, Annex I, 2.12.4.1).	**No classification. Conclusive.**
Oxidising liquids	2.13	Not applicable.	**No classification** (Not applicable)**.**
Oxidising solids	2.14	Negative test in EEC A.17 but hazard class not adequately tested.	**No classification. Inconclusive data.**
Organic peroxides	2.15	Not applicable.	**No classification** (Not applicable)**.**
Corrosive to metals	2.16	No data available.	**No classification due to lack of data.**

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute Oral Toxicity

The DS proposed acutely toxic by the oral route in Category 4 (H302: Harmful if swallowed). There were two guideline compliant (OECD TG 420, 1987) acute oral studies available as shown in the table below:

Table: *Summary of the Acute oral toxicity studies*

Study, guideline, animal strain	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
OECD TG 423 GLP: Yes Crl:WI(GIx/BRL/Han)BR	Quinoclamine (99.0%) Vehicle: 1% methyl cellulose	3/sex/dose (200, 500 mg/kg bw) 3 x females (2000 mg/kg bw)	200 < LD_{50} < 500 mg/kg bw	(B.6.2.1/01 RAR 2018) Anon. (2002)
OECD TG 420 GLP: Yes Slc:Wistar (SPF)) Females only	Quinoclamine (98.3%) Vehicle: 0.5% methyl cellulose	Sighting study: 1 x female/dose (300, 2000 mg/kg bw) Main study: 4 x females (300 mg/kg bw)	300 < LD_{50} < 2000 mg/kg bw (females)	(RB.6.2.1/02 RAR 2018) Anon. (2016)

All animals at the highest dose of 2000 mg/kg bw died (time of death after dosing: between 2 h and 2 days), and showed a number of clinical signs including dyspnoea, palpebral closure, piloerection, discoloured urine (pink) and lethargy from 1 h after dosing onwards.

Generalised clinical signs were evident in lower dose groups and subsided within a few days. During the first week of the observation period the majority of animals lost body weight, failed to gain body weight or achieved only modest body weight gains. All rats gained weight between Day 8 and day 15.

The DS proposed classification as Acute Tox 4 (H302: Harmful if swallowed) based on an LD_{50} for oral toxicity between 300 and 2000 mg/kg bw. The DS also proposed an oral ATE value of 500 mg/kg bw (based on Table 3.1.2 of Annex I to the CLP Regulation).

Acute Dermal Toxicity

The DS proposed no classification. There was one guideline compliant (OECD TG 402) and GLP study available from 2002. Quinoclamine (99.0%) was tested in 5 animals/sex at 2000 mg/kg bw, strain Crl:WI(GIx/BRL/Han)BR. No adverse clinical signs were observed. No deaths were observed.

During the first week of the observation period, the majority of animals lost body weight, failed to gain body weight or achieved only modest body weight gains. All rats gained weight between Day 8 and day 15. No macroscopic changes were observed in animals killed on Day 15.

According to the DS, the acute lethal dermal dose (LD_{50}) of quinoclamine in rats was greater than 2000 mg/kg. No classification was warranted.

Acute Inhalation Toxicity

The DS proposed no classification due to insufficient data. There was one GLP study available from 1986. 5 males and 5 females were exposed in a 4-h, whole body exposure study to a test atmosphere containing 0.79±0.07 mg/L (Mean±SD), the highest attainable concentration of Quinoclamine (98.1%). About 40% (w/w) was found to have an aerodynamic diameter <5.5 µm, i.e. the respirable fraction of the generated dust was found to be about 40%. The DS considered the study to be of limited usefulness when comparing against the most recent guideline version (2009) due to the low level of respirable particles (about 60% by weight of the test substance in the chamber air was > 5.5 µm with a further 20% exhibiting a particle size range of 3.5 – 5.5 µm). The acute inhalation LC_{50} for male and female rats in the study was determined to be >0.32 mg/L.

Comments received during consultation

There was one comment following consultation. One member state competent authority (MSCA) agreed that no conclusion on classification and labelling for acute inhalation toxicity could be decided noting that whole body exposure was acceptable according to the guideline in place when the study was performed. They stated quinoclamine cannot be allocated to a toxicity category according to the CLP guidance.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

According to the CLP criteria, the test substance should be **classified Acute Tox. 4** (300 mg/kg bw < acute toxicity estimate ≤ 2000 mg/kg bw). The corresponding converted oral **ATE value is 500 mg/kg bw** (based on Table 3.1.2 of Annex I to the CLP Regulation). RAC agrees with the DS proposal.

Acute Dermal Toxicity

RAC agrees with the DS. The LD_{50} of Quinoclamine in rats was greater than 2000 mg/kg. **No classification is warranted**. The ATE is considered > 2000 mg/kg bw.

Acute Inhalation Toxicity

RAC agrees with the DS in that no classification can be proposed for quinoclamine. The LC_{50} for male and female rats in the study could not be determined, it is reasoned to be >0.32 mg/L (4-hrs, respirable fraction, i.e. 40% of the highest attainable concentration 0.79 mg/L, after being corrected for the respirable fraction of the generated dust). The study was an old one and cannot be judged according to the current guideline (OECD TG 403, 2009). **No classification is proposed due to inconclusive data**. An ATE cannot be assigned.

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

Summary of the Dossier Submitter's proposal

The DS described the clinical signs observed in animals from the acute oral, dermal and inhalation studies reported in the most current (2018) renewal assessment report (RAR) submitted by the rapporteur Member State (RMS) to the EFSA. All effects noted were of a general systemic nature

and found to be transient, mostly resolving by the end of the first week after single dose exposure. Effects on body weight parameters were the most consistent effect noted.

No specific target organ toxicity was noted which were not already covered by other hazard classes. Some severe effects were noted at the highest concentrations in the oral studies, but these were associated with the presence of lethality. There were no specific respiratory tract irritant or narcotic effects observed for quinoclamine.

The DS did not propose classification for STOT SE.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance, which are not covered by the other hazard classes. STOT SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality. These criteria have not been met with quinoclamine and consequently RAC agrees with the DS proposal for **no classification for STOT SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

A single GLP study from 1986 was described by the DS and was originally described as having been performed according to Japanese MAFF standards. No further detail was available regarding technical guidance compliance. In total, 6 female New Zealand White rabbits each received dermal treatments of 0.5 g of quinoclamine for 4 h under semi-occlusive conditions. Treated sites were assessed for signs of reaction to treatment at 1, 24, 48, 72 and 168 h after removal of the patches.

No dermal irritation was observed at any time point in 5 rabbits during the study. One rabbit exhibited slight erythema (score 1) one hour after removal of the patches and excess test material, but not at any other timepoint thereafter. The DS considered the study acceptable though it pointed out a few discrepancies with respect to compliance with OECD TG 404.

The DS did not propose classification because quinoclamine did not fulfil the CLP classification criteria as irritating to the skin.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

In the skin irritation test conducted with quinoclamine no oedema/eschar was noted, and the mean value for erythema was below 2.3. Furthermore, no inflammation persisted to the end of the observation period. Quinoclamine does not fulfil the CLP classification criteria as irritating to

skin, RAC agrees with the DS and proposes **no classification for skin corrosion and irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

A single GLP study from 1985 was described by the DS and was originally described as having been performed according to Japanese MAFF standards. No further detail was available regarding technical guidance compliance. In total, 9 female New Zealand White rabbits each received 0.1 mL of quinoclamine placed in the lower conjunctival sac of the right eye. The treated eyes of the first group of 6 rabbits were not rinsed after dosing with the test substance. This group was the one most suitable for assessment of eye classification according to CLP. The DS considered the study acceptable and described marked effects in the eyes of the treated rabbits, noting that all effects were reversed by day 14 after dosing.

The DS agreed with the RAR assessment that the test substance had a moderate irritant effect in the eye, with the effect being reversible. The DS proposed classification into category 2 for eye irritation.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

Classification as an eye irritant category 2 is required when a mean Draize score at or above 1 (corneal opacity or iritis) or 2 (conjunctival redness or conjunctival oedema) is observed from gradings at 24, 48 and 72 h following installation of the test substance in at least 4 out of 6 animals and which fully reverse within the observation period of 21 days.

The individual eye irritation scores in this study (*Anon. 1985*) from the 6 animals in the unrinsed group clearly meet the criteria for a category 2 classification but not for a category 1 classification (table below).

Table: *Mean values for ocular lesions 24, 48 and 72 h after instillation*

Animals	**Corneal opacity**	**Iridial lesions**	**Conjunctival**	
			Redness	**Chemosis**
1. F421	0	0	0.3	0.7
2. F424	1.7	1	2	3
3. F425	1	1	2.7	2.3
4. F428	0	0	1	0.7
5. F430	1	1	2.3	2.7
6. F432	2	1	2.3	3.3
CLP Criteria: Eye Irrit. (Cat. 2)	≥ 1	≥ 1	≥ 2	≥ 2
CLP Criteria: Eye Dam. (Cat. 1)	≥ 3	> 1.5	na	na

In the eye irritation test conducted with quinoclamine, mean scores for conjunctival chemosis and redness was ≥ 2 in 4 of the 6 animals of the unrinsed group, corneal opacity was ≥ 1 in 4 of the

6 animals and iris irritation was = 1 in 4 of the 6 animals. Thus, quinoclamine fulfils the CLP classification criteria for eye irritation. RAC supports the DS conclusion, **classification is proposed with Eye Irrit. 2; H319 ("Causes serious eye irritation").**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS described the skin sensitisation potential of quinoclamine from a single, GLP and OECD TG 406 compliant guinea pig maximisation test (GPMT) study from 2001. Several screening studies were performed. The main study had 5 females in the control group and 10 females in the test group. The intradermal injection was 1% (m/v) quinoclamine in arachis oil and/or adjuvant, the topical induction was 55% (m/m) quinoclamine in arachis oil and the challenge application was 7.5 and 15% (m/m) in arachis oil. Quinoclamine elicited a positive response, indicative of skin sensitisation (delayed contact hypersensitivity) in eight of the ten test animals following the challenge application (i.e. an 80% response).

The DS stated the results fulfilled the criteria for sensitisation with sub-categorisation in category 1A.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

According to CLP Regulation 3.4.2.2.4, a response in at least 30% of the animals is considered positive when an adjuvant type guinea pig test method for skin sensitisation is performed. Quinoclamine caused a positive response in 80% of the animals in a GPMT test with an intradermal induction of 1%. Thus, quinoclamine fulfils the CLP classification criteria as a skin sensitiser.

In addition, classification into sub-categories may also be considered but is only allowed if data are sufficient to exclude one sub-category over another. According to the CLP criteria for sub-categorisation 1A (≥30% responding at ≤0.1% intradermal induction dose or ≥*60% responding at >0.1% to ≤**1%** intradermal induction dose*), quinoclamine qualifies into category 1A with an 80% response at a 1% intradermal induction dose.

The setting of a specific concentration limit (SCL) is based on potency; SCLs are generally applied for the most potent skin sensitisers classified in 1A. Potency, on the basis of the Guinea Pig Maximisation Test, is defined in table 3.7 of the CLP guidance (2017). Quinolamine is considered a "strong" sensitiser and the general concentration limit of 0.1% (w/v) shall apply.

RAC agrees with the DS and proposes **Skin Sens. 1A; 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

The DS summarised 16 repeated dose toxicity studies in different species (rat, dog, rabbit, and mice) and of different durations, including carcinogenicity, developmental and reproductive toxicity studies. The DS proposed to classify Quinoclamine as STOT RE 2; H373 ("May cause damage to blood system and kidney through prolonged or repeated exposure") with no route specified, based on findings indicating haemolytic anaemia noted in the dog (90-day and 2-year studies), supported by findings on blood noted in the rat (28-day and 90-day studies), and findings in the kidney (tubular nephrosis) noted in the dog (2-year study). The repeated dose studies were largely performed with administration via the oral route. Some dermal studies were also included but no studies were available where the inhalation route had been used.

The DS gave an extensive and detailed description of all the available repeat dose studies it considered in evaluating the information on specific target organ toxicity – repeated exposure. These included short-term, sub chronic and chronic, mostly GLP studies but several of these were found to be either pre-guideline or lacking in detail as to what guideline they adhered to. The available reports varied in their acceptability and quality, some were only of a supportive or indicative nature due to their age, lack of guideline compliance or lack of key investigations or, as in the case of the dose range finding studies, used too few animals to provide sufficiently robust data (see tables 2.6.3.1-1 and 2.6.3.1-3 of the CLH report). Some of the pre-guideline studies were repeated with more up-to-date investigations.

According to the DS, there were no significant toxic effects observed in the available studies at or below the guidance values that would support classification of quinoclamine as STOT RE 1; H372.

The DS, having assessed all the available repeated dose toxicity studies, considered the following to be relevant in supporting a classification of quinoclamine as STOT RE 2 (H373):

1. the oral (dietary) 28-day CD rat study (Anon. 2002; B.6.3.1.1/01),
2. the oral (dietary) 90-day CD rat study (Anon., 2003; B.6.3.2.1/02),
3. the oral (dietary) 90-day SD rat study (Anon., 1972; B.6.3.2.1/01),
4. the oral (capsule) 90-day dog study (Anon., 2002; B.6.3.2.2/01),
5. the oral (dietary) 2-year dog study (Anon., 1976; B.6.3.3.1/01).

According to the DS, these studies support changes in blood indicating haemolytic anaemia (increased reticulocyte counts; reduced haemoglobin at ≥10% in combination with red-, brown- or dark coloured urine and presence of amorphous debris in urine; haemosiderin deposition in the spleen; increased haemopoiesis in the bone marrow and spleen together with an increase in iron-containing pigment in the liver) as well as effects on the kidney in dogs (renal tubular nephrosis; tubular nephropathy with fibrosis and renal tubular regeneration).

The DS proposed classification in STOT RE 2; H373 ("May cause damage to blood system and kidney through prolonged or repeated exposure") with no route of exposure or a specific concentration limit (SCL) specified.

Comments received during consultation

No comments received.

Assessment and comparison with the classification criteria

The DS proposed STOT RE 2 based on findings in the blood and kidney. The DS specified 5 key studies upon which it based the STOT RE 2 classification proposal. RAC concurs with the DS assessment but also adds an additional 2 studies from the developmental toxicity database. Effects **relevant** for classification at the effective dose (ED) are summarised and compared with equivalent Guidance Values in the Table below. Other studies described in the CLH report have been omitted from the opinion because they are redundant with respect to the main studies (e.g. dose-range finding studies) or simply do not satisfy the classification criteria (i.e. relevant effects were seen at high dose values in excess of those ranges for category 1 or 2 classification). Further in-depth and detailed information of these other studies may be found in the background document and/or CLH report.

***Table*:** *Summary of major effects in relevant repeated dose toxicity studies for consideration of STOT RE classification*

Study reference	**Effective dose (ED) expressed as quinoclamine (mg/kg/d)**	**Length of exposure**	**Equivalent guidance values**	**Classification supported by the ED**
B.6.3.1.1, Study 1 Anon. 2002 Acceptable from a regulatory point of view 5/sex/dose	<u>Highest Effective Dose:</u> 84/90 mg/kg bw/day (M/F) <u>Critical effects:</u> ↓haemoglobin; M: 8%, F: 13% ↓RBC; M: 12%, F: 13% ↓PCV; M: 6%, F: 11% - Normal or slight ↑ MCV ↑RDW; M: 37%, F: 47% ↑reticulocytes; M: 170%, F: 238% ↑Ret/ABS; M: 138%, F: 196% ↑urinary colour change, distinct, dose response ≥48/44 mg/kg bw/day; M/F ↑kidney histopathology in males.	Oral (dietary) 28-day CD rat	30<C≤300 mg/kg bw/day	Cat. 2
B.6.3.2.1, Study 2 Anon., 2003 Acceptable from a regulatory point of view 10/sex/dose	<u>Effective Dose:</u> 57/75 mg/kg bw/day (M/F) <u>Critical effects:</u> ↓haemoglobin; M: 10%, F: 11% ↓RBC; M: 13%, F: 15% ↓PCV; M: 11%, F: 11% - Normal or slight ↑ MCV ↑RDW; M: 16%, F: 11% ↑reticulocytes; M: 82%, F: 135% ↑Ret/ABS; M: 59%, F: 95% ↑urinary colour change, distinct, dose response ≥14/18 mg/kg bw/day; M/F ↑spleen abs. wt. M: 44%, F: 22% ↑spleen rel. wt. M: 71%, F: 48% ↑kidney histopathology in males.	Oral (dietary) 90-day CD rat	10<C≤100 mg/kg bw/day	Cat. 2
B.6.3.2.1, Study 1	<u>Highest Effective Dose:</u> 62/65 mg/kg bw/day (M/F) <u>Critical effects:</u>	Oral (dietary) 90-day SD rat	10<C≤100 mg/kg bw/day	Cat. 2

***Table*:** *Summary of major effects in relevant repeated dose toxicity studies for consideration of STOT RE classification*

Study reference	**Effective dose (ED) expressed as quinoclamine (mg/kg/d)**	**Length of exposure**	**Equivalent guidance values**	**Classification supported by the ED**
Anon., 1972 Supportive 5/sex/dose	↑haemosiderin; M: 10/10, F: 10/10 ↑spleen abs. wt. M: 58%, F: 18% ↑spleen rel. wt. M: 63%, F: 35% ↑kidney abs. wt. M: 20%, F: -% ↑kidney rel. wt. M: 11%, F: 17% ↑kidney histopathology in males.			
B.6.3.2.2, Study 1 Anon., 2002 Acceptable from a regulatory point of view 4/sex/dose	<u>Highest Effective Dose:</u> 30/30 mg/kg bw/day (M/F) <u>Critical effects:</u> ↓haemoglobin; M: 18%‡, F: 19%‡ ↓RBC; M: 19%‡, F: 19%‡ ↓PCV; M: 14%*, F: 14%† slight ↑ MCV (6%*/5%*; M/F) -RDW; no change ↑reticulocytes; M: 187%‡, F: 175%‡ ↑Ret/ABS; M: 118%‡, F: 125%‡ ↑T. Bili; M: 111%*, F: 100% ↑spleen abs. wt. M: 21%, F: 56% ↑spleen rel. wt. M: 24%, F: 75% -no significant kidney histopathology.	Oral (capsule) 90-day dog	10<C≤100 mg/kg bw/day	Cat. 2
B.6.3.3.1, Study 1 Anon., 1976 4/sex/dose	<u>Highest Effective Dose:</u> 7.6/6.8 mg/kg bw/day (M/F) <u>Critical effects:</u> ↓haemoglobin; M: 18%, F: 19% ↓RBC; M: 22%*, F: 27%* ↓PCV; M: 16%*, F: 16%* -MCV - N/A -RDW; N/A -reticulocytes; N/A -Ret/ABS; N/A ↓T Bili; M: 56%, F: 37%* ↑spleen abs. wt. M: *-23%*, F: 55% ↑spleen rel. wt. M: *-17%*, F: 71% -no significant kidney histopathology.	Oral (dietary) 2-year dog	1.25<C≤12.5 mg/kg bw/day	Cat. 2
B.6.6.2.1, Study 4 Anon., 2002 Acceptable from a regulatory point of view 24F/dose	<u>Highest Effective Dose:</u> 75 mg/kg bw/day (F) <u>Critical effects:</u> ↑ hydronephosis; F: 3/263 (1.1% incidence, outside HCD; 0.2%, 14/6208) in the top dose only.	Oral (gavage) 14-day dosing rat developmental toxicity study	64<C≤640 mg/kg bw/day (Haber's rule)	Cat. 2

***Table*:** *Summary of major effects in relevant repeated dose toxicity studies for consideration of STOT RE classification*

Study reference	Effective dose (ED) expressed as quinoclamine (mg/kg/d)	Length of exposure	Equivalent guidance values	Classification supported by the ED
B.6.6.2.2, Study 4 Anon., 2002 Acceptable from a regulatory point of view 24F/dose	<u>Highest Effective Dose:</u> 30 mg/kg bw/day (F) <u>Critical effects:</u> ↑ hydronephosis; F: 2/124 (1.6% incidence, outside HCD; 0.06%, 2/4233) in the top dose group (30 mg/kg/day) and 1/160 in the mid dose (17.5 mg/kg/day).	Oral (gavage) 21-day dosing rabbit developmental toxicity study	4.5<C≤45 mg/kg bw/day (Haber's rule)	Cat. 1

Changes in haematological parameters and indicators of anaemia

RAC agrees with the DS that the primary target organ of quinoclamine after repeated exposure is the blood system. Several changes in haematological parameters were noted across all species indicating that quinoclamine may cause haemolytic anaemia at high dose levels.

In the 28-day oral toxicity study in CD rats (Anon., 2002), males at ≥500 ppm (≥44 mg/kg bw/day) and in females at ≥500 ppm (≥48 mg/kg bw/day) clear statistically significant effects were noted in haematological values indicative of haemolytic anaemia. Distinct changes in urinary colour were also noted at ≥500 ppm (≥48/44 mg/kg bw/day; M/F), becoming much darker with treatment. The presence of amorphous debris within red/darkened urine also indicates haemolytic anaemia.

In the 90-day oral toxicity study in CD rats (Anon., 2003), extensive haematological examinations revealed clear statistically significant effects indicative of haemolytic anaemia in the top dose group animals (males at 800 ppm or 57 mg/kg bw/day and in females at 800 ppm or 75 mg/kg bw/day). Treatment related effects on spleen weight were observed in high (57 mg/kg bw/day) and intermediate (14 mg/kg bw/day) male groups. Microscopically, an increase in haemopoiesis, pigment and congestion were present in the spleen of high dose animals. It was noted that 9/10 high dose females had dark straw-coloured urine and 8/10 males also had dark straw-coloured urine, with the remaining males (2/10) having very dark straw-coloured urine.

In the 90-day oral toxicity study in SD rats (Anon., 1972), haematological examinations were limited but there was an increased incidence of haemosiderin deposition in the spleen of males and females treated at 1000 (62/65 mg/kg M/F) and 200 ppm (14/13 mg/kg bw/day M/F). At both doses, 10/10 males and 10/10 females were affected compared to 3/10 males and 5/10 females from the respective control groups. Significant increases in spleen weight were also noted with mid and high dose groups and together with the increased deposition of haemosiderin suggest accelerated erythrocyte destruction.

In the 90-day oral (capsule) toxicity study in dogs (Anon., 2002), extensive haematological examinations revealed clear and consistent, statistically significant effects indicative of haemolytic anaemia in the top dose group animals (males and females at 30 mg/kg bw/day). There was also an increase in the group mean total bilirubin concentration of high dose animals compared to controls (111% [$p<0.05$] and 100% above controls for males and females respectively). The spleen weight of high dose females was increased but statistical significance was not attained. There was increased haemopoiesis in the bone marrow and spleen along with an increase in iron-containing pigment in the liver indicating low grade haemolytic anaemia.

Coloured urine was observed in treated animals throughout the course of the study but was not characterised in detail. The original compound was described as an orange powder (purity 99%) and there was no attempt to further explain the coloured urine by the original study authors.

In the 2-year oral (dietary) toxicity study in dogs (Anon., 1976), one male at 250 ppm died during the first year of treatment (week 45), due to a severe urinary tract infection which was not attributed to compound administration. The 250ppm dose group (7.6 mg/kg bw/day and 6.8 mg/kg bw/day for males and females respectively) was the highest effective dose group considered to satisfy the criteria for consideration of STOT RE 2. Effects were more pronounced and statistically significant in the 1000 ppm dose groups, but this dose level is outside the concentration criteria for consideration of STOT RE 2. Interestingly, reductions in HB (-25%/-32% M/F) at the 13-week timepoint at 1000 ppm clearly support STOT RE 2 if we compare against the guidance value for a standard 90-day study. Histopathological findings showed increased congestion and extramedullary haematopoesis in the spleen for females only at the relevant dose for classification but the low number of animals here (3) do not make this a robust observation. Both males and females were affected at the highest dose though this level is too high for support of classification.

Coloured urine was observed in treated animals throughout the course of the study but was not characterised in detail. This discoloration was attributed to the presence of the test material, or metabolite in the urine according to the original study report. The original compound was described as a dark, orange powder (purity 98.5%). The authors made no connection with haemolytic anaemia and the colour of the animals' urine.

Effects on the kidney

RAC agrees with the DS that an additional target organ of quinoclamine after repeated exposure is the kidney. Several histopathological changes were noted and may be considered as adverse changes indicative of toxicity and beyond normal organ adaptation but there was no obvious or remarkable organ disfunction recorded.

In the 28-day oral toxicity study in CD rats (Anon., 2002), limited histopathological changes in the kidneys were noted in males at ≥500 ppm (≥44 mg/kg bw/day) in the form of eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium (5/5 animals vs 4/5 in controls); while at 1000 ppm (90 mg/kg bw) there were also eosinophilic hyaline droplets in the cytoplasm of the proximal tubular (5/5) in addition to minor papillitis (2/5 vs 0/5 in controls) and basophilic cortical tubules (5/5 animals vs 3/5 in controls).

In the 90-day oral toxicity study in CD rats (Anon., 2003), there was an increase in pigment in the kidney of high dose females and of hyaline droplets in intermediate and high dose males and a minor increase in focal nephropathy in the kidney of both sexes in the high dose group (males at 800 ppm or 57 mg/kg bw/day and in females at 800 ppm or 75 mg/kg bw/day). It is noted that the eosinophilic hyaline droplets observed in the cytoplasm of the proximal tubular epithelium was present in all animals in all dose groups. However, an increase in the severity or grade of this histopathological feature was observed in intermediate and high dose males. There were no statistically significant increases in kidney weight with dose.

In the 90-day oral toxicity study in SD rats (Anon., 1972), histopathological changes in the kidneys were noted in males; there was an increase in eosinophilic hyaline droplets in the cortical epithelium (10/10 animals vs 0/10 in controls) and an increase in both absolute (+20%) and relative (+11%) kidney weight relative to controls.

In the 90-day oral (capsule) toxicity study in dogs (Anon., 2002) there was a small increase in kidney weight (high dose females only), associated microscopically with increased lipofuscin in the proximal tubular epithelium of some high dose animals. The histopathological evidence was weak and more suggestive of an adaptive phenomenon probably due to increased membrane

turnover arising from test article elimination. There were no other macroscopic or microscopic renal changes or significant changes in clinical chemistry parameters suggestive of overt toxicity to the kidneys.

In the 2-year oral (dietary) toxicity study in dogs (Anon., 1976), the kidney, amongst other organs, was mainly affected at the highest dose (1000 ppm) which was outside of the criteria for STOT RE 2. Tubular nephrosis was only noted in 1/4 females at 250 ppm and in both sexes at the top dose, healed areas of nephrosis (both sexes) were noted at 1000 ppm, dilated renal tubules noted in males at 1000 ppm, cystic tubules (both sexes) noted at 1000 ppm, and papillitis noted in one female.

Critically the severest form of renal pelvic cavitation or hydronephrosis was observed in foetuses from a rat developmental toxicity study (Anon., 2002) and a rabbit developmental toxicity study (Anon., 2002). Instances at the top dose in each case were outside the historical control data (HCD) supplied in the original study reports. Adjustment of the dose criteria for consideration of STOT RE 1 and 2 due to the shortened exposure period according to Haber's rule indicates that STOT RE 2 is supported by the rat study, while STOT RE 1 is supported by the findings at 30 mg/kg bw/day in the rabbit study.

Conclusion on classification

There were generally no significant toxic effects observed in the available studies at or below the guidance values (table 3.9.2 of Annex I: 3.9.2.9.6 to the CLP Regulation) that would support classification of quinoclamine as STOT RE 1 (H372) except for the 2002 rabbit main developmental toxicity study.

When assessing the available information, RAC considers the use of expert judgement and a weight of evidence approach essential. This assessment includes a comparison with recommended guidance values (which take into account the duration of exposure and the dose/concentration which produced the effect(s) (see section 3.9.2.9 and Table 3.9.1 of CLP). From the dataset, the majority of the short-term/sub-chronic/chronic studies warrant STOT RE 2 classification.

RAC recognises that several other target organs were affected in many studies (and not just the 7 key organs outlined in the table "Summary of major effects in relevant repeated dose toxicity studies" above), to different extents, especially the liver but mainly at high dose levels. The only clear and consistent response within the scope of STOT RE 2 criteria was on blood parameters and red blood cells and the kidney. The findings of reduced haemoglobin (>10%) in combination with effects on the spleen (increased extent of pigment and haemopoiesis and increased organ weight and congestion) and other haematological parameters (such as increased levels of reticulocytes) which indicate haemolytic anaemia. This effect is considered relevant for human health and noted in all the key studies to occur within the critical range of doses for STOT RE 2 classification. The DS also suggested the kidneys as a major target organ. The significance of the effects on this organ from the 7 key studies outlined in the table "Summary of major effects in relevant repeated dose toxicity studies" (above) are overall convincing, especially when RAC notes multiple species are affected and both rat and rabbit foetuses from prenatal toxicity studies display an increased incidence of hydronephrosis above what is expected from the normal background. Therefore, RAC recommends classification with STOT RE 2 with reference to blood and kidneys, i.e. **STOT RE 2; H373 ("May cause damage to the blood system and kidneys through prolonged or repeated exposure")**.

RAC notes that the DS did not specify a route of exposure. RAC agrees with the DS not to indicate the route of exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that quinoclamine was tested in a range of GLP and OECD guideline compliant *in vitro* and *in vivo* genotoxicity assays (table below), details of which were supplied in tables 2.6.4-1 and 2.6.4-2 of the CLH report.

In vitro assays included:

- 1 × *in vitro* Ames test (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*), 2002; negative.
- 1 × *in vitro* mammalian cell gene mutation test in mouse lymphoma L5178Y cells, 1989; negative.
- 1 × *in vitro* mammalian chromosome aberration test in Human lymphocytes, 1987; positive in presence of S9 mix.

In vivo assays included:

- 1 × mouse micronucleus test in bone marrow (strain LACA), single intraperitoneal (i.p) doses of 125, 250 and 500 mg/kg, (15 animals/sex/dose) 1989; negative. A minimum of 1000 polychromatic erythrocytes (PCE) were counted for each animal This study was limited since it could not be shown that the target tissue was reached (no depression of the immature to mature erythrocyte ratio). There were no measurements of the plasma or blood levels of the test substance in the study. No cytotoxicity was seen in bone marrow. No ADME data using the same route and the same species were available.
- 1 × rat unscheduled DNA Synthesis (UDS) test in hepatocytes (strain Crl:CD BR rats). Groups of 5 male rats were administered a single oral dose of quinoclamine at 800 or 2000 mg/kg, by oral gavage (Anon., 1996). The animals were divided into two test groups, one sacrificed after 12-14 h and one after a shorter period of 2-4 h. There was no induction of UDS in hepatocytes isolated *ex vivo* approximately 12-14 or 2-4 h after dosing. Test was negative. The DS noted that the *in vivo* UDS test alone should not be considered sufficient for the follow up of positive results in an *in vitro* assay according to the 2016 EFSA technical review on general recurring issues in mammalian toxicology.

Table: *RAC Summary of genotoxicity tests with Quinoclamine*

Study	Result	Methods and acceptability	Reference
***In vitro* studies:**			
Bacterial mutagenicity	negative	GLP, OECD 471 (1997), acceptable *Salmonella* Strains: TA1535, 100, 1537, 98 Other: *E. coli* WP2 uvrA strain	Beevers (2002)
Mammalian cell mutagenicity	negative	GLP, OECD 476, acceptable L5178 mouse lymphoma. The locus investigated was for Ouabain (OUA) resistance.	Asquith (1989)
Clastogenicity	negative in absence of S-9 mix positive in presence of S-9 mix	GLP, OECD 473 (1983), acceptable study with equivocal results Human lymphocytes	Asquith (1987)
***In vivo* studies:**			
UDS	negative	GLP, OECD 486, acceptable Male rat (Crl:CD BR rats) hepatocytes	Anon. (1996)
Micronucleus	negative	GLP, OECD, acceptable Mouse (LACA) bone marrow	Anon. (1989)

In vitro results

(1) Quinoclamine did not induce point mutations in bacteria or mammalian cells *in vitro*.

(2) The *in vitro* cytogenetic assay in human lymphocytes (*in vitro* chromosome aberration test) was positive in the presence of metabolic activation. The positive control compounds induced highly significant increases in aberration frequency and demonstrated that the test method and cells used were sensitive to the effects of known clastogens and that the S9-Mix was capable of metabolising an inactive precursor to a genotoxic intermediate. Quinoclamine is not a clastogen in the absence of S9 under the condition of this test. A positive result has been obtained from cells from one of the two donors used in this assay, it cannot be precluded that Quinoclamine may have clastogenic potential. The chromosome aberration test did not achieve the levels of cytotoxicity required by the current guidelines and the equivocal response in the presence of S9-mix was not further investigated. This test is considered inconclusive by RAC.

In vivo results

(1) The *in vivo* micronucleus (MN) test in the mouse was considered limited by the DS, since bone marrow exposure was not shown in the study and no measurement of the plasma or blood levels of the test substance was included in the study. The MN test found only a non-significant dose-related increase at 24 h that was within the background variability, hence it can be considered negative.

(2) An *in vivo* UDS test was available. However, the negative results in the *in vivo* UDS test were not considered sufficient by the DS to overrule the positive results in one of the *in vitro* tests. This decision by the DS was based on the EFSA 2016 publication following a pesticides peer review

meeting on general recurring issues in mammalian toxicology where they discussed genotoxicity testing and follow up on positive *in vitro* results[1].

Conclusion of the DS

There were no studies in germ cells. According to the DS, there was insufficient data to conclude on the mutagenicity of quinoclamine. The DS proposed no classification.

Comments received during consultation

There was one comment following consultation. One MSCA agreed with the dossier submitter that no conclusion on classification and labelling for genotoxicity/germ cell mutagenicity could be drawn because the data were inconclusive.

Assessment and comparison with the classification criteria

No human data are available for quinoclamine, therefore a classification with Muta. 1A is not supported. Data are not available illustrating the induction of mutagenic effects in germ cells (a criterion for Category 1B). RAC does not propose classification with Muta. 1A or B. RAC considered Muta. 2 in respect of the available information.

If *in vitro* testing provides one or more positive results, an *in vivo* follow-up study would be expected. Clastogenic substances induce structural chromosomal aberrations through breaks in DNA. The *in vitro* mammalian chromosome aberration test in Human lymphocytes (Asquith, 1987) used two human donors. Quinoclamine showed activity *in vitro* in one of the donors, but not the other, and it remains unclear why the donors reacted differently. It could thus be argued that the test was inconclusive and the results equivocal or that the test should have been repeated. Follow-up *in vivo* tests conducted in mice and rats indicated that Quinoclamine had no genotoxic activity detectable in these test systems up to concentrations of 500 mg/kg and 2000 mg/kg, respectively. Further analysis however, questioned whether these follow-up tests were sufficiently reliable.

In line with the DS, RAC considers the follow-up *in vivo* tests insufficient to fully alleviate the concern of the positive *in vitro* clastogenicity test. The *in vivo* micronucleus test in the mouse failed to demonstrate that the target tissue was exposed but RAC recognises that the substance is widely distributed in rats after gavage administration. Intraperitoneal administration is generally assumed to lead to a higher systemic availability compared to gavage so the route of administration in this case is not particularly problematic. General toxicity in the MN test and systemic toxicity in the mouse carcinogenicity study may be interpreted as supporting evidence for systemic availability of the substance in the mouse. The DS noted that the negative results in the *in vivo* UDS test were not considered sufficient to overrule positive results in the *in vitro* mammalian clastogenicity test. The DS formed the opinion that there was insufficient data to address the concerns of the positive *in vitro* test and thereby flagged a data gap in the standard battery for genotoxicity testing of quinoclamine. It must be pointed out however that amongst

[1] EFSA (European Food Safety Authority), 2016. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2016:EN-1074.

many toxicologists, the *in vivo* UDS assay may still be a valid and acceptable test if performed correctly, but alone and especially in this case considering the shortcomings of the *in vivo* MN test, it may not be sufficient since the UDS test is restricted to the detection of primary DNA repair in liver cells. However, RAC recognises that alternate methodologies could have been investigated further such as a Transgenic Rodent Somatic or a Germ Cell Gene Mutation Assay and/or *in vivo* Comet assay.

There was no attempt to compare quinoclamine with structurally related substances in the CLH report. The structure of quinoclamine contains two alerts (quinone, aromatic amine) but quinoclamine is best described as a benzenoid polycyclic aromatic hydrocarbon and any comparison with a simple monocyclic quinone such as p-benzoquinone or a monocyclic aromatic amine like aniline is limited and does not contribute to the dataset for quinoclamine. There was no evidence that metabolism of quinoclamine gives rise to monocyclic quinones or aromatic amines.

The single *in vivo* mutagenicity study is negative and does not show any major deficiencies. The *in vitro* data is mixed but overall inconclusive. Quinoclamine produced only a weak response in the available carcinogenicity studies. Concerns remain and more extensive testing would have been appropriate. Based on the available information, RAC supports the conclusion of the DS and proposes **no classification for mutagenicity due to inconclusive data.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two GLP compliant long-term oral (dietary) toxicity/carcinogenicity studies were available to the DS: a 2-year combined chronic toxicity/carcinogenicity study in the Crl: CD(SD)BR rat (*Anon., 1991,* study initiation date May 1987) and an 18-month carcinogenicity study in the CD-1 mouse (*Anon., 1993,* study initiation date May 1990). Technical guideline compliance was not stated in either original study report but broadly follow OECD TG 453 and 451 and both studies were acceptable according to the evaluating Member States in the RAR submitted in 2018 to EFSA. Study details were summarised in Table 2.6.5-1 in the CLH report.

Following treatment with quinoclamine, the main features noted in rats included epithelial hyperplasia, urinary bladder transitional cell papillomas and adrenal benign pheochromocytoma. The main features noted in mice included malignant lymphoma and histiocytic sarcoma.

Rat 2-year dietary toxicity/oncogenicity study

In a rat GLP-compliant, chronic toxicity/carcinogenicity dietary study (*Anon., 1991*), treatment with quinoclamine did not reduce the survival of rats up to the highest doses tested. Crl: CD (SD) BR strain rats were broadly allocated to two groups. The chronic toxicity group were divided into treatment groups and scheduled kills were conducted after 27, 53, 79 weeks treatment for 10 animals/sex/dose group and at study termination after 104 weeks treatment for 20 animals/sex/dose group. The carcinogenicity group were divided into treatment groups and dosed until study termination after 104 weeks treatment for 50 animals/sex/group.

Table: *Carcinogenicity groups mean dose received (mg/kg/day)*

Dietary concentration of quinoclamine (M/F) ppm	0	4	52	676
Males	0	0.21	2.8	37.6
Females	0	0.28	3.7	49.4

Non-neoplastic findings

Dose level selection was based on results from a 90-day dietary study at concentrations of 0, 31.3, 125, 500 and 2000 ppm in groups of 10 male and 10 female Sprague-Dawley rats. Lethality was noted in 2000 ppm animals and significant toxicity in 500 and 2000 ppm groups.

Treatment related clinical signs were confined to orange fur staining (related to the colour of quinoclamine and/or urinary metabolites) affecting all high dose animals (676 ppm) from week 2 onwards. General toxicity was displayed by significantly lower body weight gain in top dose females (27-28%) along with slight but significant reductions in food consumption at various time points during the study (but not at study termination). The kidney and urinary system were clear target organs with small effects also observed for the blood and pancreas.

The most striking treatment related effect was in respect of epithelial hyperplasia in the urinary tract (table below). Histological changes were initially confined to urinary bladders of top dose (37.6/49.4 mg/kg bw/day; M/F) animals (week 26) and comprised epithelial hyperplasia and chronic inflammatory changes. As the study progressed, similar lesions appeared in occasional intermediate dose animals and were more widespread, affecting the ureters, urethras, and kidneys in addition to the urinary bladders for both the chronic and carcinogenic cohorts. The effects were similar in both sexes.

There was a clear treatment-related increase in the incidence of epithelial hyperplasia particularly in high dose group animals and to a lesser extent in intermediate dose group animals. Squamous metaplasia of the hyperplastic urinary epithelium was seen in 3 high dose females, along with a single polyp (table below).

A number of other effects of treatment were evident in the kidney. In high dose group animals (37.6/49.4 mg/kg bw/day; M/F), quinoclamine caused cortical scarring and papillary necrosis in both sexes including papillary focal necrosis. Hyperplasia of the pelvic and papillary epithelium was present in the majority of top dose animals and a proportion of intermediate dose animals. Papillary necrosis, which was bilateral in most cases, was accompanied by haemorrhage, and characterised by a total loss of the tip of the papillae in top dose animals.

There was a clear increase in the incidence of epithelial hyperplasia in the ureters and to a lesser extent in the urethra of high dose and intermediate dose group animals. Another very striking effect was on pancreatic acinar atrophy where a large increase in severity was seen in both sexes from the top dose groups. Minimal acinar atrophy was already present across all dose groups but moderate to marked atrophy was notable in the top dose groups.

Epithelial hyperplasia was also present in the adrenal medulla but evidence for a treatment related response was considered to be weak and presented no correlate with the appearance of benign pheochromocytomas.

Table: *Non-neoplastic pathology at the end of the study (carcinogenicity cohort)*

Parameter/Dose (mg/kg bw)	**Males**				**Females**			
	0	**0.2**	**2.8**	**37.6**	**0**	**0.3**	**3.7**	**49.4**
Urinary bladder:								
Total: number examined	50	49	47	47	50	49	47	47
Epithelial hyperplasia	3	2	5	**41**	1	1	6	**46**
Squamous metaplasia	0	0	0	0	0	0	0	**3**
Polyp	0	0	0	0	0	0	0	**1**
Adrenal medulla:								
Total: number examined	50	24	28	47	50	29	27	50

Medullary hyperplasia	7	4	3	7	3	3	0	6
Kidneys:								
Total: number examined	50	49	48	48	50	50	50	50
Epithelial hyperplasia[1]	0	0	2	**4**	0	0	0	**11**
All grades of hyperplasia	2	5	12	**39**	2	0	10	**34**
Papillary necrosis	0	1	0	**9**	0	0	0	**3**
Papillary focal necrosis	0	2	1	**12**	0	0	0	0
Cortical scarring	3	3	2	**11**	0	0	1	**6**
Ureters:								
Total: number examined	47	21	26	38	48	20	21	47
Epithelial hyperplasia (any)	0	1	2	**19**	0	0	**5**	**21**
Urethra:								
Total: number examined	44	33	35	41	42	18	13	36
Epithelial hyperplasia	1	0	0	4	1	1	0	**6**
Pancreas:								
Total: number examined	50	28	29	46	48	20	23	50
Acinar atrophy[1]	3	1	5	**19**	0	0	1	**16**

[1] moderate and marked grades (not minimal); **Bold** text = significantly increased relative to controls

Neoplastic findings

According to the DS in the CLH report, neoplastic changes were noted in the urinary bladder (benign transitional cell papilloma noted in males at 37.6 mg/kg bw/day and in females at 49.4 mg/kg bw/day) and adrenals (increased incidence of benign pheochromocytoma noted in both sexes at the top dose), as shown in the table below. No other differences in tumour incidence (apart from a reduction in benign pituitary adenomas in top dose animals) or type were seen which were dependent on the dose levels of quinoclamine.

***Table*:** *Neoplastic findings at the end of the study (carcinogenicity cohort)*

Parameter/Dose (mg/kg bw)	**Males**				**Females**			
	0	**0.2**	**2.8**	**37.6**	**0**	**0.3**	**3.7**	**49.4**
Urinary bladder:								
Total: number examined	50	49	47	47	49	48	49	50
Transitional cell papilloma	0	0	0	**4**	0	0	0	**6**
Adrenal medulla:								
Total: number examined	50	24	28	47	50	29	27	50
Pheochromocytomas	8	4	2	14	1	0	0	4

Bold text = significantly increased relative to controls

(i) Neoplastic changes in the urinary bladder

Treatment related neoplastic changes in the carcinogenicity study were confined to benign transitional cell papillomas in the urinary bladder of 4/47 (9%) males and 6/50 (12%) females in the top dose groups (table "Neoplastic findings at the end of the study", above). The tumours were characterised by discrete exophytic epithelial masses with branching papillary processes supported by a fibrovascular core. These tumours appeared to have developed from a base of hyperplastic epithelium showing changes similar to the non-neoplastic epithelial hyperplasia seen in both high and intermediate dose group animals. No epithelial cellular atypia was seen and there was no neoplastic invasion of subepithelial connective tissues or muscle. The DS noted the

significant occurrence of epithelial hyperplasia throughout the urinary tract and squamous metaplasia associated with epithelial hyperplasia in two top dose females (table "Non-neoplastic pathology at the end of the study", above). There was no evidence for the development of malignant tumours in the urinary bladder.

According to the DS, the incidence of benign transitional cell papillomas in the urinary bladder of males (9%) was outside of the HCD from the performing laboratory – the maximum incidence from a single study group was 2%; in total 1 animal was affected out of a total of 349 animal controls, number of studies not reported. There was no HCD available for female rats.

Results from the chronic toxicity cohorts supported the findings in the carcinogenicity cohorts. Transitional cell papillomas in association with epithelial hyperplasia in the urinary bladder were also found at study termination but direct comparisons were limited because far fewer animals (relative to the carcinogenic dietary cohort groups) from each dose group were examined.

The DS considered the increase in the incidence of rat transitional cell papillomas in the urinary bladder biologically significant and in support of classification of quinoclamine as Carc. 2.

(ii) Neoplastic changes in the adrenal medulla

There was an apparent increase in the incidence of benign pheochromocytomas of the adrenal medulla in top dose animals (table "Neoplastic findings at the end of the study", above). A high spontaneous tumour incidence was already noted in the concurrent controls (16% in males). Some limited HCD was available, but this was not described in detail in the original study reports (number of studies unknown, dates of studies unknown, total animals examined from control groups = 350). In top dose animals the incidences were significantly outside (by approximately 2-fold) the ranges for benign pheochromocytoma from the performing laboratory HCD as presented in the original study report and pesticide RAR. The trend was statistically significant ($p<0.05$) for combined sexes. According to the DS, the incidence of benign pheochromocytomas in males was 29.8% and 8% in females compared to individual study backgrounds of 6-16% in male rats and 0-4% in females. There was no evidence from the chronic toxicity cohorts to support this finding in the main carcinogenicity cohorts. The DS noted that substantially fewer animals were examined in the top dose groups of the chronic toxicity cohort and that this was a factor in being unable to detect an effect on the adrenal medulla in this cohort.

The DS considered the increase in the incidence of rat benign pheochromocytomas of the adrenal medulla biologically significant and in support of classification of quinoclamine as Carc. 2.

Mouse 18-month dietary carcinogenicity study

In a mouse GLP-compliant, carcinogenicity dietary study (Anon., 1993), treatment with quinoclamine appeared to reduce the survival of mice up to the highest doses tested though the DS noted that the mortality in the study was exceptionally low, especially in the concurrent controls when comparisons were made against the HCD. Animals (50 animals/sex/group) were divided into treatment groups and dosed until study termination after 80 weeks treatment.

Table: *Mean dose received (mg/kg/day)*

Dietary concentration of quinoclamine (M/F) ppm	0	3	30	300
Males	0	0.38	3.82	40.2
Females	0	0.44	4.48	46.4

Non-neoplastic findings

The reasons for dose level selection were not described.

No statistically significant changes in body weights were noted. General toxicity was associated with clinical signs (orange fur staining) noted in both sexes at ≥ 0.38 mg/kg bw/day, increased mortality noted in both sexes at ≥ 3.82 mg/kg bw/day, statistically significant reduced bodyweight gain (33% in males, 30% in females) noted at ≥ 40.2 mg/kg bw/day, changes in organ weights noted at ≥ 3.82 mg/kg bw/day (increased relative liver weight noted in females at the top dose, increased relative kidney weights (n.s.) noted in males at ≥ 3.82 mg/kg bw/day and in females at the top dose, increased relative heart and brain weights noted in females also at the top dose).

There was no clear treatment-related increase in the incidence of epithelial hyperplasia as observed in rats treated with quinoclamine. Effects on the kidney were reported (cortical scarring and hydronephrosis in males and females at the top dose) along with many other generalised effects in several different organ systems.

Neoplastic findings

A few significant findings were noted in the RAR (2018) and CLH report. According to the DS, there were weakly statistically significant ($p<0.05$) positive trends for "adrenal spindle cell tumour or hyperplasia" (RAC notes that there was no evidence for adrenal spindle cell tumours associated with any dose of quinoclamine), malignant lymphoma in females (but not males) and histiocytic sarcoma (only in the case of both sexes combined).

Table: *Neoplastic findings from the mouse study*

Parameter/Dose (mg/kg bw)	**Males**				**Females**			
	0	**0.38**	**3.82**	**40.2**	**0**	**0.44**	**4.48**	**46.4**
Lympho-reticular tissues:								
Total: number examined	50	50	50	50	50	50	50	50
Malignant lymphoma:	1	2	3	0	3	11	7	12
Histiocytic sarcoma	0	0	0	1	0	1	1	2
Adrenals:								
Total: number examined	50	50	50	50	50	50	50	50
Spindle cell adenoma:	0	0	0	0	1	0	0	0
Pheochromocytomas	0	0	1	0	1	0	0	0

(i) Neoplastic changes in the Adrenal cortex

Treatment related hyperplastic changes in the adrenal cortex were noted with high background levels. Spindle-cell hyperplasia showed a significant incidence in top dose males only (18/50 vs 11/50 in controls). Incidences were much higher in females in all tested groups, including controls, with no dose response. A single, spindle-cell adenoma was only seen in one female from the control group. The DS concluded here was no evidence of tumour formation in the adrenal gland due to treatment.

(ii) Neoplastic changes in lympho reticular tissues (a)

In females there was a marginally significant ($p<0.05$) positive trend in malignant lymphoma due mainly to a higher incidence at the top dose only (12/50 cases, 24%) than in the controls (3/50 cases, 6%). There was no clear dose-response relationship. There was no evidence of an effect in males. HCD for Crl:CD-1(ICR)BR (VAF PLUS) mice were reported showing that the occurrence of malignant lymphoma in background data of females ranged from 2 to 11 cases (4-22%). Additional HCD for the CD-1 mouse for the performing laboratory indicated malignant lymphoma in females ranged from 0-38%. The DS agreed with the conclusions in the RAR that the increased trend for malignant lymphoma in mice should not be dismissed.

(iii) Neoplastic changes in lympho reticular tissues (b)

No occurrence of histiocytic sarcoma was reported for the concurrent controls. A marginally significant ($p<0.05$) trend was only apparent if incidence data for the sexes were combined and compared with quinoclamine dose. There was no clear dose response relationship and incidences in the treated groups was low (1/50 cases or 2% for males in the top dose group and 2/50 cases or 4% for females in the top dose group). The histiocytic sarcoma incidence was within background levels when compared with the available HCD. The HCD occurrence of histiocytic sarcoma reported by the original study authors found that this tumour ranged from 0 to 1 case in males (0-2%) and 0-6 cases in females (0-12%). Additional HCD for the CD-1 mouse for the performing laboratory indicated histiocytic sarcoma ranged from 0-3.6% in males, and 0-8% in females. The DS concluded that this was a chance finding due to an unusually low incidence in the controls in this study.

Summary of the DS proposal

According to the DS the most relevant tumour types for discussion of classification were:

- Benign transitional cell papillomas in urinary bladder in the Crl:CD(SD)BR rat
- Benign phaeochromocytoma in adrenals in the Crl:CD(SD)BR rat
- Malignant lymphoma in female mice (Crl:CD-1 (ICR) BR strain)

There was no treatment related increase in rat tumours observed in the chronic toxicology cohorts at interim kills (Weeks 26, 52, 78). The DS interpreted this to mean there was no evidence for reduced tumour latency. The DS considered the malignant lymphoma in the mouse as biologically relevant, even with the high variability noted within the available HCD range. The incidence (24%) was slightly above the range of the HCD included in the study report (22%) but within the HCD provided by the applicant (0-38%). The effect showed a statistically significant trend, which could not be dismissed by the DS.

The DS proposed a classification for quinoclamine as a carcinogen in Category 2 based on benign tumours (benign transitional cell papillomas in urinary bladder and benign pheochromocytoma in adrenals) noted in the rat and supported by the occurrence of malignant lymphoma noted in the mouse. The DS did not consider the data from the mouse together with the rat data sufficient to propose Carc. 1B classification.

Comments received during consultation

There was only one comment from an MSCA. They did not find the incidence of malignant lymphoma in mice convincing and noted the high spontaneous tumour incidence for adrenal pheochromocytoma in male rats. Overall, they considered the evidence to be borderline for classification with Carc. 2 or no classification. It was not clear which option was preferred by the MSCA.

Assessment and comparison with the classification criteria

Classification in category 1A/1B

There was little evidence to suggest quinoclamine is genotoxic, and there is no apparent mode of action data available to explain the urinary bladder benign tumours observed in rats, therefore this tumorigenic response is assumed to be potentially relevant to humans.

Classification in category 1A is not appropriate in this case as there is no human data. Classification in category 1B is also not considered appropriate as the evidence from the animal data is not considered sufficiently robust. Though it appears that two species show evidence of

tumorigenicity, the data from the malignant lymphoma incidence in the mouse 80 week study is considered weak at best. The remaining tumours are benign in nature and occur in the rat and show no reduction in latency, thus it is most appropriate to consider classification in category 2 or no classification.

Classification in category 2 or no classification

Benign transitional cell papillomas in urinary bladder in the Crl:CD(SD)BR rat

This effect constituted the strongest evidence for consideration of classification. The tumorigenic response was confined to the top dose group in both males (37.6 mg/kg bw/day) and females (49.4 mg/kg bw/day).

***Table**: Neoplastic findings at the end of the study (carcinogenicity cohort)*

Parameter/Dose (mg/kg bw)	**Males**				**Females**			
	0	**0.2**	**2.8**	**37.6**	**0**	**0.3**	**3.7**	**49.4**
Urinary bladder:								
Total: number examined	50	49	47	47	49	48	49	50
Transitional cell papilloma	0	0	0	**4**	0	0	0	**6**

Treatment related neoplastic changes in the carcinogenicity study were confined to benign transitional cell papillomas in the urinary bladder of 4/47 (9%) males and 6/50 (12%) females in the top dose groups. The response was limited to a single species with no evidence of a treatment related occurrence in mice; the tumours were benign with no evidence for progression to malignancy and there were no alterations in tumour latency. There was a particularly clear and strong treatment-related increase in the incidence of epithelial hyperplasia, especially in respect of the urinary tract, in high dose group animals and to a lesser extent in intermediate dose group animals (table "Non-neoplastic pathology at the end of the study", above). There was no evidence for a treatment related irritant effect on the affected epithelium and the tumours were considered to be of human relevance. There was no mechanistic evidence to suggest a lack of relevance for human health.

The HCD supports the concurrent controls in this study (Anon., 1991, study initiation May 1987); this tumour type is very rare.

In house HCD was reported in the original study report but only for male rats; there were no HCD for females. In total, 1 animal was affected out of a total of 349 control animals, but number of studies not reported. The highest individual study incidence was 2%, a single animal affected in one study control group.

More detail about the general background of this tumour type can be found from the 1992 report from Charles River laboratories[1]. There were 19 x 24 month studies with initiation dates between April 1984 and Feb 1989. There were a total of 1250 males and 1249 female control animals. The incidences were:

[1] Lang (1992) Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl:CD BR rat. Charles River Laboratories.

Males: 1/1250, (mean 0.08%); 1 animal was positive in a single study out of 19, maximum background incidence in a single study was 1%.

Females: 1/1249, (mean 0.08%); 1 animal was positive in a single study out of 19, maximum background incidence in a single study was 1.4%.

The general background incidence of benign transitional cell papillomas in the CD rat is such that a single incidence would be cause for concern. Coupled with the fact that the urinary tract epithelium is under strong hyperplastic pressure without knowing the basis for such an effect, the significance of 4 incidences of adenoma in males and 6 incidences in females from the top dose groups (38 - 49 mg/kg bw/day) is considered substantial and human relevance cannot be disregarded. RAC agrees with the DS and supports classification of quinoclamine as a carcinogen in Category 2 (H351 "Suspected human carcinogen").

Benign pheochromocytoma in adrenals in the Crl:CD(SD)BR rat

There was an apparent treatment-related increase in the incidence of benign pheochromocytomas of the adrenal medulla in high dose animals. The DS did not put much weight into this finding. The effect was greater in males than in females but in both cases the top dose group animals had noticeably higher incidences of this benign tumour than the respective control groups (14/47 (29.8%) in males and 4/50 (8%) in females compared with the control incidences of 8/50 (16%) in males and 1/49 (2%) in females respectively). The response was limited to a single species with no evidence of a treatment related occurrence in mice; the tumours were benign with no evidence for progression to malignancy and there were no alterations in tumour latency.

This tumour type is variable and quite common. In house HCD was reported in the original study report. Individual study backgrounds of 6-16% were reported in male rats with a mean incidence of 12% (42/349 control animals) and 0-4% in females with a mean incidence of 2.3% (8/350 control animals); the number of studies was not reported.

More detail about the general background of this tumour type in the same strain of rat can be found from the 1992 report from Charles River laboratories[1]. There were 19 x 24 month studies with initiation dates between April 1984 and Feb 1989. There were a total of 1249 males and 1258 female control animals. The incidences were:

Males: 188/1249, (mean 15%); all studies had incidences greater than zero and the individual background incidence for each study ranged from 4-30%.

Females: 49/1258, (mean 3.9%); the individual background incidence for each study ranged from 0-14.5%.

[1] Lang (1992) Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Crl:CD BR rat. Charles River Laboratories.

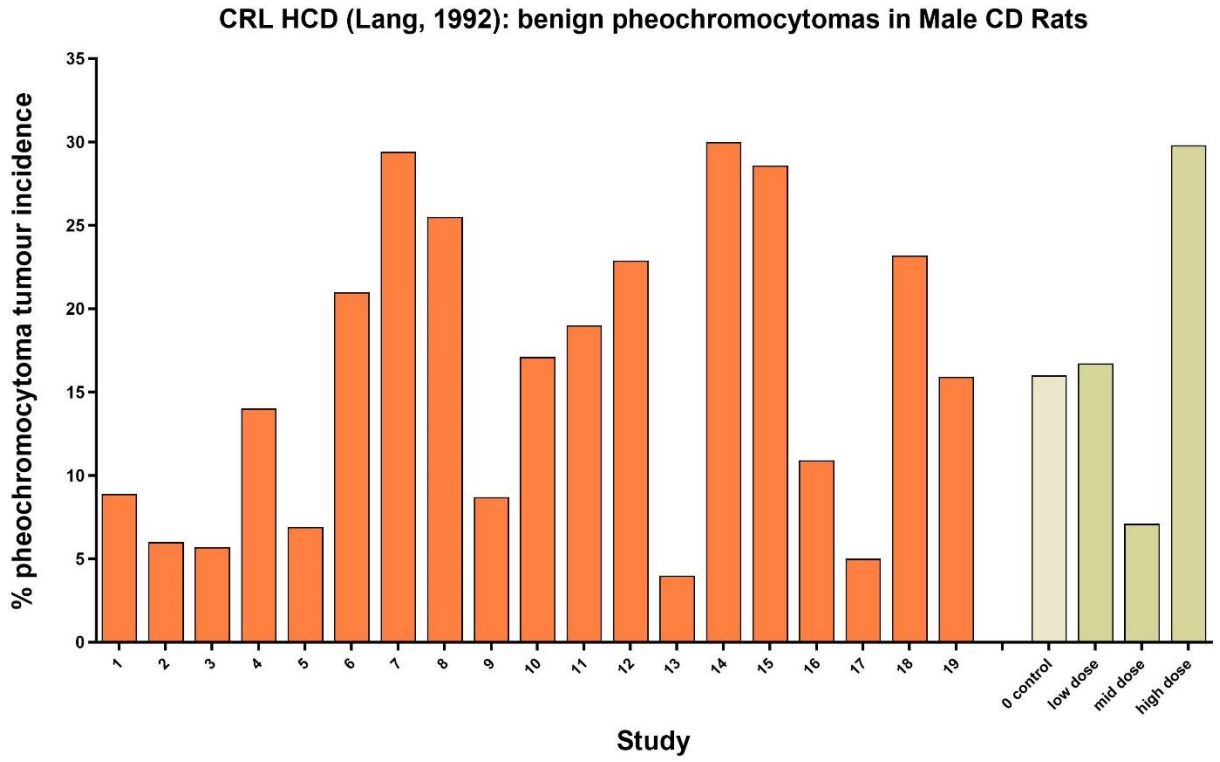


Figure: CRL study historical control data taken from Lang, 1992 which reported the incidence of spontaneous benign pheochromocytomas of the adrenal medulla in Crl:CD (SD) BR Rats from several control groups. This graph illustrates the high background and variable incidence of this tumour type in males of this strain of rat. The incidence of benign pheochromocytomas of the adrenal medulla from Anon., 1991 in the different treatment groups are illustrated at the end of the graph.

Even though the response in males is nearly double that in the concurrent controls, it remains just within the upper bound limit (30%) of the more general HCD published in 1992 from CRL. A visual plot of the CRL data in males (figure above) shows how variable this tumour type can be. Similarly, the response in females is also highly variable though the overall incidences are lower in females relative to males (figure below). The evidence from the quinoclamine carcinogenicity study suggests a weak carcinogenic response, though it must be noted that these effects occurred with relatively low exposures to quinoclamine in the top dose groups (38-49 mg/kg bw/day). Alone, the data on the incidences of adrenal pheochromocytoma are not sufficient for classification but may be regarded to support an overall case for classification into category 2 when taken together with the urinary bladder tumours.

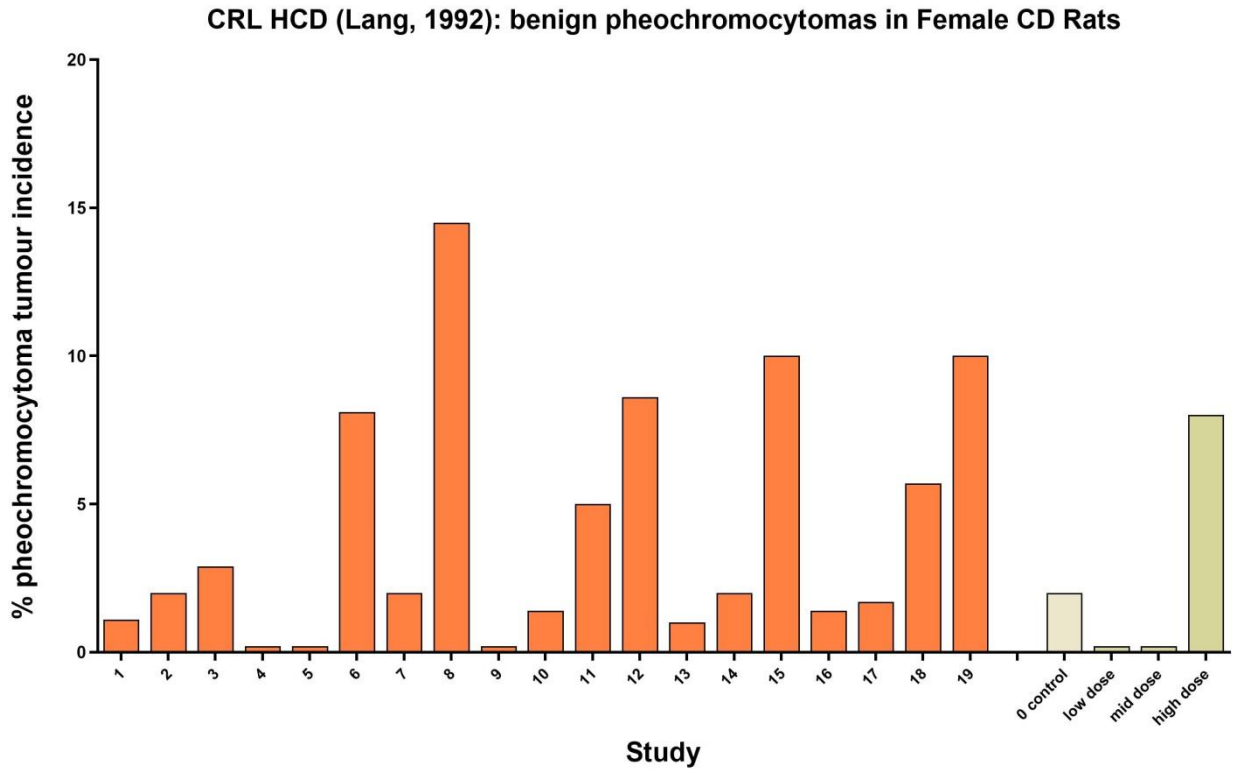


Figure: CRL study historical control data taken from Lang, 1992 which reported the incidence of spontaneous benign pheochromocytomas of the adrenal medulla in Crl:CD (SD) BR Rats from several control groups. This graph illustrates the high background and variable incidence of this tumour type in females of this strain of rat. The incidence of benign pheochromocytomas of the adrenal medulla from Anon., 1991 in the different treatment groups are illustrated at the end of the graph.

Malignant lymphoma in female mice (Crl:CD-1 (ICR) BR strain)

Malignant lymphoma was noted in the female mouse (Anon., 1993). There was a marginally significant ($p<0.05$) positive trend in malignant lymphoma due mainly to a higher incidence at the top dose only (12/50 cases, 24%) when compared with the controls (3/50 cases, 6%). There was no clear dose-response or treatment relationship. The low dose group had 11/50 cases (22%) and the intermediate dose had 7/50 cases (14%). This tumour was confined to a single species and males did not show any convincing evidence of a treatment related effect.

This tumour type is variable and quite common in female CD-1 mice. In house HCD was reported in the original study report. This showed that the background occurrence of malignant lymphoma in females ranged from 2 to 11 cases (4 – 22% incidence) from a total of 9 studies (table below).

Table: *Historical control data for Malignant lymphoma. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) female mice from carcinogenicity studies (original study report data)*

Study no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Lympho reticular tissues:									
Total: number examined:	50	50	50	50	50	50	50	50	50
Malignant lymphoma:	6	3	2	3	2	8	3	7	11
% incidence	12	6	4	6	4	16	6	14	22

Note: years of study not reported.

Further HCD was presented in the original DAR and the CLH report from the performing laboratory. Eleven studies were conducted from 1991 to 1994 with a background occurrence of malignant lymphoma in females ranging from 0 to 19 cases (0 – 38% incidence). (table below).

Table: *Historical control data for Malignant lymphoma. Neoplastic historical control information in female CD-1 mouse for the performing laboratory (1991-1994).*

Study end year	**1991**	**1992**	**1992**	**1992**	**1992**	**1993**	**1994**	**1994**	**1994**	**1994**	**1994**
Lympho-reticular tissues:											
Total: number examined	50	60	60	50	50	50	55	55	25	25	50
Malignant lymphoma:	3	6	6	12	6	19	4	0	3	3	9
% incidence	6	10	10	24	12	38	7.3	0	12	12	18

The DS did not exclude a substance related effect. The HCD data together with the actual test data from the Anon., 1993, study indicated that no firm conclusion could be made with respect to the occurrence of malignant lymphoma in female CD-1 mice and in contrast to the DS, RAC does not place much weight on the occurrence of this tumour type. RAC notes that there were no incidences of tumour in the high dose males and that the tumour incidence in the female low dose group and top dose group was similar even with a dose difference more than 100 fold. RAC finds no evidence for a treatment related increase in malignant lymphoma.

Other tumour types noted in mice

Further types of tumour are considered: (1) histiocytic sarcoma and (2) adrenal cortex tumours.

The DS noted that when treatment results for both sexes were combined, the trend for histiocytic sarcoma (3 cases in total at the top dose) was marginally significantly ($p<0.05$) positive. This statistical treatment is erroneous as the HCD indicates a different background incidence between males and females, females having the greater spontaneous incidence rate. The incidences in the top dose groups (males 1/50; females 2/50), while greater than the concurrent controls (0/50) were nonetheless well within the HCD supplied from the performing laboratory (the next 2 tables below).

Table: *Historical control data for Histiocytic sarcoma. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) female mice from carcinogenicity studies (original study report data)*

Study no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Lympho reticular tissues:									
Total: number examined	50	50	50	50	50	50	50	50	50
Histiocytic sarcoma:	0	0	3	3	1	6	3	2	3
% incidence	0	0	6	6	2	12	6	4	6

Note: years of study not reported.

Table*: Historical control data for Histiocytic sarcoma. Neoplastic historical control information in female CD-1 mouse for the performing laboratory (1991-1994).*

Study end year	**1991**	**1992**	**1992**	**1992**	**1992**	**1993**	**1994**	**1994**	**1994**	**1994**	**1994**
Lympho reticular tissues:											
Total: number examined	50	60	60	50	50	50	55	55	25	25	50
Histiocytic sarcoma:	0	3	3	2	0	1	1	0	1	0	4
% incidence	0	5	5	4	0	2	1.8	0	4	0	8

RAC agrees with the DS, that histiocytic tumours found in the mouse study were a chance finding and there was little evidence to suggest a treatment related response.

The DS noted hyperplastic changes in the adrenal cortex. Spindle-cell hyperplasia was extensive in both sexes, particularly females, but with no clear relationship to treatment. According to the US NTP online histopathology atlas, spindle or fusiform type A cells may be one of the two types of cells present in subcapsular hyperplastic lesions of the adrenal cortex. In abundance, adrenal spindle cells give rise to early hyperplastic lesions. Focal subcapsular hyperplasia is considered a proliferative lesion that if progresses it may, following subsequent enlargement and appearance of larger, round or polygonal type B cells lead to adenoma, but rarely to carcinoma. Spindle cell tumours can form subcapsular adenomas and invade the cortex, so it is always worth looking for adrenal cortical adenomas in addition to spindle cell adenomas. There was no evidence of progression to spindle cell adenomas or cortical adenomas. In contrast to the rat, there was no evidence for increased incidences of pheochromocytomas.

Summary and Conclusion of the significance of the tumour findings

Sections 5.1 through to 5.4 of this document have outlined the data and evidence for consideration of classification into category 2. It may be summarised as follows: benign transitional cell papillomas in urinary bladder and pheochromocytoma in adrenals were only found in one species (rat) and in one study. Quinoclamine induces benign tumours (transitional cell papillomas in urinary bladder) in Crl:CD(SD)BR rats of both sexes. The pheochromocytomas typically displayed a highly variable but sufficiently high spontaneous background incidence from HCD that it is difficult to conclude or dismiss if a weak treatment related response occurred. The incidence of malignant lymphoma was noted in one species only (mouse) and was within the HCD and is not considered to be treatment related.

The CLP guidance acknowledges that for tumours to be recognised as being treatment related and relevant to human health, several factors must be taken into account using a weight of evidence approach.

These factors are presented and summarised in the table below.

***Table**: Significance of the tumour findings for human relevance.*

Finding	Observation	Significance
Tumor type	1. Rodent papillomas in urinary bladder 2. Benign pheochromocytoma in adrenals 3. Malignant lymphoma, mouse	Mild Mild High
Background Incidence	1. Benign transitional cell papilloma has a very low background incidence. 2. Benign pheochromocytoma is quite variable with incidences reaching beyond 30% in control animals. 3. Malignant lymphoma is highly variable, study incidences do not exceed HCD. Low dose and top dose incidence in females are similar.	High Low. Low.
Tumors at multiple sites	In rat, possibly Yes.	High
Progression of lesions to malignancy	No evidence for progression. The malignant lymphoma is not considered treatment related.	Low
Reduced tumour latency	No	Low
Response in both sexes	Yes (benign transitional cell papilloma). Pheochromocytomas show greater effect in males.	Mild
Tumors in one or multiple species	Single species (significant effects confined to rats only)	Low
Structural similarity to other carcinogens	It is a member of the quinone class of organic molecules of which anthraquinone is also a member.	Low
Routes of exposure	Oral (dietary).	High
Local Absorption toxicokinetics comparable for humans	No information.	Low
Confounding effect by excessive toxicity	No. Only small doses were investigated in the top dose groups. In the mouse study the survival was adversely affected but there was no clear explanation for this effect.	High
Metastases	No (no evidence)	Low
Dose-related increase	No (tumours at high dose only)	Low
Mode of Action and human relevance	Unknown MoA. Cannot dismiss human relevance.	Mild
Genotoxicity	Insufficient evidence.	Low

RAC is of the opinion that there is sufficient evidence to support classification for carcinogenicity based primarily on the occurrence of benign transitional cell papillomas in urinary bladder in the rat supported by the increased incidences of adrenal pheochromocytoma. Histopathology showed no classic signs of urinary bladder irritation and inflammation (presence of calculi with lymphocytic infiltration) except for a notable epithelial hyperplasia throughout the urinary tract. There was some evidence for preneoplastic changes: squamous metaplasia of the hyperplastic urinary epithelium was seen in 3 high dose females, along with a single polyp.

RAC notes that the HCD for the rat pheochromocytomas and mouse malignant lymphomas indicates a high level of variability and high levels of incidence which makes it difficult to discern a treatment related effect in these cases though for pheochromocytomas the evidence is considered weak rather than insignificant.

RAC agrees with the DS and proposes a classification of quinoclamine as **Carc. 2 (H351 "Suspected human carcinogen")** based on conclusive evidence in rats.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Summary of data from the studies on reproductive toxicity

The DS proposed **no classification for fertility and sexual development** based on the limited results obtained in a single, pre-guideline and pre-GLP, dietary 2-generation study in the SD rat (B.6.6.1, Study 1, Anon., 1975).

The DS described 9 developmental toxicity studies in total, in both rats and rabbits, including the associated range-finding studies, in addition to a single dermal exposure developmental toxicity study using the rat. All studies claimed GLP compliance but were not stated to have been performed according to international guideline protocols. The DS noted the following developmental anomalies as the basis for **proposing classification for development in Repr. 2; H360d:**

1. Aortic arch malformations in rats and rabbits
2. Skeletal malformations in rabbits, i.e. abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, misaligned thoracic vertebral arch
3. Hydronephrosis in rats and rabbits
4. Significantly reduced foetal weight in rats (7-12*% less than controls) and non-significantly reduced foetal weight in rabbits (5%), in the presence of reduced maternal bodyweight gain.
5. Post-implantation loss in both rabbits (22.4 – 61%) and with a smaller effect in rats (10.7 – 24.5%) outside of the HCD range at the top dose level.

<u>Adverse effects on sexual function and fertility</u>

A single Sprague-Dawley rat, 2-generation reproductive toxicity study (RAR Vol. 3, B.6.6.2/01, Anon., 1975) was briefly described. The study was not GLP-compliant and pre-dated the establishment of OECD technical guidelines. Altogether, four generation litters (F1a, F1b, F2a, F2b) were produced because a second mating was introduced for each generation. Offspring from the first mating trials (F1a, F2a) were maintained only up to weaning on lactation day (LD) 21 and then sacrificed. Offspring from the second mating trials were either taken by caesarean section at the end of the gestation period or delivered and maintained through weaning or for 5 weeks (F1b) or 3 months (F2b) postweaning.

Groups of 25 male and 25 female rats received quinoclamine (98.5%) mixed in their daily feed at concentrations of 1, 25 and 500 ppm through two successive generations which corresponded to intake exposures of:

- 0/0, 0.07/0.07, 1.6/1.7 and 30.9/37.0 mg/kg bw/day for F0/F1 males and
- 0/0, 0.08/0.08, 1.9/2.0 and 37.0/43.8 mg/kg bw/day for F0/F1 females.

The DS described the study as limited and noted several deviations when compared with OECD TG 416. These were:

i. No evaluation of the oestrus cycles was performed for either generation.
ii. No examination of sperm parameters was performed for either generation.
iii. Gestation length was not specified.
iv. Organs were not weighed.
v. Vagina, testis, epididymides, seminal vesicles, prostate, and coagulating gland were not investigated microscopically.
vi. Detailed testicular histopathology was not performed.
vii. Post-lactational ovary (primordial and growing follicles) histopathology was not performed.
viii. For the offspring, age at vaginal opening or PPS for the F1or F2 was not determined.
ix. Housing conditions (temperature and humidity in experimental room) was not specified in study report.
x. The study report did not include information on statistical analysis.

Based on the available data from the 2-generation study the DS concluded that treatment with the test substance did not affect mating performance or fertility of the male and female parental animals. There were no consistent effects noted on parental food consumption, survival rates and parturition indices or postnatal and postweaning survival. Some maternal effects were noted as reductions in body weight of 9-10% and this effect was paralleled with lower offspring weights of similar magnitude at weaning in all filial generations (F1a: 13% and 7% in males and females; F1b: 14% and 9%; F2a: 8% and 9%; F2b: 11% and 5% in males and females, respectively).

There were no indications of a teratogenic effect on foetuses. No deaths or signs of compound induced toxicity were observed in the F1b or F2b generation offspring maintained for five weeks and three months postweaning, respectively. The only significant offspring effect of note with an apparent dose related increase was an increased absolute incidence of grey lung cysts, which was confined to the F2b offspring at necropsy (11, 18, 29 and 39 in the controls, low, mid and top dose groups respectively). The F1b generation did not show any observable gross pathology at necropsy. The relevance of this finding was unclear. The finding was not observed in the offspring of the F1b generation reared for 5 weeks or in other available toxicological studies on quinoclamine. It may have been symptomatic of an infectious aetiology as lung lesions in laboratory rats were quite common but there were no further details to elaborate on the observed lesions. The DS did not consider this effect relevant for reproductive toxicity classification.

Adverse effects on development

The DS provided extensive descriptions within the CLH report of 9 studies available for the assessment of developmental toxicity spanning the years 1986 to 2002. Amongst these was 1 rat dermal embryo-foetal development study (Anon., 1996) which tested quinoclamine up to 600 mg/kg bw/day, in which minimal maternal toxicity was observed and no effect was seen on the development of the foetuses. The remaining 8 studies were comprised of rat and rabbit main prenatal developmental studies and their associated range-finding studies from 1986 and similarly in updated studies performed in 2002.

The DS summarised all the main effects from each study in table 2.6.6.2-1 and table 2.6.6.2-3 in the CLH report. For clarity RAC presents the following summary table outlining each of the 9 studies assessed for developmental toxicity:

***Table**: Summary of the studies considered for developmental toxicity.*

Study type/ species	Dose levels (mg/kg bw/day)	Comments
Study 01: SD Rat teratology range finding study. GLP: Yes; Guideline: No	[0, 8, 50, 80, 200, 500] 0.25% gum tragecanth Dosing GD7-17 necropsy GD20	Ref: RAR Vol. 3, B.6.6.2.1/01 (Anon. 1986/1989) #33 5 x F/dose dose 200-500 → 60-100% lethality Limited study, possible indication of embryo lethality
Study 02: SD Rat teratology study. GLP: Yes; Guideline: No	[0, 5, 20 and 75] 0.25% gum tragecanth Dosing GD7-17 necropsy GD20	Ref: RAR Vol. 3, B.6.6.2.1/02 (Anon. 1986) #25 24 x F/dose Visceral malformations top dose group.
Study 03: SD Rat teratology range finding study. GLP: Yes; Guideline: No	[0, 10, 50, 100] 1% w/v methylcellulose Dosing GD6-19 necropsy GD20	Ref: RAR Vol. 3, B.6.6.2.1/03 (Anon. 2002) #34 7 x F/dose
Study 04: SD Rat teratology study. GLP: Yes; Guideline: Yes	[0, 5, 20 and 75] 1% w/v methylcellulose Dosing GD6-19 necropsy GD20	Ref: RAR Vol. 3, B.6.6.2.1/04 (Anon. 2002) #26 24 x F/dose
Study 05: NZW rabbit teratology range-finding study. GLP: Yes; Guideline: No.	[0, 8, 20, 50]* 0.25% gum tragecanth Dosing GD6-18 necropsy GD28	Ref: RAR Vol. 3, B.6.6.2.2/01 (Anon. 1986 and addendum, Anon., 1989). #28 2-5 x F/dose
Study 06: NZW rabbit teratology study. GLP: Yes; Guideline: No.	[0, 2.5, 7.5, 22.5] 0.25% gum tragecanth Dosing GD6-18 necropsy GD28	Ref: RAR Vol. 3, B.6.6.2.2/02 (Anon. 1986) #27 16 x F/dose
Study 07: NZW rabbit prenatal developmental range-finding study. GLP: Yes; Guideline: No.	[0, 5, 17.5, 30] 1% w/v methylcellulose Dosing GD7-28 necropsy GD29	Ref: RAR Vol. 3, B.6.6.2.2/03 (Anon. 2002) #35 7 x F/dose
Study 08: NZW rabbit prenatal developmental study. GLP: Yes; Guideline: No.	[0, 5, 17.5, 30] 1% w/v methylcellulose Dosing GD7-28 necropsy GD29	Ref: RAR Vol. 3, B.6.6.2.2/04 (Anon. 2002) #29 24 x F/dose
Study 09: SD Rat dermal teratology study. GLP: Yes; Guideline: No	[0, 5, 100, 600] 1% w/v Tween 80 Dosing GD6-15 necropsy GD20	Ref: RAR Vol. 3, B.6.8.2/01 (Anon. 1996) #30 25 x F/dose

*(also [80→8, 200→20, 500→50], doses too high, reduced after 1 day).
Key studies are **emboldened in black** (#02, #04, #06, #08)

In both the rat and the rabbit oral embryo-foetal development toxicity studies, it is clear from the DS assessment there was evidence of maternal toxicity and (in contrast to comments by industry and third party commentators), primary rather than secondary embryo-foetal toxicity with retardation of foetal development, as indicated by increased post-implantation loss, reduced foetal body weights and retarded foetal ossification in both rats and rabbits. The DS also summarised extensive commentary by the industry applicant regarding developmental effects triggered by transient undernutrition of the pregnant animals. It is important to note that no mechanistic evidence was available to the DS to substantiate any of the claims in this commentary and therefore it remains speculative.

The DS evaluated the maternal toxicity and effects on the foetuses from all studies. The DS identified the main developmental abnormalities and assessed the relevance of:

1. Aortic arch malformations noted in both species that could not be explained by maternal toxicity.
2. Skeletal abnormalities (abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, and misaligned thoracic vertebral arch, scoliosis and sternebral fusion).
3. Hydronephrosis (a term reserved for extreme renal pelvis cavitation) and misshapen kidney → adverse but not sufficient for classification.
4. Foetal growth retardation and minor foetal variations.
5. Abortion/implantation loss/intrauterine death.
6. Subcutaneous oedema → adverse but not sufficient for classification.
7. Hyperextension of limb or paw, spina bifida → adverse but not sufficient for classification.

DS assessment of the relevance of abnormalities

From the list of noted developmental abnormalities the DS concentrated on aortic arch malformations, skeletal abnormalities, hydronephosis, foetal growth retardation and implantation loss for assessing classification for development.

1. Aortic arch malformations

Aortic arch malformations were noted in several studies (table above: #02, #05, #06) in both species, and could not be explained by maternal toxicity. The defect was considered severe and relevant for classification for reproductive toxicity, although the incidences of aortic arch malformations were low and the effect was not reproducible in the later rat and rabbit 2002 studies (table above: #07, #08). Conclusion: the finding is relevant for classification.

2. Skeletal abnormalities

The DS described several types of skeletal variations in both rats and rabbits and malformations in rabbits only. The variations were similar in both species and consistent with retardation of foetal development. Malformations were seen in rabbits but did not present as a uniform or consistent set of specific target effects. Several malformations were seen in the 1986 rabbit main developmental study (Anon., 1986, #06) of which only sternebral fusion noted in 3 animals in the top dose group (22.5 mg/kg bw/day) may be considered relevant and treatment related; the incidence (2.6%) was outside the background data of the laboratory in 1985 (0.7%). The effect was not repeated in the later (2002) study which tested quinoclamine at a slightly higher dose (30 mg/kg bw/day). Conclusion: Effects observed in the Anon., 1986 study (table above: #06) are relevant for classification.

3. Hydronephrosis

The DS considered hydronephosis a malformation. If this were true, then it would be additional evidence for supporting classification. The DS commented extensively on the incidences of hydronephosis in both the rat and rabbit developmental toxicity studies (particularly in the table above: studies #04, #08). The incidences were outside the limited HCD. However, the effect, though adverse, is considered a variation by RAC, originating from either direct or indirect effects of conditions affecting the urinary conduit. Conclusion: the DS considered it sufficiently adverse for supporting classification.

4. Foetal growth retardation

Reduced foetal weight was noted in the rat (statistically significant, reductions up to 12%) and rabbit (not statistically significant). Conclusion: the DS did not consider this effect supported classification.

5. Implantation loss/intrauterine death

Many of the studies supported an effect on implantation loss. The DS noted a dose-related increased post-implantation loss in rabbits (Anon., 2002, #07, #08 with limited support from 1986, #05). In the rat studies (Anon., 2002; #03, #04) the incidence of post-implantation loss (11% compared to 5% in control; #04) was not statistically significant but was higher than in the supplied background data. The DS considered the post implantation loss treatment related and adverse but not sufficient for classification.

According to the DS, the main effects noted for proposing classification were visceral and structural abnormalities (aortic arch abnormalities, skeletal abnormalities [abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, misaligned thoracic vertebral arch], and kidney effects i.e. hydronephrosis, misshapen kidney (one single case noted in rabbit) and increased incidence of kidney pelvic cavitation (noted in rabbit).

The DS proposed classification of quinoclamine as toxic for reproduction in Category 2, H361d ("Suspected of damaging the unborn child").

Adverse effects on or via lactation

The DS drew on information provided by the limited 2-generation study in rats. There was little information to suggest an effect through lactation. There was no human evidence indicating a hazard to babies during the lactation period, or data on absorption, metabolism, distribution and excretion studies that indicate a likelihood that the substance was present in potentially toxic levels in milk, and no data on residue levels and transfer into milk. Limited reductions in pup weight were noted in the 2-gen study but were considered not to present any clear evidence of an adverse effect via lactation.

Comments received during consultation

There was a single comment from one MSCA in support of the DS' proposal for Repr. 2; H361d. The MSCA agreed with the justification outlined by the DS in the CLH report regarding multiple effects in two species over several studies.

Assessment and comparison with the classification criteria

Assessment of the data on adverse effects on sexual function and fertility

The DS assessed the Anon., 1975 rat 2-generation study appropriately. Many deficiencies were identified which call into question the acceptability and usefulness of this study. Based on the limited available data from the 2-generation study the DS concluded that treatment with the test substance did not affect mating performance or fertility of the male and female parental animals and there was no indication of a teratogenic effect in any of the offspring. RAC notes the poor dosing regimen employed in this study with a dose spacing insufficient to detect any anomalies between the control group and the top dose group. Dose levels of 0, 0.1, 2.0 and 31-43 mg/kg bw/day approximately were utilised. Follow up parameters for pubertal and other parameters in offspring were lacking. The increased absolute incidence of grey lung cysts confined to the F2b offspring at necropsy (11, 18, 29 and 39 in the controls, low, mid, and top dose groups

respectively) may have been symptomatic of an infectious aetiology. There were no detailed investigations into this lesion.

Histopathological changes

In its assessment of endocrine disrupting properties, the DS noted some histopathological changes limited to two repeat dose studies, the dietary 2-year dog study and the rat dietary chronic/carcinogenicity study. These changes were noted in the testis (dog) and uterus (rat), and female mammary gland (rat). There was no clear effect pattern and the DS did not attribute much significance to the effects.

In the 2-year dog study gonadal changes were noted at 1000 ppm [27/29 mg/kg bw/day M/F] (aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis in 3/3 animals vs 0/3 controls in males and lack of of cyclic activity in 3/3 animals vs 0/3 controls, and follicular cysts in 1/3 animals vs 0/3 controls in females).

In the rat chronic/carcinogenicity study there was reduced mammary acinar development and secretion seen in the top dose (676 ppm, 49.4 mg/kg bw/day) females at 104 weeks with no remarkable abnormalities. The study authors ascribed this to lower food consumption in this group of animals.

In the uterus, hydrometra (retention of fluid or blood in the uterus) was seen at week 26 (1, 1, 3, 4 for the control, low-, mid- and high dose, respectively). The finding was also seen at 52 weeks in 1 animal at the top dose. No further detail was provided.

The significance of these effects is uncertain, especially in light of the absence of histopathological data on these effects from the other repeated dose studies and the reproductive toxicity studies.

The DS proposed **no classification for fertility and sexual development** based on the limited results available from the 2-generation study. RAC notes the age and lack of investigated parameters with reference to an updated guideline compliant study and considers the present study insufficient on which to base any kind of robust scientific conclusion. RAC agrees with the classification proposal by the DS, for **no classification for fertility and sexual development** based on **inconclusive data**.

Assessment of the data on adverse effects on development

There were 9 studies available within the CLH report for the assessment of developmental toxicity, spanning the years 1986 to 2002. These were summarised briefly in the table "Summary of the studies considered for developmental toxicity" above. Dosing in all studies was relatively low due to excessive maternal toxicity found in rats at levels > 100 mg/kg bw/day and rabbits ≥ 80 mg/kg bw/day. The range-finding studies are mainly indicative of potential effects because of the low numbers of animals used (typically 5-7 per dose group) but in some cases they support the 2 main effects seen with treatment: increased post implantation loss and visceral and skeletal developmental abnormalities. Each study is summarised below in terms of the most serious effects that may be considered for developmental classification. The DS has described in detail the many skeletal variants in the CLH report. These are not discussed below.

Study 01: Rat teratology range finding study (Anon., 1986)

Doses [0, 8, 50, 80, (200, 500)] mg/kg bw/day

Maternal effects:

Limited study, indicative only. Maternal toxicity was unacceptable and excessive at the 200 and 500 mg/kg bw/day levels. At 80 mg/kg bw/day and lower there was minimal maternal toxicity. This was expressed mainly as small reductions in food consumption and body weight. Necropsy in the 80 mg/kg bw/day dose group and below were uneventful. Post implantation loss was evident

in the surviving dams (2) of the 200 mg/kg bw/day group but overall, there was no firm pattern for embryo lethality. The mean number of live foetuses and the sex distribution of the foetuses were not affected by treatment.

Litter effects:

There was a minor reduction of foetal body weight in the 80 mg/kg bw/day dose group (-8%). Implantation loss in the 8 mg/kg bw/day dose group was skewed because of total litter loss for 1 dam. Subsequent investigations (Anon., 1989) that focused on teratogenicity found only one foetus from the 80 mg/kg bw/day dose group with multiple major malformations. There was no evidence for a substance related or dose related effect.

Study 02: Rat main prenatal developmental study (Anon., 1986)

Doses [0, 5, 20, 75] mg/kg bw/day

Maternal effects:

There were no deaths and no clinical signs observed in any group. Maternal toxicity was minimal, expressed as a reduction in body weight gain (-25%) with a similar reduction in food consumption (-25 to -14%) during gestation in the top dose group only. Necropsy revealed an enlarged spleen in top dose females (4/24) and a single incidence in dams at 20 mg/kg bw/day. There were no effects of treatment at any dose level on implantation or on post-implantation losses. The mean number of live foetuses and the sex distribution of the foetuses were not affected by treatment. Other pregnancy data was similar amongst all dose groups to controls.

Litter effects:

There was a minor reduction of foetal body weight in the top dose group (-7%). Malformations of the aorta (4 foetal cases of absent innominate artery [brachiocephalic artery in humans], 3 cases in litter 89, 1 in litter 81 and a single case of interrupted aortic arch, litter 92) and reversal of thoracic organs (2 cases of situs inversus, litters 95 and 96) were observed in the top dose group. A single foetal incidence of absent innominate artery was also recorded for the mid dose group. There were zero incidences in the concurrent controls for these malformations. There were no HCD from the performing laboratory for the aortic malformations. Background data for *Situs inversus* was reported in the original study report. A single case (0.06%) was reported from 2171 control foetuses from 167 litters, dating from 1985, corresponding to a maximum incidence of 0.4% in one study. The present study has 2 cases in the high dose group.

The RMS had reported HCD from a collection of other laboratories and compiled by MARTA from studies conducted from 1989-1992 from Crl:CD BR rats and published by Charles-River laboratories in 1993[1]. Maximum incidences were reported in the DAR tables, but these figures were not representative of the average foetal incidence and did not solely focus on 20-day gestational studies such as the one by Anon., 1986. RAC noted the average foetal incidence for absent (agenesis) innominate artery was 0.049% and interrupted aortic arch 0%. The data was compiled from 154 studies, 3240 litters with a total of 22,892 foetuses. The incidences of aortic arch malformations in the Anon., 1986 study far exceed those of the HCD published in the CRL 1993 report.

[1] Lang (1993) Historical Control Data for Development and Reproductive Toxicity Studies using the Crl:CD®BR Rat. Compiled by MARTA. Charles River Laboratories.

Table: *Incidence of foetuses with malformations. % incidence in parentheses*

	Dose level (mg/kg bw/day)				
Malformation	**0**	**5.0**	**20**	**75**	**HCD**
Aortic arch:					
- absent innominate artery	-	-	1 (0.4)	4 (1.5)	0.05%[1]
- interrupted aortic arch	-	-	-	1 (0.4)	0%[1]
Situs inversus	-	-	-	2 (0.8)	0.06 (0.4% max)[2]
No of foetuses examined	273	285	273	263	915
No of litters examined	21	20	21	21	122

[1] Compiled by MARTA from studies conducted from 1989-1992 from Crl:CD®BR rats and published by Charles-River laboratories. There was no inhouse HCD.
[2] A single case (0.06%) was reported from 2171 control foetuses from 167 litters, dating from 1985. Inhouse data: unknown.

Study 03: Rat teratology range finding study (Anon., 2002)

Doses [0, 10, 50, 100] mg/kg bw/day

Maternal effects:

One control female was killed for humane reasons after an accident on the first day of dosing (rubber catheter lodged in the oesophagus). One high dose female died on GD 20, possibly due to maldosing. Maternal toxicity was evident with reduced bodyweight gain noted in dams of all treatment groups (18%, 27%, 41% in dams of 10, 50 and 100 mg/kg bw/day groups, respectively), reduced food consumption noted at ≥50 mg/kg bw/day, reduced gravid uterus weight (17%) noted in dams at 100 mg/kg bw/day. There was evidence of increased embryo lethality (early intrauterine deaths) with increased post-implantation loss noted at ≥50 mg/kg bw/day [2.8, 3.0, 6.2 and 10.7% at 0, 10, 50, and 100 mg/kg bw/day, respectively]. Limited HCD was supplied in the original study report; 6 studies, undated but derived from the animals used in the performing laboratory reported a range of 4.0 – 6.5% for background % post-implantation loss. The mean number of live foetuses was slightly less than controls in the top dose group and the sex distribution of the foetuses were not affected by treatment.

Litter effects:

Sex ratio and mean placental weight were unaffected by treatment. There was a minor non-significant reduction in mean foetal weight and mean litter weight by 16% and 12% respectively in the top dose group. There were no foetal malformations due to treatment.

Study 04: Rat main prenatal developmental study (Anon., 2002)

Doses [0, 5, 20, 75] mg/kg bw/day

Maternal effects:

There were no mortalities and few clinical signs of note associated with treatment. Necropsy at GD20 was uneventful. There was some evidence of maternal toxicity. Mean corrected body weights by GD20 were significantly reduced in the top (-22%) and mid dose (-13%) groups and food consumption was also impacted with significant reductions (13-44% less than controls) throughout the gestational dosing period (GD6-19). Mean gravid uterus weight was also significantly reduced in the top dose group (-30%) and mid dose group (-15%). Pregnancy rate was unaffected by treatment. One female in the high dose group showed total embryo-foetal loss. This animal had 14 implantations, all of which were early intrauterine deaths. In the high dose group, there was an increase in the incidence of post-implantation loss compared to the control group [5.0, 5.6, 6.0 and 11.0% at 0, 5, 20, and 75 mg/kg bw/day, respectively]. While this increase was not statistically significant, it was higher than expected from the current background

data (4.0%-6.5%) and was due to a higher than expected increase in the mean number of early intrauterine deaths (1.1 in the top dose group relative to the background range of 0.6 to 0.9). Mean sex ratios and mean placental weight were unaffected by treatment.

Litter effects:

In the 20 and 75 mg/kg bw/day dose groups, mean foetal weights were significantly lower (-7 to -12% respectively) than control and the effect was dose-related. Mean litter weight was also significantly reduced in the mid dose group (-13%) and top dose group (-29%) relative to the control group. The overall foetal incidences of malformations increased with dose: 1 in the control group, 2 in the low dose group (2 litters), 5 in the mid dose group (3 litters) and 6 in the high dose group (3 litters). Even though several abnormalities were noted in the high dose group they mainly occurred as single incidences except for hydronephrosis (3 cases in 2 litters, 1.1% foetal incidence, 0% in controls) which lay outside the mean for the HCD range (0.2% based on 14 cases out of 6208 control foetuses). The assignment of hydronephrosis in the RAR (2018) as a malformation is curious; RAC certainly considers it an anomaly and a variant rather than a malformation. This is also in line with the lexicon of the DevTox Nomenclature Information System found at https://www.devtox.org/. It may be classed as a malformation if it arises as a consequence of renal paraenchymal necrosis or other developmental changes that result in a change to the urinary tract that impedes normal urinary flow but there was no histological evaluation performed to clarify if changes to the urinary tract were responsible for the hydronephosis. Overall, there was no firm evidence for a dose related increase in any malformation.

<u>Study 05: Rabbit dose-range finding study (Anon., 1986/89)</u>

Doses [0, 8, 20, 50, (80, 200, 500)] mg/kg bw/day

Maternal effects:

The original design of the study was to also dose five animals in extra groups at 80, 200 or 500 mg/kg bw/day. Because of severe toxicity elicited at the highest dose level, the doses were reduced after one dose to 8, 20 or 50 mg/kg bw/day, respectively. These groups were not reliable for assessment and were kept separated from the original 8, 20 and 50 mg/kg dose groups. However, they supported the effect of increased post-implantation loss with increasing dose.

At 8, 20 and 50 mg/kg bw/day (main dose groups and not those reduced because of toxicity), there was little evidence of maternal toxicity. Body weights were similar to controls. Food consumption was significantly reduced for the 50 mg/kg bw/day group during GD6-10 only and recovered thereafter. Necropsy at GD28 was uneventful. There was no apparent effect of treatment on pre-implantation. However, the rabbit displayed increased post-implantation loss in a positive dose dependent manner [8.7, 9.1, 31.1 and 61.0% at 0, 8, 20, and 50 mg/kg bw/day, respectively] but the number of animals per dose group [pregnant: 5, 2, 2 and 3, respectively] were variable and low. The results are indicative and not robust.

Litter effects:

Despite the increase in dead implantations, there was no growth retardation apparent in the surviving foetuses. Treatment with quinoclamine at 50 and 20 mg/kg bw/day appeared to be associated with major foetal malformations. At 20 mg/kg bw/day there were 3 affected foetuses in total: 2 affected foetuses in one litter and 1 in another. One showed spina bifida and interrupted aortic arch, one showed spina bifida alone and one showed malrotated hind limb. At 50 mg/kg bw/day interrupted aortic arch (1 animal) and left kidney agenesis (1 animal) were noted in separate litters. The malformations are more significant because only a small number of foetuses were available for examination [41, 18, 13 and 10 at 0, 8, 20, and 50 mg/kg bw/day, respectively].

***Table**: Incidence of foetuses with malformations (% incidence in parentheses)*

	Dose level (mg/kg bw/day)				
Malformation	**0**	**8.0**	**20.0**	**50.0**	**HCD 1985***
Interrupted aortic arch	-	-	1 (7.7)	1 (10.0)	5 (0.5)
Spina bifida	-	-	2 (15.4)	-	2 (0.2)
Kidney agenesis	-	-		1 (10.0)	--
Malrotated limb	-	-	1 (7.7)	-	--
No of foetuses examined	41	18	13	10	915/708
No of litters examined	5	2	2	2	122/109

* background data from the performing laboratory, comprised of both control and inactive treatment animals. Range only available for combined animals. No detail for individual studies.

Study 06: Rabbit main prenatal developmental study (Anon., 1986)

Doses [0, 2.5, 7.5, 22.5] mg/kg bw/day

Maternal effects:

There were no mortalities and no clinical signs associated with treatment. There was little evidence of maternal toxicity. Body weights and food consumption were only slightly impacted; though there was a significantly reduced bw gain on GD6-9 in the top dose group, the overall effect was minor by the end of gestation. Necropsy at GD28 was uneventful. There were no effects of treatment at any dose level on implantation or on pre- or post-implantation losses.

Litter effects:

Mean foetal weight was slightly but not statistically significantly lower in the top dose group relative to the control group. Malformations were noted at 22.5 mg/kg bw/day. In total, 9 foetuses from 6 litters were affected compared with 3 foetuses from 3 control litters. The malformations consisted of scoliosis (1 animal), spina-bifida (3 animals, 3 litters), aortic arch (2 animals, same litter), major sternebral fusions (3 animals, same litter) and hyperextension of limb or paw (1 animal). All malformations except fused sternebra were within the foetal *HCD range* presented for the performing laboratory using the same strain of rabbits and dating from 1985. However, no detail was available for individual studies and the upper limit of this range cannot be put into context. Except for scoliosis, the mean incidence of all malformations was outside the mean foetal HCD. Conclusion: **sternebral fusion** noted in 3 animals in the top dose group (22.5 mg/kg bw/day) may be considered relevant and treatment related as may the other malformations noted because of the uncertainty with respect to the HCD.

***Table**: Incidence of foetuses with malformations (% incidence in parentheses).*

	Dose level (mg/kg bw/day)				
Malformation	**0**	**2.5**	**7.5**	**22.5**	**HCD 1985***
Aortic arch	1 (0.8)	-	-	2 (1.7)	5/915 (0.5)
Spina bifida	-	2 (1.7)	-	3 (2.6)	2/915 (0.2)
Scoliosis	-	-	1 (0.9)	1 (0.9)	4/708 (0.8)
Fused sternebrae (major fusion)	-	-	-	3 (2.6)	1/708 (0.1)
Hyperextension of limb or paw	-	2 (1.7)	1 (0.9)	1 (0.9)	--
No of foetuses examined	127	118	110	116	915/708
No of litters examined	16	16	16	16	122/109

* background data from the performing laboratory, comprised of both control and inactive treatment animals. Range only available for combined animals. No detail for individual studies.

Study 07: Rabbit dose-range finding study (Anon., 2002)

Doses [0, 5, 17.5, 30] mg/kg bw/day

Maternal effects:

One female in each of the low (5 mg/kg bw/day) and intermediate (17.5 mg/kg bw/day) dose groups and two in the top (30 mg/kg bw/day) dose group aborted. All other animals survived to scheduled necropsy. There were no clinical signs due to treatment. Maternal toxicity was minimal, expressed as a reduction in body weight gain (corrected, -12.5%) at the mid and top dose groups. Mean gravid uterus weight was unaffected by treatment. An increased incidence of post-implantation loss (mainly due to late intrauterine deaths) was noted at 30 mg/kg bw/day [14.9, 14.8, 14.1, and 22.4% at 0, 5, 17.5, and 30 mg/kg bw/day, respectively]. The mean number of live foetuses were not affected by treatment.

Litter effects:

Mean foetal weight was slightly but not statistically significantly lower in the top dose group relative to the control group. A common foetal malformation, arthrogryposis (multiple joint contractures), was noted in one control and one high dose foetus only. This was not related to the administration of the test compound.

Study 08: Rabbit main prenatal developmental study (Anon., 2002)

Doses [0, 5, 17.5, 30] mg/kg bw/day

Maternal effects:

One high dose female was sacrificed *in extremis* on Day 18 of gestation. One low dose female died due to a dosing error. One control female and two in each of the treated groups aborted in late gestation. All other animals survived to scheduled necropsy. There were no clinical signs due to treatment. Maternal toxicity was minimal, expressed as a reduction in body weight gain (corrected, -7 to -9%) at the mid and top dose groups. Corrected mean body weight at GD29 was only 5% reduced in the top dose group relative to controls. Mean gravid uterus weight was reduced 19% by treatment in the top dose group. A significantly increased incidence of post-implantation loss (due to both early and late intrauterine deaths) was noted at 30 mg/kg bw/day [4.8, 13.6, 15.2, and 24.9% at 0, 5, 17.5, and 30 mg/kg bw/day, respectively]. This was much greater than the limited HCD supplied in the original study report (range 7.6 – 14.1%, 6 studies, 123 litters, dates not clear but dating from 1994 to present study). The mean number of live foetuses per doe was reduced by treatment [9.5, 9.8, 8.4, and 7.8 at 0, 5, 17.5, and 30 mg/kg bw/day, respectively], HCD range was 8.9 to 9.7 from 6 studies, 123 litters, dates not clear but dating from 1994 to present study. Pregnancy rate was unaffected by treatment. One female in the low dose group and two in each of the intermediate and high dose groups showed total embryofoetal loss.

Litter effects:

There was significantly reduced litter weight noted at 30 mg/kg bw/day (-24% relative to control group) though the mean foetal weight was only marginally affected (-5%). There was increased foetal variants across all groups but abnormalities (hydronephrosis), originally described as a malformation, were noted at 17.5 mg/kg bw/day and 30 mg/kg bw/day. The malformation profile was different to those observed in the Anon., (1986) study and did not show any dose response relationship. Some single instances of skeletal malformation were seen in one foetus from the top dose group, but no convincing treatment related effect was apparent. Hydronephrosis (originating from either direct or indirect effects of conditions affecting the urinary conduit) may be considered the only relevant abnormality rather than malformation of note in the present study. The supplied

HCD was not described in detail and was difficult to assign any relevance with respect to the 2002 study.

***Table**: Incidence of foetuses with abnormalities (% incidence in parentheses)*

	Dose level (mg/kg bw/day)				
Abnormality	**0**	**5**	**17.5**	**30**	**HCD ***
Hydronephrosis	-	-	1 (0.6)	2 (1.6)	2 (0.05)
No of foetuses examined	200	176	160	124	4233
No of litters examined	21	18	19	16	?

* background data from the performing laboratory, comprised of control animals only, dates unknown, number of litters unknown.

Study 09: Rat dermal embryo-foetal development study (Anon., 1996)

Doses [0, 5, 100, 600] mg/kg bw/day

Maternal effects:

Dermal administration of quinoclamine resulted in encrusted skin at the application site in animals at a dose level of 100 mg/kg (10/25) and 600 mg/kg (19/25). All animals survived to scheduled necropsy. Necropsy observations were unremarkable except for effects on the skin. Maternal toxicity was minimal, expressed as a significant reduction in body weight gain (GD 6-16, -31%) at the top dose along with significant reductions in food consumption at GD6–9 (-20.5%) and at GD6-16 (-8%). There were no effects of treatment on post-implantation loss. The mean number of live foetuses and the sex distribution of the foetuses were not affected by treatment.

Litter effects:

Mean foetal weight was not affected by treatment with quinoclamine. There were no test item related malformations or variations.

Conclusion and comparison with the CLP criteria

Quinoclamine toxicity has proven to be problematic in determining its potential reproductive toxicity. The rat 2-generation study was insufficient to investigate fertility and sexual development, but a number of studies were available to assess developmental toxicity. The difficulty with quinoclamine is the steep dose response relationship for excessive maternal toxicity that limits the dose that can be tested in rats and rabbits in prenatal teratology studies. Rats appear to tolerate up to 100 mg/kg bw/day and rabbits appear to tolerate up to 50 mg/kg bw/day with slight to mild maternal toxicity by way of decreases in bw gain and feed consumption and small effects on foetal bw, sometimes with moderate reductions in gravid uterine weight. The problem is that biologically significant effects (and sometimes statistically significant effects) occur at these and lower dose levels, albeit at low incidences. This makes the occurrence of severe effects even more noteworthy but does not reduce the uncertainty with regard to quinoclamine having direct teratological significance. For these reasons, a classification for development in category 1 may be disregarded; the evidence is not robust enough. The main developmental rabbit studies in particular could have been dosed higher, certainly to 40-50 mg/kg bw/day instead of 22-30 mg/kg bw/day, there were indications of post implantation loss and malformations that could have been better investigated and concerns either confirmed or eliminated if the doses employed were slightly higher. Another factor that complicates this assessment is the lack of appropriate HCD. Some data from the performing laboratories are available but from a limited number of studies, other data is published from a large collection of laboratories and compiled by Charles River Laboratories where the dates of the studies are not reported or are not ideal for comparison with the quinoclamine studies. The concurrent controls remain the most important and primary comparison group for any effect. The supplied HCD often lacks detail required to interpret the incidences in a meaningful way.

Taking a very general overview of all the studies it can be seen that embryo lethality and malformations are featured (table below), so it would appear there is an effect with treatment, but it is not always consistent and the dosing ranges and top dose levels employed along with minor differences in the time of exposure may be partly accountable for this. This introduces uncertainty into any classification proposal which then becomes a borderline one. This is the case for quinoclamine.

***Table**: Overview of (severe) adverse effects in the developmental studies.*

Study	Date	Species	Embryo lethality	Malformations
#01: Pre	1986/1989	Rat (SD)	Suspected, P	No
#02: Main	1986	Rat (SD)	No.	Yes, A[1,2], S
#03: Pre	2002	Rat (SD)	Yes, P	No.
#04: Main	2002	Rat (SD)	Yes, P	No. H (see text, study #04)
#05: Pre	1986/1989	Rabbit (NZW)	Suspected, P	Suspected, A[2], K, SB,
#06: Main	1986	Rabbit (NZW)	No.	Yes, A, FS
#07: Pre	2002	Rabbit (NZW)	Yes, P	No.
#08: Main	2002	Rabbit (NZW)	Yes. P	No. H
#09: Main	1996	Rat (SD)	No.	No.

Suspected = limited data, no dose response, or low number of animals
P = post implantation loss
A = aortic arch malformation
S = Situs inversus
SB = Spina bifida
H = hydronephrosis
K = kidney agenesis
FS = fused sternabrae
[1] absent innominate artery,
[2] interrupted aortic arch,

The results of the developmental toxicity studies suggest that the following effects may be considered as part of a weight of evidence for classification purposes:

i. malformations (aortic arch) in rats and rabbits (study #2, #05, #06),
ii. malformations (situs inversus) in rats (study #2),
iii. malformations (kidney agenesis, Spina bifida, fused sternabrae) in rabbits (study #06),
iv. increased post implantation loss in rats and rabbits (study #3, #04, #07, #08 with positive indications from #01, #05).
v. Hydronephrosis in rats and rabbits support STOT RE 2 as a minimum (study #04, 08).

RAC makes special note of hydronephrosis. Hydronephrosis may be defined as marked dilation of the renal pelvis and calices, secondary to obstruction of urine flow, usually combined with destruction of the renal parenchyma. The destruction of the renal parenchyma must be confirmed histologically. The latter investigation did not take place in the supplied studies (rat #04, rabbit #08). The lack of histopathological confirmation of destruction of the renal parenchyma in these studies makes the classification as a malformation doubtful. However, applying Haber's rule as an approximation for adjusting for dosing with respect to the period of exposure and standard 90-day oral studies, it can be seen that the incidence of hydronephosis (which in itself is recognised as a

severe abnormality and occurs outside the HCD range), satisfies the criteria for at least STOT RE2 in rats and STOT RE1 in rabbits (see STOT RE section).

Classification conclusion

There was no information on the potential of quinoclamine to adversely affect development in humans and therefore classification in Category 1A is not warranted.

Classification in Category 1B (presumed human reproductive toxicant) should be largely based on data from animal studies that provide clear evidence of an adverse effect on development in the absence of other toxic effects. Alternatively, if occurring together with other toxic effects, the adverse effect on reproduction should also be considered not to be a secondary non-specific consequence of other toxic effects. RAC concludes that the whole data package available for quinoclamine does not provide robust, clear evidence of developmental toxicity for classification in Category 1B and is therefore, not appropriate. Severe maternal toxicity was reported at ≥ 200 mg/kg bw/day in rats (dev tox study #01) and ≥ 80 mg/kg bw/day in rabbits (dev tox study #05), and developmental effects were not always reproducible, consistent or significantly above the HCD in both species (rat and rabbit).

Regarding classification in category 2 (suspected human reproductive toxicant), RAC considers the fact that increased embryo lethality (post implantation loss) and the presence of malformations in two species (aortic arch), skeletal malformations in rabbits (fused sternabrae) with Spina bifida, and situs inversus in rats are all of concern. The incidences are low, and, in some cases, a higher dose could have been tested. This creates a degree of uncertainty upon which RAC must consider either classification in category 2 for developmental effects or no classification.

RAC considers the weight of evidence to support the classification of quinoclamine for developmental toxicity (Cat. 2). In conclusion, RAC agrees with the DS and proposes **toxic for reproduction in Category 2, H361d ("Suspected of damaging the unborn child")** based on conclusive data.

Adverse effects on or via lactation

Limited reductions in pup weight were noted in the 2-gen study but were considered not to present any clear evidence of an adverse effect via lactation. There was no data about residue levels and their transfer into milk. RAC agrees with the **DS not to classify for effects via lactation on the basis of inconclusive data**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance has low potential for bioaccumulation and is not rapidly degradable. The Dossier Submitter proposes to classify the substance with Aquatic Acute 1, M=10 based on an E_rC_{50} of 0.029 mg/L for algae from a formulation test (range 0.01 - 0.1 mg/L) and with Aquatic Chronic 1, M=10 based on a NOEC of 0.00213 mg/L for fish based on the active substance test (range 0.001 - 0.01 mg/L).

Degradation

Quinoclamine was considered to be hydrolytically stable at environmentally realistic pH values and temperatures. One study on hydrolysis of quinoclamine was available (OECD TG 111). Study conditions were pH 4, 7 and 9, and temperatures 50 and 74ºC. At pH 4 and 7 (50ºC), <10% hydrolysis had occurred after 5 days and the study was terminated. At pH 9, the study was prolonged 14 days to enable estimation of the DT_{50}. The DT_{50} was 9 days, and this was extrapolated to 360 days at 20ºC. A single hydrolysis product was identified as HCN (2-chloro-3-hydroxy-1,4-naphthalenedione) which accounted for 50% AR after 9 days at pH 9, 50ºC. No data was available to assess if HCN would fulfil the criteria for classification as hazardous to the aquatic environment.

The photochemical half-lives from the two aquatic photolysis studies were estimated to be in the range of 4.2 – 42.9 days in natural sunlight. Several unknown photolysis products were formed, two of which were identified as phthalic acid and 2-carboxybenzaldehyde, both present as >10% AR (applied radioactivity). According to the data presented in the CLH report, phthalic acid would not fulfil the criteria for classification as hazardous to the environment. Neither of the photolysis products was classified for environmental effects in the ECHA C&L Inventory.

In the ready biodegradability study performed according to the Draft OECD TG No 301 "CO_2 Evolution test" (1990), no significant CO_2 evolution was observed over 28 days. The test concentrations used were 18 and 35 mg/L. It was noted that part of the test substance remained un-dissolved in the medium (water solubility of quinoclamine is 19.8 mg/L, 20ºC). The conclusion that quinoclamine is not readily biodegradable was still considered valid by the DS.

In the available mineralisation in surface water study (OECD TG 309), two test concentrations were used and the results indicated the degradation might be dose dependent. The results from the low dose experiment are considered as more representative. At study end (day 61), mineralisation reached 29.5% AR at the high dose (100 µg/L) and 50.7% AR at low dose (10 µg/L). In sterile samples (dosed at 100 µg/L), quinoclamine remained stable throughout the test. Nine metabolites were observed. HCN (2-chloro-3-hydroxy-1,4-naphthalenedione) was observed as max 5.2% AR and 2-chloro-1,4-dimethoxy-3-aminonaphthalene was observed as max 6.2% AR (both on day 61, low dose). The remaining seven metabolites were individually present only as <5% AR. Single first order (SFO) DT_{50}s were determined to be 30.6 days (low dose) and 121 days (high dose).

There were two water/sediment studies available, results are presented in the table below. Quinoclamine was relatively rapidly distributed to the sediments in both studies. Several metabolites were identified in both studies but only AN (2-amino-1,4-naphthalenedione) was identified. AN was observed >10% in both studies. SFO-SFO $DegT_{50}$ for AN in the total systems were determined to 22.7 days (river) and 47.8 days (pond). There is no data on AN available to compare with the CLP criteria and derive an environmental classification.

Table: *Results from the water/sediment studies*

	OECD TG 308 Day 60, study end	BBA Guideline Part IV 5-1 Day 105, study end	BBA Guideline Part IV 5-1 Day 56
Mineralisation, % AR	river system 25.7 pond system: 11.8	ditch system: 15.5 river system: 30.8	ditch system: 18.8 river system: 27.4
Non-extractable residues,% AR	river system: max 67.9 pond system: 82.4	ditch system: 80.6 river system: 67.1	ditch system: 73.1 river system: 62.1
SFO DT50, total system	river system: 7.0 days pond system: 8.9 days	ditch system: 6.5 days river system: 6.1 days	

The DS concluded that quinoclamine is considered as hydrolytically stable at environmentally realistic temperatures and pH values. The substance was not readily biodegradable in 28-day test for ready biodegradability. The primary degradation products cannot be demonstrated to not fulfill the classification criteria for hazards to the aquatic environment and therefore primary degradation cannot be used to conclude the substance is rapidly degradable. In a surface water simulation test half-life was longer than 16 days and ultimate degradation did not reach >70% within 28 days. In two studies on biodegradation in water/sediments half-lives for primary degradation in the total systems were shorter than 16 days but ultimate degradation did not reach >70% within 28 days in the systems. In conclusion, the DS determined quinoclamine to be not rapidly degradable.

Bioaccumulation

There is no experimental BCF available. The log P_{ow} measured with an HPLC method was 1.58 at pH 11 (30°C). The DS is of the opinion that the effect of pH is not relevant as quinoclamine has no measurable dissociation constant. An experimental log P_{ow} of 2.12 (experimental database match) and an estimated log P_{ow} of 1.50 were obtained from Episuite v.4.11 (LOGKOW v. 1.68). The DS concluded that quinoclamine has a low potential for bioaccumulation.

Aquatic toxicity

Table: *Summary of data considered by the DS for classification of quinoclamine (studies considered not relevant by RAC in italics)*

Species	Test substance	Test type	Endpoint	Toxicity mg a.s./L	Reference
Toxicity to fish					
Oncorhynchus mykiss	Quinoclamine 98.5%	Semi-static OECD TG 203, GLP	**LC_{50}, 96-h**	**0.044** (48-h, gm) mm 52-76.5 % of nominal	Anonymous 43 1991a Report 912043117 (In DAR 2007)
Oncorhynchus mykiss	Quinoclamine 98.3%	ELS 90-d Flow-through OECD TG 210, GLP	NOEC **EC_{10}**	0.00213 (nom) **0.0024** (nom) mm 80-120% of nominal	Anonymous 50 2015 Report AGK-001/4-43/E
Toxicity to invertebrates					
Chironomus riparius	*Quinoclamine, >95%*	*24-d Water-sediment (spiked water) OECD draft TG 1998, GLP*	*Emergence (b NOEC(aq) EC_{10}(aq)*	*0.063 (nom) 0.052 (nom)*	*Kleiner 2000a Report 991048113 (In DAR 2007)*
Daphnia magna	*Mogeton 50% WG*	*Static 48 h OECD TG 202*	*EC_{50}*	*1.03 (nom)*	*Heintze 1998b Report 98001/01-AADm*
Toxicity to algae					
Navicula pelliculosa *Poorly reliable but supportive*	*Quinoclamine >95%*	*72-h Static OECD TG 201, GLP*	*NOEC E_rC_{10} E_rC_{50} E_bC_{10} E_bC_{50}*	*0.07 0.115 0.468 0.06 0.185 (72-h meas.)*	*Barth 2000 Report 991048121 (In DAR 2007)*

Scenedesmus subspicatus	*Mogeton 50% WG*	*72-h* *Static* *OECD TG 201*	*NOEC* *LOEC* *EC_{10}* *E_rC_{50}* *E_bC_{50}*	- *0.014* - *0.029* *0.014* *(gmm)*	*Dengler 1998* *Report* *98001/01-AASs*
Toxicity to macrophytes					
Lemna minor	Quinoclamine >95%	7-day Semi-static OECD draft TG 1997, GLP	NOEC E_rC_{10} E_rC_{50} E_YC_{10} E_YC_{50}	0.04 0.05 0.11 0.03 0.09 gmm 60-76% of nom.	Kleiner 2000b Report 991048122 (In DAR 2007)
Lemna minor	*Mogeton 50% WG*	*7-day* *Semi-static* *OECD TG 201*	*NOEC* *ErC_{10}* *ErC_{50}* *EyC_{10}* *EyC_{50}*	*Not determined (<0.05)* *0.0453* *0.116* *0.0309* *0.0711* *nominal*	*Juckeland 2008* *Report* *08 10 48 013 W*
Myriophyllum spicatum	Quinoclamine, 98.3%	14-day Semi-static OECD TG 238 draft 2013, GLP	NOEC ErC_{10} ErC_{50} EyC_{10} EyC_{50} Root number EC_{10} EC_{50}	0.0086 0.0108 0.1347 0.0018 0.0613 0.0044 0.0515 gmm 2.57-43.4% of nom.	Juckeland 2015 Report 14 10 48 008 W

gm = geomean concentration
mm = mean measured concentration
nom = nominal concentration
(b - No effects on development rate were observed in this study.

There is one reliable acute toxicity study on quinoclamine available for fish (OECD TG 203). No analyses were made after 72 and 96 h and consequently no geomean over the whole exposure period could be determined. It was nevertheless considered reasonable to assume that the 48-h values were representative for the mean concentrations during the whole study. The concentration-response curve was steep with 0% and 90% mortality in two subsequent concentration levels. Therefore, the DS calculated the LC_{50} as a geomean between the levels causing 0% and 90% mortality, resulting in a 96-h LC_{50} of 0.044 mg/L for *Oncorhynchus mykiss*, instead of using the geometric mean of LC_{100} and LC_0 to calculate the LC_{50}.

For chronic toxicity to fish, there is a reliable 90-day ELS test available on *Oncorhynchus mykiss* (OECD TG 210). The 90-day nominal NOEC and EC_{10} were 0.00213 mg/L and 0.0024 mg/L, respectively. The measured concentrations were 80-120% of nominal.

There is no acute invertebrate data for the active substance. A nominal 48-h EC_{50} of 1.03 mg/L is available from a formulation (Mogeton 50% WG) study on *Daphnia magna*. No test description was available in the CLH Report or the Annexes.

For chronic invertebrate toxicity, there is a *Chironomus riparius* 24-day water-sediment study available on the active substance. The nominal aquatic EC_{10} was 0.052 mg/L. The DS regarded this as a key study for invertebrates. Four replicates with 20 larvae each per control and concentration were exposed for 24 days under static conditions. Tested concentrations were 0,

0.063, 0.125, 0.25, 0.50 and 1.0 mg a.s./L (nominal). The water-sediment system was spiked into the overlying water. After 1 h, 7 and 24 days, samples were taken for analysis of the test concentrations in the overlaying water, pore water, and sediment. At the same time, test concentrations in overlaying water, sediment and pore water were verified at three treatment levels (the lowest and the two highest). Measured concentrations of quinoclamine declined rapidly in the overlaying water, with 69% of the nominal initial concentrations after 1 h and no recovery at the end of the study. From the measurements of day 7 onwards the majority of the recovered residues were found in the sediment. Effect values were expressed as nominal concentrations. Significant effects on emergence were observed at 0.125 mg a.s./L. No effects on development rate were observed.

The only available algae test on quinoclamine was a 72-h *Navicula pelliculosa* test (OECD TG 201) where measured concentrations were 56-98% of the nominal concentrations with the lowest values at the end of the test, indicating decreasing concentrations with time. Effect values were expressed as measured concentration after 72 h. The study was not considered valid since the validity criteria as stipulated in the test guideline of a mean CV (%) of <35% was not fulfilled (actual value 43%). Nonetheless, the study was used as supportive information.

There is also a study on a formulated product (Mogeton 50% WG) (OECD TG 201) available on *Scenedesmus subspicatus* giving an E_rC_{50} of 0.029 mg/L (geometric mean). No NOEC could be derived from the study. No test description is available in the CLH Report or its Annex.

There are two reliable macrophyte studies available on quinoclamine. In the 7-day *Lemna minor* test the E_rC_{50} was 0.11 mg/L and the E_rC_{10} was 0.05 mg/L, expressed as geometric mean measured concentrations. In the 14-day sediment free *Myriophyllum spicatum* study, the E_rC_{50} was 0.1347 mg/L and the E_rC_{10} was 0.0108 mg/L. The most sensitive endpoint in this test was root number where the EC_{50} was 0.0515 mg/L and the EC_{10} was 0.0044 mg/L.

For *Lemna minor* there was also a test with formulation (Mogeton 50% WG). In this OECD TG 221 study, the nominal E_rC_{10} was 0.0453 mg/L and the nominal E_rC_{50} was 0.116 mg/L. No test description is available in the CLH Report or the Annexes.

Comments received during consultation

There were comments from three Member States (MS). One MS supported the proposed classification. They also noted dichlone as an impurity in the active substance. The DS had the view that the small dichlone amounts present would not have an impact on the classification of the substance.

Many detailed comments were given by the other two MS.

Given the significant partitioning from water to the sediment phase over the study period, the *Chironomus riparius* study was not considered reliable for hazard classification. An issue on which the DS agreed. Additionally, based on a *Daphnia* study presented in the DAR but not in the CLH Report, it was concluded in the comments that invertebrates are not the most acutely sensitive species. It was also proposed to use the surrogate approach for chronic invertebrate toxicity due to lack of chronic data on invertebrates. The CLH Report states there are no indications from the available data that the co-formulants in the product are more toxic or increase the toxicity of quinoclamine to aquatic organisms. An MS thought this information to be relevant as the DS proposes that the acute classification is based on a study using the formulation. The DS answered that the conclusion was based on results from comparable studies with active ingredient and formulation.

The use of the toxicity data based on the formulated product (Mogeton 50% WG) was not considered appropriate for classification purposes by one MS. According to them, the classification of a substance is generally based on test data from the substance itself. In studies conducted with

formulated products, it cannot be excluded that effects can at least partially be attributed to other constituents of the formulations. The DS has not provided a justification as to why the studies conducted with the formulation are adequate to conclude on the active substance. The DS also thought that the OECD TG 238 *Myriophyllum spicatum* study was not suitable for classification purposes.

The DS acknowledged that the use of formulation data for classification purposes would need further discussion. They also agreed that discussion may be needed on selection of the key study for aquatic macrophytes.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS and considers quinoclamine as not rapidly degradable based on:

- The substance was not readily biodegradable in OECD TG 301 test where no significant CO_2 evolution was observed over 28 days.
- DT_{50}s in the surface water simulation test (OECD TG 309) were 30.6 days (low dose) and 121 days (high dose). Mineralisation reach 50.7% AR and 29.5% AR at low and high dose, respectively. Several metabolites were observed. Since the ultimate degradation was not achieved in < 16 days, no rapid degradation was shown in the test.
- In the two water/sediment studies (OECD TG 308) quinoclamine was relatively rapidly distributed to sediment. The SFO DT_{50}s for the total system were < 16 days. Mineralisation reached a maximum of 30.8 % AR. Non-extractable residues were from 62.1 to 82.4 % AR. Several metabolites were observed but not all identified. There is no information available to determine if the metabolites fulfil the criteria for classification as hazardous to the aquatic environment. Consequently, no rapid degradation was shown.
- the substance was considered hydrolytically stable at environmentally realistic pH values and temperatures (OECD TG 111)
- The primary degradation products cannot be demonstrated to not require classification for aquatic hazards.

Bioaccumulation

No experimental fish bioconcentration study is available. The available log Pow values of 1.58 (HPLC method), 2.12 (experimental, LOGKOW v. 1.68) and 1.50 (estimated, LOGKOW v. 1.68) are below the classification cut-off value of 4. Consequently, RAC agrees with the DS that quinoclamine has a low potential for bioaccumulation.

Aquatic toxicity

The DS proposed classification based on data derived using formulations of quinoclamine and gave the following reasons for accepting the studies:

- There are no indications from the available data that the co-formulants in the products are more toxic or increase the toxicity of quinoclamine to fish.
- Since the available data indicate a comparable toxicity of the active substance and the formulation.

However, RAC notes that full aquatic toxicity data is not available for any of the co-formulants. Co-formulants serve different purposes in the formulated products and might have an effect on the overall toxicity of a product. Consequently, while RAC accepts there may be circumstances where data derived using formulations may be used for classification, as data on the co-formulants is not available here, RAC does not find it appropriate to use such data for the

classification of quinoclamine for aquatic hazards. Available data derived using technical quinoclamine will be used instead.

Acute Aquatic Toxicity

There was one reliable acute fish study available. The measured 48h LC_{50} was 0.044 mg/L for *Oncorhynchus mykiss*.

There was no reliable acute invertebrate data available for classification based on the active substance.

There was reliable data available on macrophytes *Lemna minor* and *Myriophyllum spicatum*. The lowest acute toxicity value for *Lemna minor* was a 7d E_rC_{50} of 0.11 mg/L. For *Myriophyllum spicatum* the lowest value was a 14d EC_{50} for root number of 0.0515 mg/L.

Myriophyllum spicatum study is further discussed under chronic aquatic toxicity (below).

Chronic Aquatic Toxicity

There was one chronic fish study available. The nominal 90d EC_{10} was 0.0024 mg/L for *Oncorhynchus mykiss*. The measured concentrations were 80-120% of the nominal.

The only chronic invertebrate study was a 24d water/sediment study with *Chironomus riparius*. RAC is of the opinion that the study is not reliable for classification due to significant partitioning from water to sediment and due to nominal concentrations being used although the concentration of the substance declined substantially towards the end of the test. Consequently, there is no reliable chronic invertebrate data available for classification.

There was reliable data available for the macrophytes *Lemna minor* and *Myriophyllum spicatum*. The lowest chronic toxicity value for *Lemna minor* was a 7d E_rC_{10} of 0.05 mg/L. For *Myriophyllum spicatum* the lowest value was a 14d EC_{10} for root number of 0.0044 mg/L.

Myriophyllum spicatum has not until recently been a widely used species in aquatic toxicity testing. There are two OECD TGs available for testing OECD TG 238 (without sediment) and OECD TG 239 (with sediment). Quinoclamine had been tested according to the OECD TG 238. The test duration was 14 days during which multiple generations are not possible which would be a normal prerequisite for chronic aquatic toxicity testing. However, the substance is a herbicide and had severe effects in the test, RAC concludes that the data is considered for both acute and chronic classification in this case. There are multiple effect endpoints reported in the test including growth rate but RAC is of the opinion that the lowest toxicity value for root number should be chosen for classification.

Comparison with the criteria

RAC concludes that the lowest acute toxicity data was the 48h LC_{50} of 0.044 mg/L for *O. mykiss*. The 14d EC_{50} for *M. spicatum* was of the same order of magnitude. There was no data on *Daphnia* but RAC notes that the ECOSAR v. 1.11 calculations indicate invertebrates not being the most sensitive trophic level for acute toxicity. In RAC's opinion, quinoclamine warrants classification as Aquatic Acute 1, M=10 (0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L).

The lowest chronic toxicity data was the 90d EC_{10} of 0.0024 mg/L for *O. mykiss*. The 14d EC_{10} of 0.0044 mg/L for *M. spicatum* was in the same order of magnitude. There is no reliable data for invertebrates. The ECOSAR v.1.11 estimation based on acute-chronic ratios indicates that invertebrates are not the most sensitive trophic level for chronic toxicity either. RAC is of the opinion that quinoclamine, as a not rapidly degradable substance, warrants classification as Aquatic Chronic 1, M=10 (0.001 mg/L < NOEC ≤ 0.01 mg/L).

RAC consequently agrees with the Dossier submitter proposal although basing the decision on data derived using technical quinoclamine rather than formulation data.

Overall, RAC agrees with the DS that quinoclamine warrants classification as **Aquatic Acute 1; H400 (M=10) and Aquatic Chronic 1; H410 (M=10)**.

In case new reliable data on aquatic toxicity of the substance on invertebrates becomes available the classification might have to be revisited.

ANNEXES:

Annex 1 The 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 RAC (excluding confidential information).