Committee for Risk Assessment
RAC

Annex 1
Background document
to the 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

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

Adopted
17 September 2020

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON QUINOCLAMINE (ISO); 2-AMINO-3-CHLORO-1,4-NAPHTHOQUINONE

European Commission

Combined Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

QUINOCLAMINE

Volume 1

sanitised

Rapporteur Member State: Sweden
Co-Rapporteur Member State: Germany

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON QUINOCLAMINE (ISO); 2-AMINO-3-CHLORO-1,4-NAPHTHOQUINONE

Volume 1

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List of Endpoints

Versions of RAR, Vol 1 Quinoclamine

Date	Reason for revision
May 2018	First version submitted to EFSA
September 2018	Revised following Echa Accordance check of CLH proposal.

Classification

Please note that the RMS has tried to use the new combined RAR/CLH template for Volume 1 (SANCO/12592/2012. rev 1.1, October 2017). This means that the structure and content of the document should serve both as a basis for decision under Regulation (EC) No 1107/2009 and as a proposal for classification under Regulation (EC) No 1272/2008.

NB: The application for renewal of Quinoclamine under EU Reg. 1107/2009 was withdrawn by the applicant shortly after submission of the RAR to EFSA in May 2018.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 EFSA peer review has taken place for the substance.

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Level 1

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

Quinoclamine is an active substance currently approved until 31 December 2018 under Commission Regulation (EC) No. 1107/2009. This renewal assessment report (RAR) was prepared to evaluate the supplementary dossier submitted by Agro-Kanesho Co. Ltd. to support the renewal of the approval of Quinoclamine. Sweden, acting as the rapporteur member state (RMS) evaluated all aspects of the application and the supplementary dossier, in accordance with the procedures specified in Commission Implementing Regulation (EU) No. 844/2012 of 18 September 2012.

The RAR provides a discussion of relevant studies submitted for the previous EU evaluation as well as relevant new studies and information generated and submitted to support the renewal. Where necessary, studies submitted for the previous EU evaluation have been re-evaluated to allow risk assessment along current standards, and to validate previous conclusions and/or calculations.

Quinoclamine is not yet subject to harmonised classification. A proposal for harmonised classification and labelling according to the CLP criteria is included in this RAR and will also be submitted to ECHA. The structure of the RAR follows the new combined template for Assessment Reports and proposals for Harmonised Classification and Labelling (CLH reports), SANCO/12592/2012, rev. 1.1 (October 2017).

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Germany, acting as Co-RMS, agreed to review the RAR before the submission to the Commission and EFSA.

1.1.3 EU Regulatory history for use in Plant Protection Products

In the EU-regulatory context, Quinoclamine was first evaluated within the programme for review of existing active substances provided for in Article 8(2) of EU Council Directive 91/414/EEC. Agro-Kanesho Co. Ltd. was the notifier and sole data submitter in support of Annex I inclusion. Sweden acted as rapporteur member state (RMS). The first draft of the previous DAR was finalised in 2005. As a result of the peer review the DAR was revised in 2007. Addenda were also produced for some of the sections of the DAR in 2007.

To support the discussions that preceded the Annex I inclusion, EFSA was given mandate to perform a peer-review and the authority delivered its conclusion on 14 November 2007 (EFSA Scientific Report (2007) 117, 1-70). The Commission then presented a Review Report (SANCO/3622/07 – rev. 1, 1 February 2008). There was no request for confirmatory data to be submitted after the inclusion in Annex I of EU Council Directive 91/414/EEC.

Quinoclamine was included in Annex I of EU Council Directive 91/414/EEC on 1st January 2009 (Commission Directive 2008/66/EC of 30 June 2008), and was subsequently approved under Regulation (EC) No. 1107/2009 (repealing Council Directive 91/414/EEC) via Commission Implementing Regulation (EU) No. 540/2011 of 25th May 2011. The current expiry date for this approval is 31 December 2018.

EFSA has published a Reasoned opinion on the review of the existing maximum residue levels (MRLs) for Quinoclamine according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2013;11(3):3141).

1.1.4 Evaluations carried out under other regulatory contexts

The RMS is not aware of any EU-evaluations of Quinoclamine carried out in the framework of other relevant EU-legislation (e.g. biocides, flavourings, food additives, cosmetics).

Quinoclamine was not included in the Inventory of Evaluations performed by the Joint Meeting on Pesticide Residues (JMPR).

No information has been provided by the applicant whether Quinoclamine has been evaluated or registered in any country outside the EU.

1.2 Applicant(s) information

1.2.1 Name and address of applicant(s) for approval of the active substance

AGRO-KANESHO CO. LTD.
7F Akasaka Shasta-East 4-2-19
Akasaka, Minato-ku
Tokyo, 107-0052, Japan

European Address:
Agro-Kanesho European Branch
Rudolf-Kinau Weg 20
21680 Stade, Germany

Primary contact:

[redacted]
DHD-Consulting GmbH
Lilly-Reich-Strasse 8
31137 Hildesheim, Germany
E-Mail: [redacted]
Tel.: [redacted]
Fax: [redacted]
Mobile [redacted]

Contact 2:

[redacted]
AGRO-KANESHO CO., LTD.
Department of Registration and Regulatory Affairs
7F Akasaka Shasta-East 4-2-19
Akasaka, Minato-ku
Tokyo, 107-0052, JAPAN
E-Mail: [redacted]
Tel.: [redacted]
Fax [redacted]

Contact 3:

[redacted]
Agro-Kanesho European Branch
Rudolf-Kinau Weg 20
21680 Stade, Germany
E-Mail: [redacted]
Tel.: 0[redacted]

1.2.2 Producer or producers of the active substance

AGRO-KANESHO CO., LTD.
7F Akasaka Shasta-East 4-2-19
Akasaka, Minato-ku
Tokyo, 107-0052, Japan

Contact:

[redacted]
Department of Registration and Regulatory Affairs

7F Akasaka Shasta-East 4-2-19
Akasaka, Minato-ku
Tokyo, 107-0052, JAPAN
E-[redacted]
Tel.: [redacted]
Fax [redacted]

Location of the manufacturing site:
See confidential Annex C in Vol. 4.

1.2.3 Information relating to the collective provision of dossiers

The RMS received an application for renewal of the approval of Quinoclamine only from Agro-Kanesho Co. Ltd. A collective provision of dossiers has therefore not been necessary.

1.3 Identity of the active substance

1.3.1 Common name proposed or ISO-accepted and synonyms

ISO: Quinoclamine
Synonyms: Quinoclamin, ACN, ACNQ, K-1616, Mogeton

1.3.2 Chemical name (IUPAC and CA nomenclature)

IUPAC: 2-amino-3-chloro-1,4-naphthoquinone
CA: 2-amino-3-chloro-1,4-naphthalenedione

1.3.3 Producer's development code numbers

Synonym for Quinoclamine: ACN technical
In toxicological reports also: Mogeton or K-1616
In phys-chem reports also: Mogeton or Mogeton techn.

1.3.4 CAS, EC and CIPAC numbers

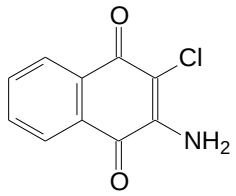
CAS-No.: 2797-51-5
EEC-No.: 220-529-2
CIPAC-No.: 648

1.3.5 Molecular and structural formulae, molecular mass

Molecular formula: $C_{10}H_6ClNO_2$

Molecular mass: 207.6

Structural formula:



1.3.6 Method of manufacture (synthesis pathway) of the active substance

For further information, please refer to the confidential Annex C in Vol. 4.

1.3.7 Specification of purity of the active substance in g/kg

The first Annex I-inclusion approved minimum purity of 965 g/kg, which is also proposed for the renewal. RMS agrees with the specification (see Volume 4 for further information).

1.3.8 Identity and content of additives (such as stabilisers) and impurities

1.3.8.1 Additives

For further information, please refer to the confidential Annex C in Vol. 4.

1.3.8.2 Significant impurities

For further information, please refer to the confidential Annex C in Vol. 4.

1.3.8.3 Relevant impurities

The first Annex I-inclusion approved a maximum content of 15 g/kg, which is also proposed for the renewal. RMS agrees with the specification (see Volume 4 for further information).

1.3.9 Analytical profile of batches

For further information, please refer to the confidential Annex C in Vol. 4.

1.4 Information on the plant protection product

1.4.1 Applicant

The same as for the active substance, see section 1.2.1 above.

1.4.2 Producer of the plant protection product

Cheminova Deutschland GmbH & Co. KG

Stader Elbstraße 28

21683 Stade

Germany

Contact:

[redacted]

Agro-Kanesho European Branch

Rudolf-Kinau Weg 20

21680 Stade, Germany

Tel.: [redacted]

E-mail: [redacted]

Location of the manufacturing site:

See confidential Annex C in Vol. 4.

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product

Trade name: Mogeton TOP

Codes: SIT 95570H

ASU 95570 H

Mogeton 50% WG

Mogeton TOP 50% WG

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product

1.4.4.1 Composition of the plant protection product

Pure active substance

content of pure active substance :	**500.0 g/kg**	**50.0% w/w**
Limits (± 25 g/kg, as given by FAO 2010):	475 g/kg	525 g/kg

Technical active substance (96.5 % purity)

content of technical active substance :	518 g/kg	51.8 % w/w
Limits (± 25 g/kg as given by FAO 2010):	493 g/kg	543 g /kg

1.4.4.2 Information on the active substances

Type	**Name/Code Number**
ISO common name	Quinoclamine
CAS No	2797-51-5
EC No	220-529-2
CIPAC No	648
Salt, ester anion or cation present	No

1.4.4.3 Information on safeners, synergists and co-formulants

For further information, please refer to the confidential Annex C in Vol. 4.

1.4.5 Type and code of the plant protection product

Water dispersible granules [Code: WG]

1.4.6 Function

Quinoclamine is a selective herbicide and algaecide.

1.4.7 Field of use envisaged

The proposed representative uses of Mogeton TOP for EU authorisation are:

- Post-emergence control of a broad spectrum of common mosses and algae occurring on golf greens.
- Post-emergence control of liverwort occurring on the substrate of container-/pot-grown ornamental crops in open field or glasshouse nurseries.

1.4.8 Effects on harmful organisms

Quinoclamine inhibits photosynthesis in algae, mosses and other plants by interfering the electron transfer on two target sides: ii) inhibiting the D1–protein complex of the Photosystem II (Hill-reaction) similar to other herbicides such as triazines or ureas, ii) binding at the electron-donating side of the Photosystem I. However, according to HRAC information, the detailed mechanism of action of Quinoclamine is unknown till now. For further reading on the mode of action, please refer to Volume 3 CA, section B.3.6.

The applicant reported, that according to investigations on mosses, the compound is rapidly absorbed by all green plant parts and by the rhizoids. Differing to higher plants, the translocation of the compound seems to be limited in

mosses. Warm and humid climatic conditions promote the photosynthesis inhibiting effects of the compound. Under favourable conditions mosses die off after some days.

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

Table 1.5.1-1. GAP for the proposed representative uses of Quinoclamine (representative formulation: Mogeton TOP 50% WG).

Member state(s)	Crop and/or situation	F G or I (a)	Pests controlled	Formulation			Application			Max. application rate per treatment			PHI (days) (c)	Remarks
				Name	Type (b)	a.s. conc.	method	Timing / season	No. / use / crop / season	kg prod. /ha	kg a.s. /ha	Water (L/ha)		
EU-N (DE)	Golf greens	F	Mosses, algae	Mogeton TOP	WG	50%	Downward spraying	April - August	1	7.5	3.75	1000	n.a.	Only on established greens
EU-N	Golf greens	F	Mosses, algae	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	7.5	3.75	1000	n.a.	Only on established greens. Spot application
EU-N	Golf greens	F	Mosses, algae	Mogeton TOP	WG	50%	Downward spraying	April - August	1	3.75	1.875	500	n.a.	Only on established greens
EU-N (DE)	Nursery stock potted plants	F	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	7.5	3.75	1000	n.a.	Pots on permeable sheets on the ground. No application on flowering plants
EU-N (DE)	Nursery stock potted plants	F	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	3.75	1.875	500	n.a.	Pots on permeable sheets on the ground. No application on flowering plants
EU-N (NL)	Nursery stock potted plants	F	Liverwort	Mogeton TOP	WG	50%	Downward spraying	April - August	1	2.88	1.44	800	n.a.	Pots on permeable sheets on the ground. No application on flowering plants
EU-N (DE)	Nursery stock potted plants	G	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	7.5	3.75	1000	n.a.	Walk-in tunnel; Pots on permeable sheets on the ground. No application on flowering plants
EU-N (DE)	Nursery stock potted plants	G	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	3.75	1.875	500	n.a.	Walk-in tunnel; Pots on permeable sheets on the ground. No application on flowering plants
EU (DE)	Nursery stock potted plants	G	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	7.5	3.75	1000	n.a.	Greenhouse; Pots on permeable sheets on the ground
EU (DE)	Nursery stock potted plants	G	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	3.75	1.875	500	n.a.	Greenhouse; Pots on permeable sheets on the ground
EU (NL)	Nursery stock potted plants	G	Liverwort	Mogeton TOP	WG	50%	Hand-held Spraying (Backpack)	April - August	1	1.62	0.81	450	n.a.	Greenhouse; Pots on permeable sheets on the ground

EU-N (northern and central Europe) includes Sweden, Norway, Iceland Finland, Denmark, United Kingdom, Ireland, Belgium, The Netherlands, Luxembourg, Germany, Poland, Czech Republic, Slovakia, Austria, Hungary, Switzerland, Estonia, Latvia, Lithuania, Romania, Slovenia and northern France, according to SANCO 7525/VI/95 (23 September 2016).
a) F = field/outdoor application; G = greenhouse/tunnel application; I = indoor application
b) WG = water dispersible granules
c) PHI = minimum pre-harvest interval

1.5.2 Further information on representative uses

Mogeton TOP is diluted in water and applied by tractor mounted sprayer or handheld sprayer.

During the evaluation, the RMS asked the applicant to justify why Mogeton TOP is intended for maximum one application per crop and season, considering that a treatment with Mogeton TOP protects crop only for a period of about 2-4 months. Moreover, the RMS considered that the number of applications per crop is not necessarily the same as the number of applications per season, if more than one crop per season is grown in the same place.

Regarding the use on golf greens, the applicant responded that only an unusually wet place would require more than one treatment per season against moss in lawn, since golf greens are planned to avoid standing water, i.e. the ground is practically always sloped. Based on this reasoning, the RMS accepted the restriction in the GAP table of maximum one application per season on golf greens.

Regarding the use in nursery stock potted plants, the applicant clarified that it is theoretically possible in the nurseries to have more than one treatment at a specific place. However, the treated plant pots contain multi-year ligneous plants (i.e. with wooden stems) – and are usually grown relatively large, hence keeping their position most or all of the season. Further, since even minor phytotoxic effects can make potted plants difficult to sell, and the orange colour of Quinoclamine is very intensive, Mogeton TOP is not used on, and not registered for herbaceous plants.

Mogeton TOP is not intended for use on areas where human food is cultivated, where livestock is grazing or where animal feeding stuffs are harvested. Therefore, no studies on residues in or on treated products, food or feed are necessary. Further, no re-entry period for livestock or withholding period for animal feeding stuffs need to be established. As the proposed representative uses refer to permanent crops (lawn) and special cultivation systems (containerized plants in nurseries), with maximum one application per year, recommendations concerning the influence of the product on succeeding crops are not considered relevant.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not relevant.

1.5.4 Overview on authorisations in EU Member States

Different formulations with Quinoclamine are widely registered in the EU for control of mosses, liverwort and algae on lawns, ways and nursery plants. Currently, solo-products with Quinoclamine for any of these purposes are authorised in Austria, Belgium, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Luxembourg,

Netherlands, Poland, Slovakia, Spain and United Kingdom. Altogether, the maximum authorised total application rate in the EU is 3.75 kg a.s./ha.

Level 2

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

2.1.1 Summary of identity

The identity of Quinoclamine is summarized in Level 1, section 1.3.

2.2 Physical and chemical properties

2.2.1 Summary of physical and chemical properties of the active substance

Quinoclamine is an orange solid (technical grade, 99.0 % purity). A melting point of 200-202 °C was determined for the technical grade substance (99.0 % purity). 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). The Henry's law constant at 20°C were calculated to be 3.05 x 10^{-5} Pa x m^3 x mol^{-1}. Quinoclamine has a calculated pKa of 0.93 (deprotonation of the amino group), since no dissociation occurred between pH 2-11. The solubility in water is 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 the 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. Because of the determination at such high pH, the value was also calculated using KOWWIN. The found value of 1.50 indicated the validity of the measured value. The log Pow of the metabolites included in the residue definition for risk assessment (surface water) were predicted using KOWWIN and were all well below 3. The RMS thus considers that experimental data is not required. All spectral data is available and acceptable for the active substance itself. Since the active substance as manufactured contains a relevant impurity (dichlone), spectral data is also required for this impurity. UV-VIS, IR, NMR and MS-data is available and acceptable for dichlone.

Since the melting point is > 40 °C, flash point was not considered applicable. The active substance is not flammable, auto-flammable nor explosive and has no oxidising properties as determined in accordance with the tests recommended in the data requirements (Reg No 283/2013).

The substance should also not be classified as a flammable substance (based on negative screening test in EEC A.10 which is valid for CLP), explosive (based on structural assessment in accordance with the waiving criteria in

CLP) or oxidizing (based on structural assessment in accordance with the waiving criteria in CLP) under CLP. No other data has been provided which permits further conclusions to be made on physical hazard under CLP.

Table 2.2.1-1. Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment
Physical state at 20°C and 101,3 kPa	Solid	Bates, M. 2000	Visual assessment
Melting/freezing point	200-202 °C	Bates, M. 2000	Measured
Boiling point	348-350 °C	Bates, M. 2000	Measured
Relative density	1.554	Bates, M. 2000	Measured
Vapour pressure	7 x 10-6 Pa at 25 °C 3 x 10-6 Pa at 20 °C	Bates, M. 2000	Measured (extrapolated)
Surface tension	72.1 mN/m	Bates, M. 2000	Measured
Water solubility	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) 20.7 mg/l ± 0.7 mg/l at pH 9 (borax buffer)	Bates, M. 2000	Measured
Partition coefficient n-octanol/water	1.58 (30 °C, pH 11) 1.50 (theoretical)	Bates, M. 2000	Measured/estimated
Flash point	Not applicable		Melting point >40 °C
Flammability	Not flammable	Bates, M. 2000	Measured
Explosive properties	Not explosive	Bates, M. 2000	Calculated/measured
Self-ignition temperature	The test material did not self-ignite below 230 °C (30 °C above the melting point).	Bates, M. 2000	Measured
Oxidising properties	Not oxidising	Bates, M. 2000	Measured
Granulometry	No data		
Solubility in organic solvents and identity of relevant degradation products	acetone: 12.2-12.8 g/l 1,2-dichloroethane: < 10 g/l ethyl acetate:< 10 g/l n-heptane:< 10 g/l methanol:< 10 g/l p-xylene < 10 g/l	Bates, M. 2000	Measured
Dissociation constant	pKa=0.93	Bates, M. 2000	Calculated since no dissociation occurs between pH 2-11.
Viscosity	Not relevant. The active substance is a solid (melting point 200-202 °C).	-	-
<u>Active substance:</u> Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV/Vis: acidic test solutions (pH: 2.5) ε = 14100 at 219 nm ε = 21500 at 266 nm ε = 2410 at 339 nm ε = 2570 at 460 nm unadjusted test solutions (pH: 6.3) ε = 14100 at 219 nm ε = 22500 at 266 nm ε = 2370 at 338 nm ε = 2550 at 458 nm alkaline test solutions (pH: 11.0) ε = 13900 at 218 nm ε = 22200 at 267 nm ε = 2230 at 339 nm ε = 2560 at 460 nm	Bates, M. 2000	Measured

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<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment</th></tr>
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<tr><td>1605</td><td>Aromatic C-C stretch</td></tr>
<tr><td>1305-968</td><td>Aromatic C-H in plane bend, C-N stretch</td></tr>
<tr><td>852-661</td><td>Aromatic out of plane bend</td></tr>
<tr><td>852-532</td><td>C-Cl strech</td></tr>
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<tr><td>7.84</td><td>Triplet</td><td>H7 or H8</td></tr>
<tr><td>7.75</td><td>Triplet</td><td>H7 or H8</td></tr>
<tr><td>6.79</td><td>Broad singlet</td><td>NH2</td></tr>
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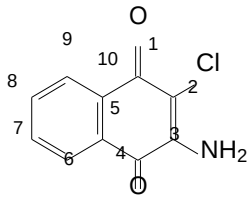

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<tr><td>176.3</td><td>1</td><td>C4</td></tr>
<tr><td>147.3</td><td>1</td><td>C3</td></tr>
<tr><td>135.6</td><td>1</td><td>C7/C8</td></tr>
<tr><td>133.7</td><td>1</td><td>C5/C10</td></tr>
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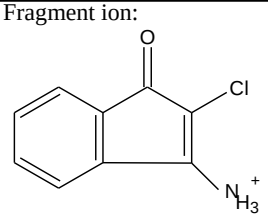
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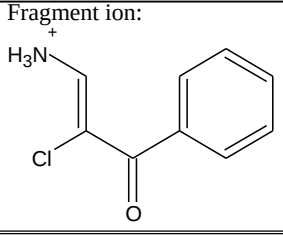
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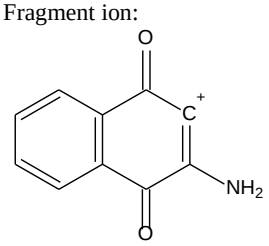
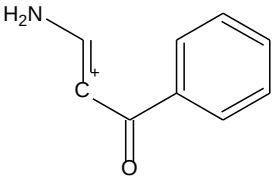
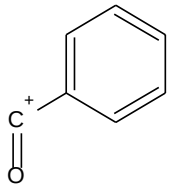
	172	Fragment ion: 		
	146	Fragment ion: 		
	105	Fragment ion: 		
Relevant impurity (dichlone): Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	UV-VIS, IR, ^{1}H- and ^{13}C-NMR and MS data are acceptable and confirms the structure of the relevant impurity.		Dardemann & Frauen 2007 (UV-VIS) Henke, 2015 (IR) Class, 2008 (NMR) Class, 2007 (MS)	Measured

2.2.1.1 Evaluation of physical hazards

2.2.1.1.1 Explosives

Table 2.2.1.1.1-1. Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Structural examination/calculation of oxygen balance/DSC (for enthalpies for exothermic processes)	Not explosive		Bates, M. 2000

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

The explosive properties of Quinoclamine were investigated by calculation of the oxygen balance and comparing bond groupings present in the molecule. The oxygen balance is 161.83 %, which is under the CLP criteria of 200 for non-explosiveness. Furthermore, Quinoclamine contains no functional groups known to confer explosive properties or explosive enhancing groups. DSC showed no exothermic processes between 20 °C and 600 °C with

enthalpies near the trigger value for explosion 500 J/g. Hence, it was considered that Quinoclamine does not present a significant risk of explosion.

2.2.1.1.1.2 Comparison with the CLP criteria

Quinoclamine should not be classified as explosive according to the CLP criteria of oxygen balance.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed.

2.2.1.1.2 Flammable gases (including chemically instable gases)

Hazard class not applicable (Quinoclamine is not a gas)

2.2.1.1.3 Oxidising gases

Hazard class not applicable (Quinoclamine is not a gas)

2.2.1.1.4 Gases under pressure

Hazard class not applicable (Quinoclamine is not a gas)

2.2.1.1.5 Flammable liquids

Hazard class not applicable (Quinoclamine is not a liquid)

2.2.1.1.6 Flammable solids

Table 2.2.1.1.6-1. Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EEC A.10	Material not highly flammable		Bates, 2000

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

One test performed in accordance with EEC A.10 is available. The substance did not ignite in the preliminary screening test and is thus not regarded as highly flammable in the sense of the test method.

2.2.1.1.6.2 Comparison with the CLP criteria

The preliminary screening test in EEC A.10 and in CLP are identical. The substance should thus not be classified as a flammable substance under CLP.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

No classification is proposed. Data is conclusive but not sufficient for classification.

2.2.1.1.7 Self-reactive substances

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No data has been provided addressing this property, and this is not required. There are no chemical groups present in the molecule associated with explosive or self-reactive properties.

2.2.1.1.7.2 Comparison with the CLP criteria

Data lacking

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed due to lack of data.

2.2.1.1.8 Pyrophoric liquids

Hazard class not applicable (Quinoclamine is not a liquid)

2.2.1.1.9 Pyrophoric solids

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

No specific data derived in accordance with the recommended test method in CLP have been provided. However, Quinoclamine has been handled in air within all studies available in the dossier and there are no reports of self-ignition (see references in all sections).

2.2.1.1.9.2 Comparison with the CLP criteria

Data (experience in handling) is conclusive but not sufficient for classification.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

2.2.1.1.10 Self-heating substances

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

Data lacking

2.2.1.1.10.2 Comparison with the CLP criteria

Data lacking

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

No classification is proposed due to lack of data.

2.2.1.1.11 Substances which in contact with water emit flammable gases

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No specific data derived in accordance with the recommended test method in CLP have been provided. However, Quinoclamine has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas.

2.2.1.1.11.2 Comparison with the CLP criteria

Data (experience in handling) is conclusive but not sufficient for classification.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification is proposed. Data (experience in handling) is conclusive but not sufficient for classification.

2.2.1.1.12 Oxidising liquids

Hazard class not applicable (Quinoclamine is not a liquid).

2.2.1.1.13 Oxidising solids

Table 2.2.1.1.13-1. Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A.17	Not oxidizing in the sense of the test method. The maximum burning rate of the reference (barium nitrate):cellulose mixture (3:2) was higher (1.19 mm/s)	The test item:cellulose 1:9, 3:7 and 4:& mixtures also burned	Bates, 2000

Method	Results	Remarks	Reference
	than the maximum burning of the test item: cellulose mixture (2:8; 0.61 mm/s)	to completion, but 5:5 and 6:4 did not.	

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

One test performed in accordance with EEC A.17 is available. The test was negative in the sense of the test method.

2.2.1.1.13.2 Comparison with the CLP criteria

The test under EEC A.17 does not utilize the same reference standard as in the test recommended under CLP (potassium bromate). Moreover, the decision logic in CLP stipulates that the reference:cellulose mixture should also be tested in 3:7 and 2:3 ratios whereas in EEC A.17 only a 3:2 mixture could be used as in this case. Hence, the decision logic cannot be followed and it cannot be fully concluded that the test substance is not an oxidizer under CLP.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

No classification is proposed due to lack of data.

2.2.1.1.14 Organic peroxides [equivalent to section 8.14 of the CLH report template]

Hazard class not applicable (Quinoclamine is not an organic peroxide)

2.2.1.1.15 Corrosive to metals

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

No data have been provided addressing this property.

2.2.1.1.15.2 Comparison with the CLP criteria

Data lacking

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to lack of data.

2.2.2 Summary of physical and chemical properties of the plant protection product

Mogeton Top is an orange water dispersible granule (WG). The product is not considered explosive (test method EEC A.14) or oxidizing (test method EEC A.17). It should not be classified for flammability based on flammability and auto-flammability (test methods EEC A.10, A.15). The product pH of a 1% aq. dispersion in distilled water is 6.33 at 23°C. The pour density is 0.56 g/mL and the tap density is 0.60 g/mL. All physical and chemical properties indicate that no particular problems are to be expected. No tank mixes are intended. There were no significant changes in the formulation upon storage at 54 °C in 2 weeks or during 2 years at ambient temperature. The formulation is thus considered stable to storage under the specified conditions and the commercial packaging used in the 2 years storage showed no sign of deformation or other material changes. One interim report (3 months) is available for a two years storage study of the formulation in the commercial packaging (HDPE) in which the relevant impurity dichlone is monitored using a validated analytical method. The final report needs to be evaluated when available (data gap).

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 energy was shown to be < 500 J/g at > 500°C (303 J/g at 530°C).	**No classification, conclusive.**
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.**

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

For evaluation of the minimum effective dose rate of Mogeton TOP, the applicant referred to five efficacy studies performed in 2006 under GEP conditions. The efficacy against mosses and liverwort was tested at three application rates: 0.5 N (1875 g a.s./ha), 0.8 N (3000 g a.s./ha) and 1 N (3750 g a.s./ha). These studies are summarised in Vol. 3 CP, Annex B.3, sections B.3.9.1 and B.3.9.2.

For moss control on lawn, the following was concluded:

- At the beginning of the assessment period (1-3 WAT) the mean efficacy value achieved 87.11% for the 0.5 N rate of Mogeton TOP compared to 92.00% for the 0.8 N rate and 95.20% for the 1 N rate.
- At the time 7-8 WAT and 12-14 WAT the efficacy of the 0.5 N rate treatment had declined to lower levels but still approximately 50%.
- In conclusion, 0.5 N rate of Mogeton TOP (corresponding to 1875 g a.s./ha) also achieved good control of common mosses on lawn 1-3 WAT after one post emergence spray application.

For liverwort in containers, the following was concluded:

- At the beginning of the assessment period (1-4 WAT) the mean efficacy value achieved 74.88% for the 0.5 N rate of Mogeton TOP compared to 83.59% for the 0.8 N rate and 92.47% for the 1 N rate.
- At the time 6-10 WAT and 12-18 WAT the efficacy of the 0.5 N rate treatment had declined to lower levels but still approximately in the range 57-41%.
- In conclusion, 0.5 N rate of Mogeton TOP (corresponding to 1875 g a.s./ha) also achieved good control of liverwort in containers 1-4 WAT after one post emergence spray application.

The (even lower) rates of 1.440 kg/ha and 0.810 kg/ha listed for The Netherlands correspond to the currently registered rates in The Netherlands.

2.3.2 Summary of information on the development of resistance

The applicant provided a resistance risk analysis based on the EPPO guideline PP 1/213(3), which is available in Vol. 3 CA, Annex B.3, section B.3.7.

Quinoclamine from the chemical group of quinones is not yet classified by HRAC and according to the applicant no evidence of resistance to Quinoclamine has been reported so far. Therefore, the actual resistance risk is regarded low and should be acceptable without special restrictions.

2.3.3 Summary of adverse effects on treated crops

No adverse effects on treated crops are known according to the applicant.

2.3.4 Summary of observations on other undesirable or unintended side-effects

No undesirable or unintended side effects are known according to the applicant.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Safe handling

Do not smoke. Keep ignition source away. Ensure good ventilation/ exhaustion at the workplace and good interior ventilation. Protect against electrostatic charges. Avoid contact with eyes and skin. Do not inhale gases/fumes/aerosols. Keep away from feedstuffs, beverages and food.

Personal protective equipment

Wear appropriate safety glasses and face shield, as well as appropriate body protection and respiratory protection.

Safe storage

Keep in a dry place between 0°C and 30°C in tightly sealed containers. Ensure good ventilation/exhaustion at the workplace. Protect from heat and direct sunlight. Provide a solvent resistant, sealed floor. Store in a way that unauthorized persons and especially children do not have access.

Transport

Table 2.4.1-1. Road/ rail transport – ADR/RID

UN number:	3077
ADR Class:	9
Packaging group:	III
Environmental hazards:	Environmentally hazardous substance, solid, N.O.S.
UN proper shipping name:	-

Table 2.4.1-2. Marine transport - IMDG

UN number:	3077
OMI/IMDG Class:	9
Packaging group:	III
Marine Pollutant:	Yes
UN proper shipping name:	-

Fire

Use water, foam, carbon dioxide, dry chemicals, or dry sand as extinguishing media. Fire-fighters should wear positive pressure, full-face self-contained breathing apparatus. Cool fire-exposed containers. No dangerous decomposition products are anticipated in the event of a fire

2.4.2 Summary of procedures for destruction or decontamination

Package the waste and contact the local authorities to make sure that the waste will be led to controlled incineration or safe waste disposal according to the official regulations.

The pyrolytic behaviour of the active substance does not need to be reported as the content of halogens of the active substance in the preparation is <60%.

No other methods of safe disposal than controlled incineration are proposed.

2.4.3 Summary of emergency measures in case of an accident

In case of inhalation move the victim to fresh air. Seek medical advice immediately.

In case of contact with skin take off contaminated clothing, and wash immediately affected area with plenty water and soap. Seek medical advice, if contact skin and cause irritation or anthema.

In case of contact with eyes, rinse immediately with plenty of water for several minutes; do not forget to remove lens. Seek medical advice, if in case of eye contact.

If swallowed, wash mouth with plenty water. Seek medical advice immediately. Do not induce vomiting.

In case of leakage or spill, wear respiratory protection. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided. Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

2.5 Methods of analysis

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1. Methods for the analysis of the active substance as manufactured

A summary of the methods applied in order to analyse the active ingredient as well as the relevant impurity Dichlone in technical grade Quinoclamine in context of five batch analyses is presented in the table below. Analytical methods for the determination of significant impurities in Quinoclamine are presented in confidential part Volume 4.

Table 2.5.1.1-1. Summary of analytical methods for technical active substance.

Matrix	Analyte	Type of method	Validation	References
Technical a.s.	Quinoclamine	(HPLC/DAD (UV)), 266 nm, external standard method	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Manella, L. (2015) 2771W
Technical a.s.	Quinoclamine	(HPLC/DAD (UV)), 266 nm, external standard method	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Marin, J. (2009) 1886W
Technical a.s.	Dichlone	(HPLC/DAD (UV)), 266 nm, external standard method	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Manella, L. (2015) 2771W
Technical a.s.	Dichlone	(HPLC/DAD (UV)), 266 nm, external standard method	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Marin, J. (2009) 1886W
	Impurities [a]			

a) Details for significant impuritites are reported in Volume 4 confidential part

Table 2.5.1.1-2. Summary of analytical methods for formulation analysis.

Matrix	Analyte	Type of method	Validation	References
Mogeton Top 50 % WG	Quinoclamine	HPLC-UV	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Dardemann, J. 2010
Mogeton Top 50 % WG	Quinoclamine	HPLC-UV	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Dardemann, J. 2010 AB 95570-PC-032A
Mogeton Top 50 % WG	Quinoclamine	HPLC-UV	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Thieu-Simchen V.A. 2016

Matrix	Analyte	Type of method	Validation	References
Mogeton Top 50 % WG	Dichlone	HPLC-UV	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Thieu-Simchen V.A., 2016
ASU 95570 H	Dichlone	HPLC-UV	Sufficiently validated with respect to specificity, linearity, accuracy and precision (i.e. in accordance with SANCO/3030/99 rev. 4)	Dardemann, J., Frauen, M., 2008
	Impurities [a]			

a) Details are reported in Volume 4 confidential part

2.5.1.2. Methods for risk assessment

The methods used for generating data for risk assessment in the dossier are presented in the tables below. Most methods are considered acceptable and it can thus be confirmed that the data generated by the methods is valid. However, for some studies related to vertebrate data validation data is missing. Since it relates to vertebrate data, which should not be repeated, no data gap has been proposed.

Table 2.5.1.2-1 Summary of analytical methods used for data generation for the active substance and its metabolites.

Matrix	Analyte	Type of method Single (S) /Multi (M)	Validation	Reference
Soil	Quinoclamine	S, HPLC/UV (254 nm), TLC (254 nm)	satisfactory	Völkel W. (2015) 20140108
Soil	^{14}C-Quinoclamine	S, HPLC/UV (254 nm), TLC (254 nm)	Not required	Adam D. (2016)
Soil	^{14}C-Quinoclamine	LSC	Not required	Bishop, L., 2003
Soil	Phthalic acid (metabolite M6)	M, HPLC/MS (m/z 164.877 → 120.800, 77.000, 88.800)	Satisfactory	Adam D. (2016)
Soil	Phthalamic acid (metabolite M9)	M, HPLC-MS (m/z 166.062 →149.000, 121.000)	Not satisfactory (one sample per fortification level only, three fortification levels)	Piskorski R., 2016
Soil	2-Oxalyl-benzoic acid (metabolite M10)	M, LC/MS/MS	Satisfactory	Fiebig S. (2016)
Soil	2-oxamoylbenzoic acid (metabolite M11)	M, LC/MS/MS	Satisfactory	Fiebig S. (2016)
Soil	Quinoclamine	M, HPLC-MS/MS (m/z 208 → 77.1 quantification, m/z 208 → 105.1; 89.1 confirmation)	Satisfactory	Dautel P. (2016) 15 10 35 2093 Dautel P. (2016) 15 10 35 2092
Soil	Quinoclamine	GC-ECD	Satisfactory	Brielbeck, B., Marx, D., 1997 RU0896 & RU0595
Soil	^{14}C-Quinoclamine	LSC	Not required	Lewis, C. J., 2000
$CaCl_2$ solution/soluble matrix of standard soil, soil	2-Amino-1,4-naphthoquinone (metabolite AN)	S, HPLC/UV (265 nm)	Satisfactory	Dardemann J. (2009)
Soil	Quinoclamine	GC-ECD	Not satisfactory(only accuracy data available)	Brosius, E. M., 1990

Matrix	Analyte	Type of method Single (S) /Multi (M)	Validation	Reference
Water	^{14}C-Quinoclamine	LSC	Not required	Lewis, C. J., 2001
Water	^{14}C-Quinoclamine	LSC	Not required	Yeomans, P., 2003
Water	^{14}C-Quinoclamine	LSC	Not required	Shah, J. F., 2006
Water	Quinoclamine	HPLC	Not satisfactory (only a calibration curve presented)	Werle, H., 1992
Surface water (biodegradation test)	^{14}C-Quinoclamine	LSC	Not required	Völkel, W., 2016
Water (degradation/metabolism study)	^{14}C-Quinoclamine	LSC	Not required	Völkel, W., 2016
Water/sediment	^{14}C-Quinoclamine	LSC	Not required	Muttzall, P. I. 1993
Air/soil	^{14}C-Quinoclamine	LSC/TLC	Not required	Reichert, N., 1994
Dose solution (Rat and human liver microsomes)	^{14}C-Quinoclamine	LC-PDA-(RAD)-MS	Not required	Piñeiro Costas, N., 2016
Gravimetric filters (used in acute inhalation in rat)	Quinoclamine	Gravimeter	Not required	Van Huygevoort, A.H.B.M., 2009
~~Dose in air (used in inhalation 4 hours study in rats)~~	~~Quinoclamine~~	~~GC-FID~~	~~Not satisfactory (no validation data)~~	[redacted] ~~1986~~
~~Dose solution (used in oral 28-day study in rat)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~2002, 2003~~
~~Diet feed (used in oral 13 week study in rat)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~2002~~
~~Dose solution (used in 28-day dermal study in rat)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~2002~~
~~Rat liver (used in unscheduled DNA synthesis/genotoxicity study)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (raw data missing for linearity and accuracy)~~	[redacted] ~~1996~~
~~Diet feed (used in oral 104 week study in rat)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~1991~~
~~Diet feed (used in oral 80 week study in mouse)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~1993~~
~~Diet feed (used in oral 4 prenatal studies in rat and rabbit (2 studies each))~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (no raw data available)~~	[redacted] ~~2002~~
~~Dose solution (used in dermal embryo study)~~	~~Quinoclamine~~	~~HPLC~~	~~Satisfactory~~	[redacted] ~~1996~~
Grass (turf)	^{14}C-Quinoclamine	LSC	Not required	Schnöder, F., 2003
~~Diet feed (used in short term toxicity study in bobwhite quail)~~	~~Quinoclamine~~	~~HPLC~~	~~Not satisfactory (raw data missing for linearity and accuracy)~~	[redacted] ~~2001~~
~~Diet feed (used in short term toxicity study in Mallard Duck)~~	~~Quinoclamine~~	~~HPLC~~	~~Satisfactory~~	[redacted] ~~2005~~
~~Diet feed (used in reproductive toxicity study in bobwhite quail)~~	~~Quinoclamine~~	~~HPLC~~	~~Satisfactory~~	[redacted] ~~., 2002~~
Diet feed (used in two fish toxicity studies)	Quinoclamine	HPLC	Not satisfactory (validation data missing)	[redacted] 1991

Matrix	Analyte	Type of method Single (S) /Multi (M)	Validation	Reference
Diet feed (used in fish toxicity study)	Quinoclamine	HPLC	Satisfactory	[redacted] 2015
Algae Diet feed (used in fish toxicity study)	Quinoclamine	HPLC	Not satisfactory (validation data missing)	[redacted] 1991
Aquatic invertebrate, *Daphnia magna*	Quinoclamine	HPLC	Satisfactory	Jahnke, M., 1994
Larvae *Chironomus riparius*	Quinoclamine	HPLC	Satisfactory	Weber, H., 2000
Larvae *Chironomus riparius*	2-amino-1,4-naphthoquinone	HPLC	Satisfactory	Juckeland, 2009
Algae *Navicula pelliculosa*	Quinoclamine	HPLC	Not satisfactory (precision data is missing)	Weber, H., 2000
Aquatic plant *Myriophyllum spicatum*	Quinoclamine	HPLC	Satisfactory	Juckeland, D., 2015
Aquatic plant *Lemna minor*	Quinoclamine	HPLC	Satisfactory	Weber, H., 2000
Water various chem/phys	Quinoclamine	HPLC	Satisfactory	Bates, 2000
Grass	Quinoclamine	HPLC	Satisfactory	Dardemann, O., Frauen, M., 2005

Table 2.5.1.2-2. Summary of analytical methods used for data generation for the plant protection product.

Matrix	Analyte	Type of method Single (S) /Multi (M)	Validation	Reference
Leaf Wash Solutions	Quinoclamine	HPLC-MS/MS	Satisfactory	Perny, A., 2016
Diet feed	Quinoclamine	HPLC	Satisfactory	Laky, V., 2008
Arthropods	Quinoclamine	HPLC-MS/MS	Satisfactory	Henkes, K., 2016
Dose solution (used in 4 fish toxicity studies)	Quinoclamine	LC-MS/MS	Satisfactory	[redacted] 2000
Dose solution (used in fish toxicity study)	Quinoclamine	LC-MS/MS	Satisfactory	[redacted] 1998
Aquatic invertebrate, Daphnia magna	Quinoclamine	HPLC	Satisfactory	Heintze, A., 1998
Algae Scenedesmus subspictus	Quinoclamine	HPLC	Satisfactory	Dangler, D., 1998
Aquatic plant Lemna minor	Quinoclamine	HPLC	Satisfactory	Juckeland, D., 2008
Dose solution (used in fish toxicity study)	Quinoclamine	LC-MS/MS	Satisfactory	[redacted] 2016
Aquatic invertebrate, Daphnia magna	Quinoclamine	LC-MS/MS	Satisfactory	Renner, P., 2016
Honeybee larvae Apis mellifera	Quinoclamine	HPLC	Satisfactory	Kleebaum, K., 2015
Soil mesofauna	Quinoclamine	HPLC-MS/MS	Satisfactory	Dautel, P., 2016
Spray solution (3.75 g a.i./L) Honeybee larvae Apis mellifera	Quinoclamine	HPLC	Satisfactory	Dardemann, J. 2009
Non-target plants, 2 studies	Quinoclamine	HPLC	Satisfactory	Friedrich, S., 2015

2.5.2 Methods for post control and monitoring purposes

New studies for post control and monitoring purposes have been evaluated in Vol. 3, section B.5.2. Suitable analytical methods for plant products are missing, which is considered as a data gap. Analytical methods for food of animal origin for monitoring purposes are not required.

An overview assessment of the submitted analytical methods for soil, water, air, body fluids and tissue showed that acceptable methods for all relevant matrices/commodity groups are available:

Soil

For monitoring purposes a HPLC/MS/MS method was validated for analyzing Quinoclamine in soil. The daughter ions m/z 105 and m/z 172 were used for quantification and confirmation purpose, respectively. LOQ for soil was determined to be 0.05 mg/kg.

Water

For monitoring purposes a HPLC/MS/MS method was validated for analyzing Quinoclamine in water (drinking water, surface water). The daughter ions m/z 105 and m/z 172 were used for quantification and confirmation purpose, respectively. LOQ for both drinking and surface water was determined to be 0.1 µg/L.

Air

As monitoring method for Quinoclamine in air we refer to a study already evaluated under 91/414/EEC, applying HPLC/MS/MS. The limit of detection was 1.5 µg/m^3.

Body fluids and tissues

For monitoring purposes HPLC/MS/MS methods were validated for analysis of Quinoclamine in both body fluids, i.e. blood and body tissues, i.e. liver and kidney. The daughter ions m/z 105 and m/z 172 were used for quantification and confirmation purpose, respectively. LOQ in blood was determined to be 0.05 mg/L. LOQ in liver and kidney was determined to be 0.01 mg/kg.

The analytical method performances were evaluated according to Guidelines SANCO/825/00 rev. 8.1.
A summary of the analytical methods covering relevant residue definitions and limits is shown in Table 2.5.2-1 below.

Table 2.5.2-1. Summary of analytical methods covering relevant residue definitions and limits.

Matrix / crop group	Analyte	LOQ	Residue limit
Food of plant origin	Methods for food of plant origin are needed to detect misuse of Quinoclamine. The lack of suitable analytical methods is therefore considered a data gap.		
Food of animal origin	Not relevant for the representative uses		
Soil	Quinoclamine	0.05 mg/kg	0.05 mg/kg (default limit)
Surface water	Quinoclamine	0.1 µg/L	2.13 µg/L (fish NOEC)
Drinking/ground water	Quinoclamine	0.1 µg/L	0.1 µg/L (EU drinking water limit)
Air	Quinoclamine	1.5 µg/m^3	9 µg/m^3 (based an AOEL of 0.03 mg/kg bw/day)

Matrix / crop group	Analyte	LOQ	Residue limit
Body fluids and tissues	Quinoclamine	Body fluids (blood): 0.05 mg/L (HPLC/MS/MS) Body tissues (kidney, liver): 0.01 mg/kg (HPLC/MS/MS)	Body fluids: 0.05 mg/L Body tissues: 0.1 mg/kg

Table 2.5.2-2. Overview of accepted residue analytical methods.

Matrix / crop group	Primary method	Analyte	Confirmatory method	Independent Lab Validation (if appropriate)
Food of plant origin	Suitable methods are missing. This is considered a data gap.	-	-	-
Food of animal origin	Not required	-	-	-
Soil	KCA 4.2/01 Nichetti S. (2015) Report No. CH-562/2015 HPLC-MS/MS	Quinoclamine	No separate confirmatory method is required	-
Drinking, Ground and Surface water	KCA 4.2/02 Nichetti S. (2015) Report No. CH-561/2015 HPLC-MS/MS	Quinoclamine	No separate confirmatory method is required	KCA 4.2/03 Johannes, J. (2016) Report No. 15033001G92601
Air	KCA 4.2 c Winbush, J. Report No. 619/152-D2149 HPLC-MS/MS	Quinoclamine	No separate confirmatory method is required	-
Body fluids and tissues	Body fluids KCA 4.2/04 Seibold, A. Report No. P3911G HPLC-MS/MS Body tissues KCA 4.2/05 Seibold, A. Report No. P3912G HPLC-MS/MS	Quinoclamine	No separate confirmatory method is required	-

2.6 Effects on human and animal health

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals

Table 2.6.1-1. Summary table of toxicokinetic studies

Method, guideline, test material, species, strain, sex	Results	Remarks	Reference
Toxicokinetics OECD TG 471 ^{14}C-Quinoclamine, purity: 99.7% Quinoclamine, purity 99.0% Vehicle: 0.3% aqueous carboxymethylcellulose Rat Sprague Dawley Crl:CD BR M, F Single oral dose: 3 or 300 mg/kg bw Repeated oral dose: 3 mg/kg bw for 3 or 5 days GLP: Yes	<u>Oral absorption:</u> Rapid (maximum concentrations in blood within 1.2 and 0.25 h (single low dose) and within 9 h and 21 h (single high dose) for males and females, respectively Extent: >82% (based on urinary and biliary excretion plus radioactivity remaining in carcass) <u>Excretion:</u> Rapid (greater portion of radioactivity excreted in urine and faeces within 24 h of dose administration (single low dose) Within 72 h (single high dose) Excretion in urine: about 62% (males) and 64% (females) (single low dose) and 49% (males) and 47% (females) (single high dose) Excretion in faeces: 23% (males) and 16% (females) (single low dose) and 36% (males) and 41% (females) (single high dose) Excretion in bile: about 20% (males) and 25% (females) (single low dose). Excretion in expired air: <1% of administered dose <u>Distribution:</u> Widely distributed. Highest radioactivity found in the carcass, GI-tract, stomach, liver and the kidneys. Radioactivity was rapidly distributed and cleared from tissues with distribution and clearance being slower in females. No evidence of accumulation of radioactivity in any tissue following either single or repeated dose administration. <u>Metabolism:</u> Extensively metabolised in the rat. The metabolites identified were mainly the product of conjugation occurring on the two carbonyl groups. There was also a hydrolysis	Acceptable Deviations from OECD TG 471: i. Four animals (two males and two females) were used in the biliary investigations (the guideline recommends four animals of the appropriate sex (or of both sexes) ii. Thyroid and skin were not collected for the evaluation of tissue distribution	RAR Vol. 3 B.6.1.1/01 Anonymous 1(2002) Report No.: 619/102-D1145 New data for Annex I renewal: **No**

Method, guideline, test material, species, strain, sex	Results	Remarks	Reference
	product of quinoclamine formed by chlorine replacement.		
Comparative *in vitro* metabolism study The United States Food and Drug Administration: Guidance for Industry: Safety Testing of Drug Metabolites (February 2008) [1(4,5,8)-^{14}C]Quinoclamine, Radiochemical purity: 99.7% Quinoclamine, purity: 99.9% Liver microsomes from male Wistar rats and male humans (pool of 25 donors) GLP: Yes	In rat and human microsomes approximately 18% of initial Quinoclamine quantity was metabolized with half-life values >60 minutes. Out of sixteen detected metabolites in rat and human microsome incubations, three unique metabolites (M7, M8 and M14) were present in the human liver microsomal incubations. Based on the percentages of total radioactivity of the chromatogram , these three unique human metabolites were each <0.5% of total radioactivity.	No existing OECD TG. Further experiments to identify the chemical structure of metabolites M7, M8 and M14 were not considered necessary since these metabolites were presented in liver microsomes of human at low concentrations (<0.5% of total radioactivity)	RAR Vol. 3 B.6.1.3/01 Anonymous 2 (2016) Report No.: 506992 New data for Annex I renewal: **Yes**

M: males
F: females

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

In vivo data:

Sprague Dawley Cr1:CD BR strain rats (Charles River, UK; 150-280 g) were dosed orally with 3 and 300 mg/kg bw [^{14}C]-Quinoclamine. The test substance was formulated in 0.3% aqueous carboxymethylcellulose. Pharmacokinetic, tissue distribution and excretion balance investigations were performed following single oral administration of nominally 3 mg/kg and 300 mg/kg body weight. A biliary excretion investigation was performed following a single administration at 3 mg/kg body weight and a tissue distribution investigation was performed following repeated oral administration at 3 mg/kg body weight. Rats were researched for blood and plasma kinetics, excretion balance, biliary excretion and tissue distribution following single and repeat oral administrations. Dose administration is given in table below:

Dose group	Frequency of dose	Study type	Dose level mg/kg	MBq/kg	Number of animals Males	Females
A1 pilot	Single	Excretion balance	300	4	2	2
A2 pilot	Single	Pharmacokinetic	300	4	2	2
B	Single	Excretion balance	3	4	4	4
C	Single	Excretion balance	300	4	4	4
D	Single	Pharmacokinetic	3	4	4	4
E	Single	Pharmacokinetic	300	4	4	4
F	Single	Biliary cannulation	3	4	2	2
G	Single	Tissue distribution	3	4	16	16
H	Single	Tissue distribution	300	4	16	16
I	Repeat	Tissue distribution	3	4	16	16

Absorption:

Absorption of radioactivity, estimated from the extent of urinary and biliary excretion plus the radioactivity remaining in the carcass, was >82%. Absorption of radioactivity was rapid (blood levels of radioactivity reached mean maximum concentrations within 1.2 h and 0.25 h in the low dose group and within 9 h and 21 h in the high dose group for males and females respectively).

Distribution:

Distribution was extensive. Radioactivity was rapidly distributed and cleared from tissues with distribution and clearance being slower in females. Repeated dose administration at the low dose level increased the rate of elimination of radioactivity. There was no evidence of accumulation of radioactivity in any tissue following either single or repeated dose administration. Highest radioactivity was found in the carcass, GI-tract, stomach, liver and the kidneys.

Excretion:

Elimination of radioactivity was rapid with the greater portion being recovered from urine and faeces within 24 h of dose administration at the low dose level and within 72 h at the high dose level.

Elimination via urine was about 62% (males) and 64% (females) (single oral low dose) and 49% (males) and 47% (females) (a single oral high dose). Faecal excretion was about 23% (males) and 16% (females) (single oral low dose) and 36% (males) and 41% (females) (single oral high dose). Excretion in bile was about 20% (males) and 25% (females) (a single oral low dose). In the pilot study it was determined that excretion of radioactivity in expired air was <1% of the administered dose.

Metabolism:

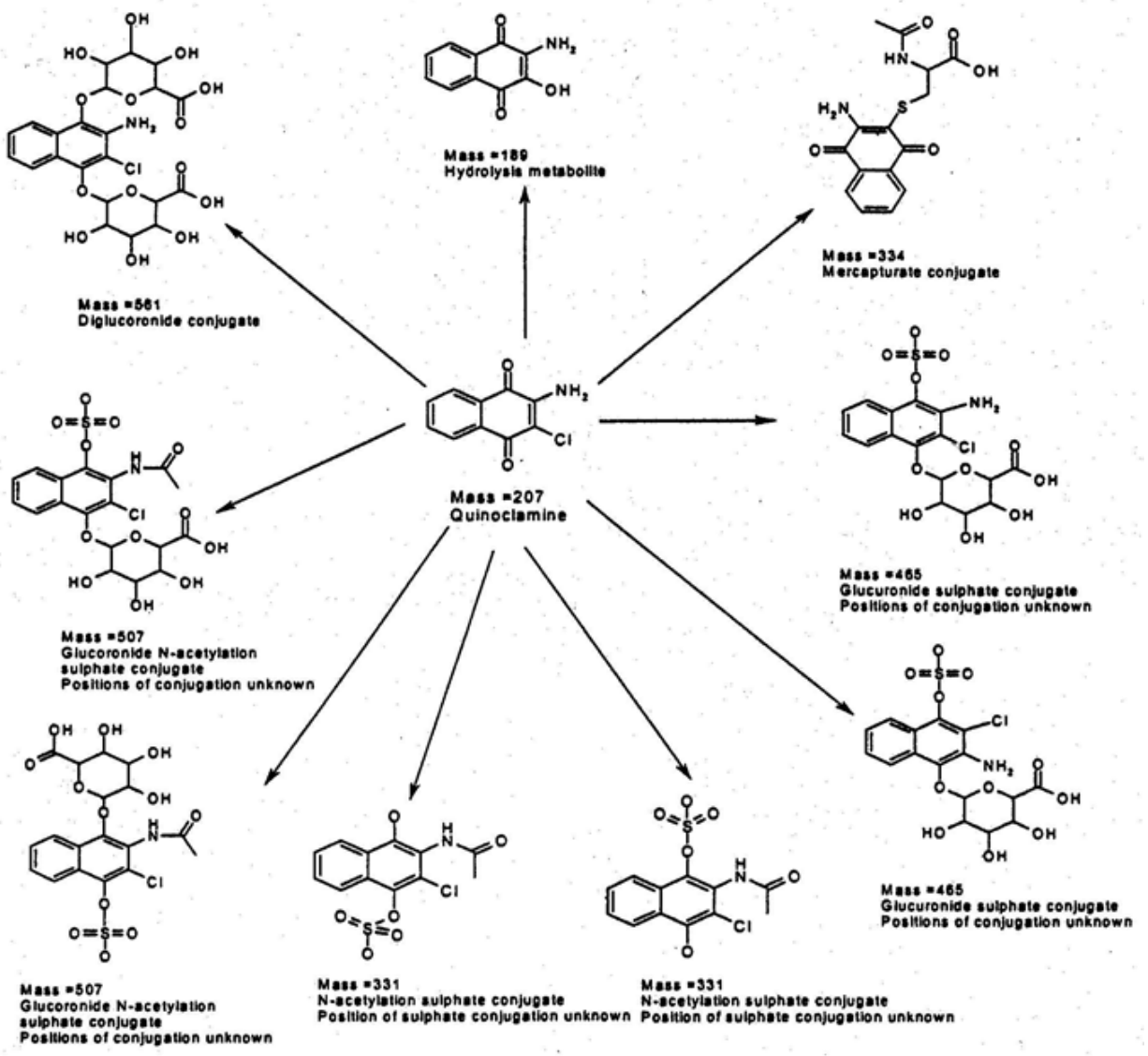
Quinoclamine was extensively metabolised in the rat. Up to 15% of the urinary radioactivity, <1% of the faecal radioactivity and up to 10% of the biliary radioactivity co-chromatographed with parent compound. At least seven regions in urine, at least eight regions in faeces and at least two regions in bile co-eluted with supplied metabolite standards but the presence of these could not be confirmed by mass spectrometry. The metabolites identified were mainly the product of conjugation occurring on the two carbonyl groups. The diglucuronide, two separate N-acetyl/glucuronide/sulphate tri-conjugates, an N-acetyl/sulphate di-conjugate and a mercapturate conjugate formed by chlorine replacement were all identified. The position of glucuronide and sulphate conjugations could not be confirmed from the fragmentation patterns. There was also a hydrolysis product of quinoclamine formed by chlorine replacement. Comparison of the respective radiochromatograms showed that all the identified metabolites seemed to be present in each matrix.

The conjugated products are larger molecules and generally polar in nature. Thus, they can be readily excreted from the body, which is shown by the large amount of excreted radioactivity. The glucuronide conjugation was the most important phase II reaction leading to a decreased toxicity of the product.

In vitro data:

In rat and human microsomes approximately 18% of initial Quinoclamine quantity was metabolized with half-life values >60 minutes. Out of sixteen detected metabolites in rat and human microsome incubations, three unique metabolites (M7, M8 and M14) were present in the human liver microsomal incubations. Based on the percentages of total radioactivity of the chromatogram, these three unique human metabolites were each <0.5% of total radioactivity. Further experiments to identify the chemical structure of metabolites M7, M8 and M14 were not considered necessary since these metabolites were presented in liver microsomes of human at low concentrations (<0.5% of total radioactivity).

Figure: Assigned structures of metabolites of [14C]-Quinoclamine following oral administration to the rat



2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route

Table 2.6.2.1-1. Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral OECD TG 423 GLP: Yes *There is no justification of the choice of vehicle*	Rat Crl:WI(GIx/BRL/Han)BR M, F 3/sex/dose (200, 500 mg/kg bw) 3 females (2000 mg/kg bw)	Quinoclamine Purity: 99.0% Vehicle: 1% methyl cellulose	200, 500, 2000 mg/kg bw Observation period: 15 days	200 - 500 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.1/01 Anonymous 3 (2002) Report No.: 619/141 New data for Annex I renewal: **No**
Acute oral OECD TG 420 GLP: Yes *There is no justification of the choice of vehicle*	Rat Slc:Wistar (SPF)) F <u>Sighting study:</u> One female/dose (300, 2000 mg/kg bw) <u>Main study:</u> Four females (300 mg/kg bw)	Quinoclamine Purity: 98.3% Vehicle: 0.5% carboxymethyl cellulose sodium (0.5 w/v% CMC-Na)	300, 2000 mg/kg bw Observation period: 14 days	300-2000 mg/kg bw (F)	RAR Vol. 3 B.6.2.1/02 Anonymous 4 (2016) Experiment No.: G427 (154-768) New data for Annex I renewal: **Yes**

M: males
F: females

Table 2.6.2.1-2. Summary table of human data on acute oral toxicity

No data

Table 2.6.2.1-3. Summary table of other studies relevant for acute oral toxicity

No data

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

The acute oral LD_{50} in male and female rats was between 300 and 2000 mg/kg bw. There was no death among animals at 200 or 300 mg/kg bw. At the dose level of 2000 mg/kg bw/day all animals died.

Clinical signs noted in rats of the Crl:WI(GIxBRL/Han)BR strain at 200 mg/kg bw included discoloured urine (from 1 hr after dosing), soft and discoloured faeces (affecting the females on Day 2 only), and anogenital soiling. Recovery of surviving rats, as judged by external appearance and behaviour, was advanced by Day 3 and

completed by Day 4. At 500 mg/kg bw all rats showed discoloured urine and faeces, diarrhoea and anogenital soiling. Isolated causes of salivation, lethargy, piloerection, prone, unkempt appearance, dark faeces and a wasted appearance was seen. Clinical signs were apparent from 1 hour after dosing. Recovery of surviving rats was advanced by Day 6 and completed by Day 9. Rats treated at 2000 mg/kg bw, which all died (time of death after dosing: between 2 hrs and 2 days), showed a number of clinical signs including dyspnoea, palpebral closure, piloerection, discoloured urine (pink) and lethargy from the 1 hour observation. The majority of surviving rats dosed at 200 mg/kg bw failed to gain body weight during the firsts week of the observation period. One male rat treated at 200 mg/kg bw had gained body weight between Day -1 and Day 8. Rats treated at 500 mg/kg bw lost large amounts of body weight between Day -1 and Day 8. All surviving rats did gain body weight between Day 8 and 15 and all rats treated at 200 mg/kg bw and the female treated at 500 mg/kg bw gained overall body weight during the observation period. No macroscopic changes were observed for the majority of animals killed on Day 15. Macroscopic examination of one male treated at 500 mg/kg bw revealed enlarged kidneys. Examination of decedents treated at 500 or 2000 mg/kg bw revealed abnormal contents (orange fluid) in the stomach and small intestine of three rats. In one rat dosed at 2000 mg/kg bw abnormal contents were also found in the large intestine and caecum and the caecum was also found to be thin. The mucosal surfaces of the stomach were also orange in two individuals treated at 2000 mg/kg bw. Dark contents were apparent in the stomach and jejunum of one rat treated at 2000 mg/kg bw. The mucosal surface of the stomach of this rat was also dark. In two rats the connective tissue in the abdominal cavity was yellow. One male treated at 500 mg/kg bw had dark contents of the bladder and a female had dark areas on the lungs.

Clinical signs noted in rats of the Slc:Wistar (SPF) strain at 300 mg/kg bw included yellow-red chromaturia (from 1 to 6 hrs after dosing), loose stools (from 2 to 5 hrs after dosing), and ptosis (from 2 to 3 hrs after dosing), but these findings were slight and transient. Body weight measurement and necropsy revealed no abnormalities in any of the animals. The one animal dosed with 2000 mg/kg bw which died five hrs after administration showed clinical signs including yellow-red chromaturia, loose stools, compound-coloured faeces, irregular respiration, salivation, lethargy, ptosis, decrease in locomotor activity, soiled fur in the anogenital region and prone position.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with oral LD_{50} of 300-2000 mg/kg bw. The LD_{50} for oral toxicity was between 300 and 2000 mg/kg bw and quinoclamine thus does fulfil the CLP classification criteria for acute oral toxicity (Acute Tox. 4). The corresponding converted oral ATE value is 500 mg/kg bw (based on Table 3.1.2 of Annex I to the CLP Regulation).

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification of quinoclamine as acutely toxic by the oral route in Category 4 (H302: Harmful if swallowed) is proposed. The corresponding oral ATE value is 500 mg/kg bw.

2.6.2.2 Acute toxicity - dermal route

Table 2.6.2.2-1. Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal OECD TG 402 GLP: Yes	Rat Crl:WI(GIx/BRL/Han)BR M, F <u>Preliminary study:</u> 2 females (2000 mg/kg bw) <u>Main study:</u> 5/sex (2000 mg/kg bw)	Quinoclamine Purity: 99.0%	2000 mg/kg bw Observation period: 15 days	>2000 mg/kg bw (M, F)	RAR Vol. 3 B.6.2.2/01 Anonymous 5 (2002) Report No.: 619/143-D6144 New data for Annex I renewal: **No**

Table 2.6.2.2-2. Summary table of human data on acute dermal toxicity

No data

Table 2.6.2.2-3. Summary table of other studies relevant for acute dermal toxicity

No data

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

The acute dermal LD_{50} in male and female rats exceeded 2000 mg/kg bw. No adverse clinical signs were observed. Principal signs of reaction to treatment were staining of the snout noted from 30 minutes or 1 hour after dosing and orange coloured urine apparent from Days 2 and 4. Recovery of rats as judged by external appearance and behaviour, was complete by Day 5. Slight erythema affecting two females between Days 4 and 9 and orange staining throughout the observation period. 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. However, one male failed to regain its pre-study body weight and the body weight gain in some individuals was only modest. No macroscopic changes were observed in animals killed on Day 15.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

According to the CLP Guidance, classification in Acute Tox. 4 (the lowest classification) is required for substances with dermal LD_{50} of 1000-2000 mg/kg bw. The LD_{50} for dermal toxicity was above 2000 mg/kg bw, and quinoclamine thus does not fulfil the CLP classification criteria for acute dermal toxicity.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed for quinoclamine.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 2.6.2.3-1. Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation Guideline: not stated in the study report GLP: Yes *The study is not acceptable due to low amount of respirable particles*	Rat Wistar M, F 5/sex	ACN technical (Quinoclamine) Purity: 98.1% Aerosol About 40% by weight of the test substance in the chamber air was 5.5 µm or less	0.79 mg/L (4 hrs, whole body) (highest attainable concentration) Observation period: 14 days	LC_{50} (4 hrs, respirable fraction): >0.32 mg/L (value corrected for the respirable fraction of the generated dust, 40%)	RAR Vol. 3 B.6.2.3/01 Anonymous 6 (1986) Report No.: TXC 3/86432 New data for the Annex I renewal: **No**

Table 2.6.2.3-2. Summary table of human data on acute inhalation toxicity

No data

Table 2.6.2.3-3. Summary table of other studies relevant for acute inhalation toxicity

No data

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

An acute inhalation toxicity study in the rat is available (study previous evaluated in DAR 2005). The acute inhalation LC_{50} for male and female rats in the study was >0.32 mg/L. No deaths were observed. Abnormal body posture, abnormal respiratory pattern and rubbing of the snout or paws against the mesh of the exposure compartment were observed in a proportion of rats exposed to quinoclamine. These signs were considered to be consistent with the response to exposure to an irritant dust. A lesion involving the cornea (keratitis) and resulting in some opacity in the eye was evident, in a proportion of rats exposed to the test substance, from Day 2 of the observation period. This sign persisted, particularly in females, during the entire observation period. There was also a marked decrease of bodyweight over a period of 5 days following exposure to quinoclamine. Subsequently the rate of bodyweight gain was similar to or in excess of that observed for the control rats. Furthermore, there was a marked to moderate reduction in food consumption for 6 days in male rats and for 7 days in female rats following exposure to quinoclamine, and water consumption was reduced for 2-7 days following exposure.
Following changes were observed during the necropsy: penis was inflamed and of swollen appearance in 2 male rats exposed to quinoclamine, and the fur and tail of all exposed rats were stained orange.
The study was conducted according to GLP but the study is considered of limited usefulness due to low amount of respirable particles (about 40% by weight of the test substance in the chamber air was 5.5 µm or less).

Furthermore, the concentration used in the study was low (0.79 mg/L, highest attainable concentration). According to the CLP Guidance (3.1.2.3.2) inhaled particles between 1 and 4 microns mean mass aerodynamic diameter (MMAD) will deposit in all regions of the rat respiratory tract. This particle size range corresponds to a maximum dose of about 2 mg/L. In order to achieve applicability of animal experiments to human exposure, dusts and mists would ideally be tested in this range in rats.

As a conclusion the study was considered not acceptable due to low amount of respirable particles, also taking into consideration that the mode of exposure was whole-body and not nose-only which is recommended in the OECD TG 403.

2.6.2.3.2. Comparison with the CLP criteria regarding acute inhalation toxicity

According to the CLP Guidance (Table 3.1.1) substances can be allocated to one of four toxicity categories based on acute toxicity by the inhalation route as shown in table below:

	Category 1	**Category2**	**Category 3**	**Category 4**
Dusts and Mists (mg/l) (4 hr testing exposure)	ATE ≤ 0.05	0.05 < ATE ≤ 0.5	0.5 < ATE ≤ 1.0	1.0 < ATE ≤ 5.0

The LC_{50} for inhalation toxicity was greater than 0.32 mg/L for both sexes. However, the study was not acceptable due to low amount of respirable particles. Of particular importance in classifying for inhalation toxicity is the use of well articulated values in the highest hazard categories for dusts and mists. Inhaled particles between 1 and 4 microns mean mass aerodynamic diameter (MMAD) will deposit in all regions of the rat respiratory tract. This particle size range corresponds to a maximum dose of about 2 mg/l. In order to achieve applicability of animal experiments to human exposure, dusts and mists would ideally be tested in this range in rats (Annex I: 3.1.2.3.2 to the CLP Regulation). Results from studies in which substances with particle size with a MMAD > 4 μm have been tested can generally not be used for classification, but expert judgement is needed in cases where there are indications of high toxicity.

2.6.2.3.3. Conclusion on classification and labelling for acute inhalation toxicity

No conclusion on classification and labelling for acute inhalation toxicity could be drawn. A **data gap** is identified for this endpoint.

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 public 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.

2.6.2.4 Skin corrosion/irritation

Table 2.6.2.4-1. Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset2 - Mean scores/animal - Reversibility	Reference
Dermal irritation	Rabbit	ACN technical (Quinoclamine) Purity: 98.1%	0.5 g/animal Examination after 1, 24, 48,	One hour after removal of the patches and excess test material, the treated sites of one of 6 rabbits showed a very slight erythema (score 1), but the effect was not	RAR Vol. 3 B.6.2.4/01

Guideline: Not stated in study report GLP: Yes	New Zealand White 6 females		72 and 168 hours	reported thereafter at 24-169 hours after patch removal. No other signs of erythema or oedema were observed in any of the test animals.	Anonymous 7 (1985) Report No.: 105/8509 New data for the Annex I renewal: **No**

Table 2.6.2.4-2. Summary table of human data on skin corrosion/irritation

No data

Table 2.6.2.4-3. Summary table of other studies relevant for skin corrosion/irritation

No data

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

In a study performed in accordance with GLP, 6 female New Zealand White rabbits each received dermal treatments with 0.5 g of quinoclamine for 4 hours under occlusive conditions. After four hours the dressing was removed and the treated areas gently cleaned using cotton wool soaked in water at 37°C. One hour after removal of the patches and excess test material, the treated sites were assessed for signs of reaction to treatment. Similar examinations were undertaken twenty four, forty eight, seventy two and one hundred sixty eight hours after patch removal. No dermal irritation was observed 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 no dermal irritation was observed throughout the remainder of the study. Orange staining of skin was noted in all animals at the 1 hr observation but not at the 24, 48, 72 or 168 hr observations.

The RMS considers the study as acceptable. It was checked for compliance with OECD TG 404 and following deviations were noted:

i. No initial testing was performed using one animal

ii. Six animals were used in the study (the guideline recommends two or three animals to be used in the case a corrosive effect is not observed)

iii. The humidity of the experimental animal room was in the range of 63-81% (the guideline recommends that the relative humidity of the experimental animal room does not exceed 70%)

iv. No raw data was available.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

According to the CLP Guidance Table 3.2.2, a substance should be classified in Category 2 (Irritant) if:

"-mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

-inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above"

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. Thus, quinoclamine does not fulfil the CLP classification criteria as irritating to skin.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed for quinoclamine.

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**.

2.6.2.5 Serious eye damage/eye irritation

Table 2.6.2.5-1. Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results - Observations and time point of onset - Group mean scores (24, 48 and 72 hours) - Reversibility**	**Reference**
Eye irritation Guideline: Not stated in study report GLP: Yes	Rabbit New Zealand White 6 females (not rinsed after dosing) 3 females (rinsed with distilled water 2 min. after dosing)	ACN technical (Quinoclamine) Purity: 98.1%	0.1 mL aliquot (weighing 0.06 g)/animal Examination after 1, 24, 48, 72, 168 and 336 hours	Scores (**unrinsed group** / rinsed group): Conjunctivae: Chemosis: **2.1** / 0.0 Discharge: **0.6** / 0.0 Redness: **1.8** / 0.6 Cornea: Opacity: **0.9** / 0.1 Iris: Iritis: **0.7** / 0.0 No reaction to treatment remained 14 days after dosing.	RAR Vol. 3 B.6.2.5/01 Anonymous 8 (1985) Report No.: 106/8509 New data for the Annex I renewal: **No**

Table 2.6.2.5-2. Summary table of human data on serious eye damage/eye irritation

No data

Table 2.6.2.5-3. Summary table of other studies relevant for serious eye damage/eye irritation

No data

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

In a study performed in accordance with GLP, 0.1 ml of quinoclamine was placed in the lower conjunctival sac of the right eye of 9 female rabbits. The treated eyes of the first group of six rabbits (6 animals) were not rinsed after dosing with the test substance. The treated eyes of the three rabbits in second group were rinsed approximately two minutes after dosing. Each eye was rinsed over a one minute period using distilled water at 37°C. Reaction to treatment was assessed 1, 24, 48, 72, 168 and 336 hours after dosing. The eyes were assessed for damage or irritation to the cornea, iris and conjunctivae using the untreated eye as a control.
The RMS considers the study as acceptable. It was checked for compliance with OECD TG 405 and following deviation was noted:
i. The humidity in the experimental room was in the range of 57-81% (the guideline recommends the relative humidity not to exceed 70%)
ii. Group of animals was used to investigate the influence of washing (this is not recommended in the guideline unless it is scientifically justified, and if a satellite group is needed, two rabbis should be used).
One hour after dosing well defined redness and severe chemosis of the conjunctivae were apparent in all six rabbits of the unrinsed group, four of these rabbits also exhibiting iris inflammation in the treated eye. Some changes in response had occurred within twenty four hours of dosing, severe conjunctival irritation maintained by two rabbits, moderate conjunctival exhibited by two rabbits and well defend conjunctival irritation present in the treated eye of the remaining two rabbits of this group. Corneal opacity was observed in the treated eye of four rabbits and iris inflammation was also apparent in four rabbits at this time. Marked reduction in irritation had occurred in two animals of the group at the forty eight hour observation but iris inflammation, corneal opacity and moderate or severe conjunctival irritation showed some decline seventy two hours after dosing and at the one hundred and sixty eight hour observation small areas of corneal opacity remained in the treated eye of two rabbits, other irritant reaction having declined. No reaction to treatment remained fourteen days after dosing.

Severe conjunctival irritation was observed in the treated eye of two rabbits of the rinsed group one hour after dosing, the remaining rabbit of the group exhibiting a well defined conjunctival response. Twenty four hours after dosing a small area of corneal opacity and slight conjunctival redness was observed in one animal and well defined conjunctival redness was observed in the treated eye of a second rabbit of the group. This reaction declined, slight conjunctival redness remaining in both animals at the forty eight hour observation. No further response to treatment was apparent.

The results indicated that the test substance had a moderate irritant effect in the eye, with the effect being reversible. Rinsing the material from the eye within two minutes of its administration markedly reduced the severity, incidence and duration of the irritant response observed.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

According to the CLP Guidance Table 3.3.2, a substance should be classified in Category 2 (Irritating to eyes)

" If it produces, at least in 2/3 animals, a positive response of:

-corneal opacity ≥1 and/or

-iritis ≥1, and/or

-conjunctival redness ≥2 and/or

-conjunctival oedema (chemosis) ≥2

calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days"

In the eye irritation test conducted with quinoclamine, mean scores for chemosis was > 2 in the unrinsed group Thus, quinoclamine does fulfil the CLP classification criteria for eye irritation.

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

Classification of quinoclamine for eye irritation in Category 2 (H319: Causes serious eye irritation) is proposed.

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").**

2.6.2.6 Respiratory sensitisation

Table 2.6.2.6-1. Summary table of animal studies on respiratory sensitisation

No data

Table 2.6.2.6-2. Summary table of human data on respiratory sensitisation

No data

Table 2.6.2.6-3. Summary table of other studies relevant for respiratory sensitisation

No data

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

No data

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

Not relevant as no data are available

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not relevant as no data are available

2.6.2.7 Skin sensitisation

Table 2.6.2.7-1. Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Guinea Pig Maximisation Test (GPMT) OECD TG 406 GLP: Yes *There is no justification of the choice of vehicle*	Guinea pig Dunkin-Hartley F 2 females (1st screening study) 2 females (2nd screening study) 3 females (3rd screening study) <u>Main study:</u> 5 females in control group and 10 females in test group	Quinoclamine Purity: 99.0% Vehicle: arachis oil	1st screening study: 0.25 – 10% w/v 2nd screening study: 10 – 55% w/w 3rd screening study: 20 – 55% w/w <u>Main study:</u> Intradermal injection: 1% m/v Quinoclamine in arachis oil and/or Freund's complete adjuvant (FCA). Topical induction: 55% m/m Quinoclamine in arachis oil. Challenge application: 7.5 and 15% m/m Quinoclamine 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 (80%). An inconclusive response was seen in one further animal and negative results in the remaining test animal.	RAR Vol. 3 B.6.2.6/01 Anonymous 9 (2001) Report No.: 619/119-D6144 New data for Annex I renewal: **No**

Table 2.6.2.7-2. Summary table of human data on skin sensitisation

No data

Table 2.6.2.7-3. Summary table of other studies relevant for skin sensitisation

No data

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

The skin sensitisation potential of quinoclamine was assessed in one study (GPMT). 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 (80%). An inconclusive response was seen in one further animal and negative results in the remaining test animal.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

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

The CLP Regulation allows classification of skin sensitisers in one hazard category, Category 1, which comprises two sub-categories, 1A and 1B. Classification into sub-categories is only allowed if data are sufficient (CLP Annex I, 3.4.2.2.1.1). Therefore care should be taken when classifying substances into Category 1B when Category 1A cannot be excluded. In such cases classification into category 1 should be considered. This is particularly important if only data are available from certain tests showing a high response after exposure to a high concentration but where lower concentrations which could show the presence of such effects at lower doses are absent (in line with some test protocols where a maximised dose should be used). The criteria for sub-categorisation based on results from Guinea pig maximisation tests are given in table below:

Sub-category	Assay	Response
1A	Guinea Pig Maximisation Test	≥30% responding at ≤0.1% intradermal induction dose or ≥60% responding at >0.1% to ≤1% intradermal induction dose
1B	Guinea Pig Maximisation Test	≥30% to <60% responding at >0.1% to ≤1% intradermal induction dose or ≥30% responding at >1% intradermal induction dose

According to table above, quinoclamine fulfils the criteria for sub-categorisation in category 1A (Guinea Pig Maximisation Test: >60% responding at 1% intradermal induction dose).

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Classification of quinoclamine as a skin sensitiser (Skin Sens. 1A, H317: May cause an allergic skin reaction) is proposed.

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).**

2.6.2.8 Phototoxicity

Table 2.6.2.8-1. Summary table of studies on phototoxicity

Method, guideline, deviations if any	Test substance	Dose levels duration of exposure	Results	Reference

In vitro 3T3 NRU phototoxicity test Balb/c 3T3 fibroblast cells (clone 31, mouse fibroblast cell line) OECD TG 432 GLP: Yes	Quinoclamine Purity: 98.3%	31.6, 10.0, 3.16, 1.00, 0.316, 0.100, 0.0316 and 0.0100 µg/mL Final concentration of DMSO in culture medium: 1.0% (v/v) Incubated with Neutral Red for approximately 3.5 h	-UV OD_{540}, -UV SEM: IC_{50}: 3.59 µg/mL +UV OD_{540}, -UV SEM: IC_{50}: 3.23 µg/mL PIF: 1.11 Quinoclamine is non-phototoxic (PIF factor of <2)	RAR Vol. 3 B.6.2.7/01 Anonymous 10 (2015) Report No.: 508771 Anonymous 10 (2015) (Report amendment number 1) New data for Annex I renewal: **Yes**

OD: optical density
IC_{50}: cell viability reduced to 50%
PIF: Photo Irritation Factor

Table 2.6.2.8-2. Summary table of human data on phototoxicity

No data

Table 2.6.2.8-3. Summary table of other studies relevant for phototoxicity

No data

2.6.2.8.1 Short summary and overall relevance of the provided information on phototoxicity

Quinoclamine technical was evaluated for phototoxicity in the *in vitro* 3T3 NRU at concentrations of 31.6, 10.0, 3.16, 1.00, 0.316, 0.100, 0.0316 and 0.0100 µg/mL. Since quinoclamine had a PIF factor below 2, quinoclamine is non-phototoxic.

2.6.2.9 Aspiration hazard

Table 2.6.2.9-1. Summary table of evidence for aspiration hazard

No data

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No data

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Not relevant as no data are available

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

Not relevant as no data are available

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE)

Table 2.6.2.10-1. Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acute oral OECD TG 423 *There is no justification for the choice of vehicle* Rat Crl:WI(GIx/BRL/Han)BR M, F 3/sex/dose (200, 500 mg/kg bw) 3 females (2000 mg/kg bw) GLP: Yes	Quinoclamine Purity: 99.0% Vehicle: 1% methyl cellulose 200, 500, 2000 mg/kg bw Observations in 15 days	LD_{50}: 200 - 500 mg/kg bw (M, F) <u>200 mg/kg bw:</u> There were no deaths. Discoloured urine from 1 hr after dosing, affecting all animals. Soft and discoloured faeces affecting the females on Day 2. Anogenital soiling in one female from 4 hrs after dosing and complete by Day 4 <u>500 mg/kg bw:</u> 1 male and two females died. All rats showed discoloured urine and faeces, diarrhoea and anogenital soiling. Isolated causes of salivation, lethargy, piloerection, prone, unkempt appearance, dark faeces and a wasted appearance were seen. Clinical signs were apparent from 1 hour after dosing. Recovery of surviving rats was advanced by Day 6 and complete by Day 9. Macroscopic examination of one male revealed enlarged kidneys. Furthermore, one male had dark contents of the bladder and a female had dark areas on the lungs. <u>2000 mg/kg bw/day:</u> All rats died. All rats showed a number of clinical signs including dyspnoea, palpebral closure, piloerection, discoloured urine (pink) and lethargy from the 1 hour observation. A rat had straub tail and was prone and another showed spasticity prior to death. A further rat appeared to have recovered from signs of toxicity by Day 2 but by Day 3 it showed palpebral closure, a wasted appearance, discoloured faeces (dark) and anogenital soiling on Day 3 and 4. Macroscopic examination of decedents revealed enlarged kidneys, abnormal contents (orange fluid) in the stomach and small intestine of three rats. In one rat abnormal contents were also found in the large intestine and caecum, and the caecum was also found to be thin. The mucosal surfaces of the stomach were also orange in two individuals. Dark contents were apparent in the stomach and jejunum of one rat. The mucosal surface of the stomach of this rat was also dark. In two rats the connective tissue in the abdominal cavity was yellow.	RAR Vol. 3 B.6.2.1/01 Anonymous 11 (2002) Report No.: 619/141 New data for Annex I renewal: No

Acute oral OECD TG 420 Rat Slc:Wistar (SPF)) F <u>Sighting study:</u> One female/dose (300, 2000 mg/kg bw) <u>Main study:</u> Four females (300 mg/kg bw) GLP: Yes *There is no justification for the choice of vehicle*	Quinoclamine Purity: 98.3% Vehicle: 0.5% carboxymethyl cellulose sodium (0.5 w/v% CMC-Na) 300, 2000 mg/kg bw Observation period: 14 days	300-2000 mg/kg bw (F) <u>300 mg/kg bw:</u> There were no deaths. Animals administered 300 mg/kg showed yellow-red chromaturia (from 1 to 6 hrs after dosing), loose stools (from 2 to 5 hrs after dosing), and ptosis (from 2 to 3 hrs after dosing), but these findings were slight and transient. Body weight measurement and necropsy revealed no abnormalities in any of the animals. <u>2000 mg/kg bw:</u> 1 animal dosed died five hours after administration, and the clinical signs observed started one hour after administration of the test substance. The observations included; yellow-red chromaturia, loose stools, compound-coloured faeces, irregular respiration, salivation, lethargy, ptosis, decrease in locomotor activity, soiled fur in the anogenital region, prone position. The animal showed loss of locomotor activity and lateral position at 4 hrs after dosing and died at 5 hrs after the dosing. No abnormal changes were observed in any organs at necropsy.	RAR Vol. 3 B.6.2.1/02 Anonymous 4 (2016) Experiment No.: G427 (154-768) New data for Annex I renewal: Yes
Acute dermal OECD TG 402 Rat Crl:WI(GIx/BRL/Han)BR M, F <u>Preliminary study:</u> 2 females (2000 mg/kg bw) <u>Main study:</u> 5/sex (2000 mg/kg bw) GLP: Yes	Quinoclamine Purity: 99.0% 2000 mg/kg bw Observation period: 15 days	>2000 mg/kg bw (M, F) There were no deaths, and clinical signs were limited to anogenital soiling from 4 hours after dosing and pink coloured urine on the liner under the cage on Days 3 and 4. Anogenital soiling was no longer apparent on Day 3 and is associated with the bandaging procedure and not to toxic effects of the test article. Discolouration of the urine could be attributed to elimination of absorbed test article. The dermal test sites were stained orange throughout the observation period. Necropsy on Day 8 revealed no microscopic changes.	RAR Vol. 3 B.6.2.2/01 Anonymous 13 (2002) Report No.: 619/143-D6144 New data for Annex I renewal: No

Acute inhalation Guideline: not stated in the study report Rat Wistar M, F 5/sex GLP: Yes *The study is limited due to low amount of respirable particles (study not accepted for the purpose of classification for acute toxicity)*	ACN technical (Quinoclamine) Purity: 98.1% <u>Form and particle size (MMAD):</u> Aerosol. About 40% by weight of the test substance in the chamber air was 5.5 µm or less <u>Dose level:</u> 0.79 mg/L (4 hrs, whole body) (highest attainable concentration) Observation period: 14 days	LC_{50} (4 hrs, respirable fraction): >0.32 mg/L (value corrected for the respirable fraction of the generated dust, 40%) There were no deaths <u>Clinical signs during the exposure:</u> Abnormal body posture, abnormal respiratory pattern and rubbing of the snout or paws against the mesh of the exposure compartment were observed in a proportion of rats exposed to ACN (technical). These signs were considered to be consistent with the response to exposure to an irritant dust. <u>Clinical signs during the observation period:</u> A lesion involving the cornea (keratitis) and resulting in some opacity in the eye was evident, in a proportion of rats exposed to ACN (technical), from Day 2 of the observation period. This sign persisted, particularly in females, during the entire observation period. Inflammation of the penis was seen in a proportion of the male exposed rats from Day 5 to Day 14 of the observation period.	RAR Vol. 3 B.6.2.3/01 Anonymous 14 (1986) Report No.: TXC 3/86432 New data for the Annex I renewal: No

Table 2.6.2.10-2. Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.2.10-3. Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Following acute oral exposure in rats clinical symptoms such as discoloured urine, soft and discoloured faeces and anogenital soiling were noted at 200 mg/kg bw. At higher doses ptosis (at 300 mg/kg bw) and salivation, lethargy, piloerection, prone, unkempt appearance, dark faeces and a wasted appearance (at 500 mg/kg bw) were noted in addition. These effects were transient in nature. Clinical signs were apparent from 1 hour after dosing. Recovery was completed by Day 4 (200 mg/kg bw) to Day 9 (500 mg/kg bw). At 2000 mg/kg bw all rats died (time of death after dosing: between 2 hrs and 2 days). These animals showed clinical signs such as yellow-red chromaturia, loose stools, compound-coloured faeces, dyspnoea, palpebral closure, piloerection, lethargy, irregular respiration, salivation, ptosis, decrease in locomotor activity, soiled fur in the anogenital region and prone position. The majority of surviving rats dosed at 200 mg/kg bw failed to gain body weight during the firsts week of the observation period. Rats treated at 500 mg/kg bw lost large amounts of body weight between Day -1 and Day 8.

All surviving rats did gain body weight between Day 8 and 15, and all rats treated at 200 mg/kg bw and the female treated at 500 mg/kg bw gained overall body weight during the observation period. Macroscopic changes were noted in one male treated at 500 mg/kg bw (enlarged kidneys). Examination of decedents treated at 500 or 2000 mg/kg bw revealed abnormal contents (orange fluid) in the stomach and small intestine of three rats. In one rat dosed at 2000 mg/kg bw abnormal contents were also found in the large intestine and caecum and the caecum was also found to be thin. The mucosal surfaces of the stomach were also orange in two individuals treated at 2000 mg/kg bw. Dark contents were apparent in the stomach and jejunum of one rat treated at 2000 mg/kg bw. The mucosal surface of the stomach of this rat was also dark. In two rats the connective tissue in the abdominal cavity was yellow. One male treated at 500 mg/kg bw had dark contents of the bladder and a female had dark areas on the lungs (RAR Vol. 3, B.6.2./01-02)

Following acute dermal administration in rats at the dose level of 2000 mg/kg bw no adverse clinical signs were noted. Principal signs of reaction to treatment were staining of the snout noted from 30 minutes or 1 hour after dosing and orange coloured urine apparent from Days 2 and 4. Recovery of rats as judged by external appearance and behaviour, was complete by Day 5. Slight erythema affecting two females between Days 4 and 9 and orange staining throughout the observation period. 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. However, one male failed to regain its pre-study body weight and the body weight gain in some individuals was only modest. No macroscopic changes were observed in animals killed on Day 15 (RAR Vol. 3, B.6.2.2/01)

Following acute inhalation administration in rats at a concentration of 0.79 mg/L (4 hrs, whole body) no deaths were observed. Abnormal body posture, abnormal respiratory pattern and rubbing of the snout or paws against the mesh of the exposure compartment were observed in a proportion of rats exposed to quinoclamine. These signs were considered to be consistent with the response to exposure to an irritant dust. A lesion involving the cornea (keratitis) and resulting in some opacity in the eye was evident, in a proportion of rats exposed to the test substance, from Day 2 of the observation period. This sign persisted, particularly in females, during the entire observation period. There was also a marked decrease of bodyweight over a period of 5 days following exposure to quinoclamine. Subsequently the rate of bodyweight gain was similar to or in excess of that observed for the control rats. Furthermore, there was a marked to moderate reduction in food consumption for 6 days in male rats and for 7 days in female rats following exposure to quinoclamine, and water consumption was reduced for 2-7 days following exposure. Following changes were observed during the necropsy: penis was inflamed and of swollen appearance in 2 male rats exposed to quinoclamine, and the fur and tail of all exposed rats were stained orange. The study was conducted according to GLP but the study was considered not acceptable for the purpose of classification for acute toxicity due to low amount of respirable particles (about 40% by weight of the test substance in the chamber air was 5.5 µm or less) (RAR Vol. 3, B.6.2.3/01).

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

Specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance, 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. The hazard class STOT SE is differentiated into STOT SE Category 1 and 2; and STOT SE Category 3.
Classification in STOT SE 1 is required for substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in this category on the basis of reliable and good quality evidence from human cases or epidemiological studies, or observations from animal studies in which significant and/or severe toxic effects of relevance to human health are seen at generally low exposure levels (Annex I: Table 3.8.1 of the CLP Regulation).
Classification in STOT SE 2 is required for substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure. Substances are classified in this category on the basis of observations from animal studies in which significant toxic effects of relevance to human health are seen at generally moderate exposure levels (Annex I: Table 3.8.1 of the CLP Regulation).

In the acute toxicity studies performed in the rat, no specific target organ toxicity were noted which were not covered by the other hazard classes. Following acute oral administration, abnormal contents (such as coloured fluids) were noted in the stomach, intestine and caecum. Furthermore, enlarged kidney (one male), small intestine (three females), and dark areas of the lungs (one female) were noted following acute oral administration. These effects were however, not considered of concern for a classification as STOT-SE since the effects were noted at doses with the presence of lethality.

Classification in STOT SE 3 is limited to transient narcotic effects and respiratory tract irritation (Annex I: Table 3.8.1 of the CLP Regulation).
The criteria for respiratory tract irritation (RTI) include "*There are currently no validated animal tests that deal specifically with RTI, however, useful information may be obtained from the single and repeated inhalation toxicity tests. For example, animal studies may provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. Such animal studies can be used as part of weight of evidence evaluation*" (Annex I: 3.8.2.2.1 of the CLP Regulation).
The criteria for narcotic effects include "*Narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure*" (Annex I: 3.8.2.2.2 of the CLP Regulation).

There were no specific respiratory tract irritant or narcotic effects observed for quinoclamine that are indicative of STOT SE 3 classification.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

No classification for STOT SE is proposed for quinoclamine.

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**.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity)

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE)

Table 2.6.3.1-1. Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
Oral 28-day study OECD TG 407 Rat Crl:CD®(SD)IGSBR M, F 5/sex/dose <u>*Deviations from OECD TG 407:*</u> *Detailed clinical observations and functional observations were not made, oestrus cycle of females was not determined, determinations of haematocrit and blood clotting time/potential were not included in the haematological examinations, thyroid hormones were not investigated, seminal vesicles were not weighed, cervix was not preserved for histopathological examination* GLP: Yes	Quinoclamine (purity: 99.0%) Oral (dietary) 0, 5, 50, 500, 1000 ppm (corresponding to 0, 0.5, 4.7, 44 and 84 mg/kg bw/day for males; 0, 0.5, 5.3, 48 and 90 mg/kg bw/day for females) 28 consecutive days	<u>5 ppm:</u> No treatment related effects <u>50 ppm:</u> No treatment related effects <u>500 ppm:</u> ↓**bw gain** (M:19%, F:21%, n.s) ↓FC (F) **-changes in haematological parameters** (↓haemoglobin (M:10%), ↓red blood cell count (M), ↓packed cell volume (M),↑reticulocytes (M),↑absolute reticulocytes (M),↑red cell distribution width (M, F), ↑haemoglobin distribution width (M, F)) -changes in biochemistry (↓alanine aminotransferase (M), ↑bilirubin (M, n.s., F, n.s.)) **-changes in urine analysis parameters** (red-, brown- or dark coloured urine (M, F), ↑amorphous debris (M, F), ↑urine volume (M)) **-changes in organ weights** (↓ thymus, (adjusted) M: 25%, F: 41%) **-histopathological changes in the kidneys** (↑eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium) (M) <u>1000 ppm:</u> ↓**bw gain** (M: 42%, F: 41%) ↓FC (M, F) **-changes in haematological parameters** (↓haemoglobin (M: 8%, F: 13%), ↓red blood cell count (M, F), ↓packed cell volume (M, n.s, F),↑reticulocytes (M, F),↑absolute reticulocytes (M, F),↑red cell distribution width (M, F), ↑haemoglobin distribution	RAR Vol. 3, B.6.3.1.1/01 Anonymous 15 (2002) Report No.: 619/148 New data for the Annex I renewal: No

		width (M, F), ↑mean platelet volume (M), ↑platelet distribution width (M), ↑prothrombin time (M), activated partial thromboplastin time (M),↑plateletcrit (F), ↑platelet (F)) -**changes in biochemistry** (↓alanine aminotransferase (M),↑bilirubin (M, n.s., F, s.s.)) -**changes in urine analysis parameters** (red-, brown- or dark coloured urine (M, F),↑amorphous debris (M, F), ↑urine volume (M)) -**changes in organ weights** (↓thymus M: 24%, n.s., F: 48%) -**macroscopical changes** (large kidney, one male only[a]) -**histopathological changes in the kidneys** (↑eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium, minor papillitis characterized by hyperbasophilia of the collecting duct epithelium, interstitial polymorph accumulation and hyperplasia of the urothelium overlying the renal papilla) (M) NOAEL (both sexes): 50 ppm (corresponds to 4.7 and 5.3 mg/kg bw/day in males and females, respectively) LOAEL (both sexes): 500 ppm (corresponds to 44 and 48 mg/kg bw/day in males and females, respectively)	
Oral 28-day study No guideline stated in study report Dog Beagle M, F 1/sex/dose *The study report was checked for compliance with OECD TG 409 adopted 21st September 1998 and the following deviations were observed:* *i. Four weeks treatment instead of 3 months* *ii. The group size (2 animals) is smaller than recommended (8 animals- four females and four males).* *iii. Haematocrit was not determined* *iv. No ophtalmological examination was performed* *v. Ornithine decarboxylase was not determined* *vi. Gallbladder and thymus were not weighed*	Quinoclamine (purity: 99.0%) Oral (capsules) 0, 3, 10, 30, 100[b] mg/kg bw/day 28 consecutive days	<u>3 mg/kg bw/day:</u> No treatment-related effects <u>10 mg/kg bw/day:</u> -clinical signs (red- or black coloured urine (F)) -changes in urinalysis parameters (↑turbidity (M)) <u>30 mg/kg bw/day:</u> **clinical signs** (red- or black coloured urine (M, F)) ↓FC -**changes in urinalysis parameters** (↑turbidity (M)) -**organ weight changes** (↑abs spleen (M: 89%, F: 51%),↑rel spleen (M: 97%, F: 58%)) -**histopathological changes** (kidneys: tubular nephropathy and transitional cell hyperplasia (M, F); urinary bladder: transitional cell hyperplasia (M, F)) <u>100 mg/kg bw/day[b]:</u> -**clinical signs** (red- or black coloured urine (M, F), vomiting (M, F), subdued on Day 3 (F)) ↓**bw loss** (Day 4: 13% (M), 18% (F)) -**poor food consumption** (M, F) -**changes in biochemistry** (Day 4: ↓sodium (M, F), ↓potassium (M, F), ↓chloride (M, F),	RAR Vol. 3, B.6.3.1.2/01 Anonymous 16 (2002) Report No.: 619/149 New data for the Annex I renewal: No

vii. Histopathological examination was limited to following organs: kidney, liver, urinary bladder GLP: Yes		↑urea (M, F), ↑total bilirubin (M, F), ↑creatinine (M, F), ↑total cholesterol (M, F)) -**organ weight changes** (↑abs liver (M: 11%, F: 20%), ↑rel liver (M: 51%, F: 44%), ↑abs spleen (11%, F: 20%), ↑rel spleen (M: 45%, F: 320%), ↑abs kidney (M, 17%, F: 15%), ↑rel kidney (M: 60%, F: 38%) -**macroscopical changes** (abnormal urinary bladder contents) (F) -**histopathological changes** (kidneys: tubular nephropathy and transitional cell hyperplasia (M, F); urinary bladder: transitional cell hyperplasia and arteritis (M, F) and epithelial necrosis (M)) *Study accepted as a range finding study only. Due to limited histopathology and low number of animals used it is not appropriate to establish a NOAEL/LOAEL. The results of the study are considered equivocal for classification.*	
Oral 90-day study No guideline stated in study report Rat Sprague-Dawley (SPPF) M, F 5/sex/dose *The study report was checked for compliance with OECD TG 408 adopted 21st September 1998 and the following deviations were observed:* *i. Housing and feeding conditions are not reported* *ii. Few animals were used in the study, 5/sex/dose (the guideline recommends 10/sex/dose)* *iii. It is not indicated in the study if detailed clinical observations were made* *iv. No ophthalmological examination was made* *v. Sensory reactivity to stimuli of different types and functional observations were not made.* *vi. Haematological examinations were limited (erythrocyte count, platelet count and measure of blood clotting time/potential was not included)* *vii. Biochemical determinations were limited (sodium, potassium, glucose, total cholesterol, urea, blood, urea nitrogen, creatinine were not included)* *viii. Urinalysis were limited (appearance, volume, osmolality,*	Quinoclamine (purity: not stated in study report) Oral (dietary) 0, 50, 200 and 1000 ppm (equivalent to 0, 3, 14, 62 mg/kg bw day in males and 0, 3, 13, 65 mg/kg bw day in females) 13 weeks	<u>50 ppm:</u> ↓FC (F: 12%) ↓water consumption (15%) (F) -changes in biochemistry (↓Albumin/Globulin ratio (M, F)) -organ weight changes (↑rel mandibular gland M: 27%, n.s.)) <u>200 ppm:</u> ↓bw gain (M: 6%) ↓FC (F: 11%) ↓water consumption (12%) (F) -changes in biochemistry (↓Albumin/Globulin ratio (M)) -**organ weight changes** (↑rel left adrenal (M: **60**%), ↑rel mandibular gland (M: 27%), ↑rel right kidney (M: 7%)) -**histopathological changes** in spleen (↑hemosiderin deposition (M, F) and kidney (hyaline droplets in the cortical epithelium (M)) <u>1000 ppm:</u> ↓bw gain (F: 7%) ↓FC (M: 5%, F: 14%) ↓water consumption (M: 23%, F: 17%) **-changes in biochemistry** (↓Albumin/Globulin ratio (M), ↑GOT (M, n.s.)) -**organ weight changes** (↑abs spleen (M:58%, F: 18%), ↑rel spleen (M: 63%, F: 35%), ↑abs liver (M: 12%), ↑rel liver (M: 16%, F: 19%), ↑abs kidney (M: 20%), ↑rel kidney (M: 11%, F: 17%), ↑rel left adrenal (M: 80%), ↑rel mandibular gland (M: 40%)) -**histopathological changes** in spleen (↑hemosiderin deposition (M, F), liver (bile duct proliferation (M, F), in kidney (hyaline droplets in the cortical epithelium (M)) NOAEL (both sexes): 50 ppm (corresponds to to 3 mg/kg bw/day)	RAR Vol. 3, B.6.3.2.1/01 Anonymous 17 (1972) Report No.: not specified New data for the Annex I renewal: No

glucose and blood/blood cells were not included) *ix Pituitary, parathyroid, thymus, oesophagus, trachea, aorta, uterus, female mammary gland, prostate, urinary bladder, peripheral nerve, skin and eyes were not preserved for histopathological examination* *x. No historical control data available* GLP: No		LOAEL (both sexes): 200 ppm (corresponds to 14 and 13 mg/kg bw/day in male and females, respectively) *Study considered limited and accepted as supportive data only*	
Oral 90-day study OECD 408 (1998) Rat Crl:CD (SD)IGSBR M, F 10/sex/dose GLP: Yes	Quinoclamine (purity: 99%) Oral (dietary) 0, 50, 200 and 800 ppm (equivalent to 0, 3.61, 13.89, 56.74 mg/kg bw/day in males, and 0, 4.56, 17.81, 74.81 mg/kg bw/day in females) 13 weeks	<u>50 ppm:</u> -clinical signs (↑fur staining) (M, F) ↓**bw gain** (Start to week 13: F 17%) ↓FC (F, n.s.) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -changes in biochemistry (↑mean alanine aminotransferase (M)) <u>200 ppm:</u> -clinical signs (↑fur staining) (M, F) ↓**bw gain** (Start to week 13: F 21%) ↓FC (F) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -**changes in myelogram data** (↓mean in eosinophils (F)↓total myelopoietic cells (F)) -**changes in haematological parameters** (↑haemoglobin distribution width (M, F),↑reticulocytes (M, F), ↑abs reticulocytes (F), ↓red blood cell count (M, F), ↓packed cell volume (F), ↓haemoglobin (F: 4%), ↑activated partial thromboplastin time (M)) -**changes in biochemistry** (↑mean aspartate aminotransferase (M, n.s.), ↑mean alanine aminotransferase (M, n.s.)) -**changes in urinalysis** (dark straw coloured urine (M, F)) - **changes in organ weights** (↑rel spleen (M: 22%), ↑rel liver (F: 11%), ↓rel thymus (F: 41%)) -**histopathological changes** in spleen (↑extent of pigment (F)), in liver (sinusoidal cell pigment (M, F)), in kidneys (↑extent of eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium (M)), in thymus (minor thymic atrophy (M, F)) <u>800 ppm:</u> -clinical signs (↑fur staining) (M, F) ↓**bw gain** (M: 20-28%, F: 27-38%) ↓FC (M, F) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -**changes in myelogram data** (↓mean in eosinophils (F), ↓total myelopoietic cells (F)) -**changes in haematological parameters** (↑red cell distribution width (M, F), ↑haemoglobin distribution width (M, F), ↑mean cell volume (F), ↑reticulocytes (M, F),	Vol. 3, B.6.3.2.1/02 Anonymous 18 (2003) Report No.: 0619/132 New data for the Annex I renewal: No

		↑abs reticulocytes (M, F), ↓packed cell volume (M, F), ↓red blood cell count (M, F), ↓haemoglobin (M: 10%, F: 11%), ↑activated partial thromboplastin time (M), ↓platelet distribution width (M), ↓ mean cell haemoglobin (F)) -**changes in biochemistry** (↑mean aspartate aminotransferase (M), ↑mean alanine aminotransferase (M, n.s.)) -**changes in urinalysis** (dark straw coloured urine (M, F)) -**changes in organ weights** (↑rel spleen (M: 44%, F: 22%), ↑rel liver (M: 10%, F: 16%), ↑brain (M), ↓rel thymus (F: 48%)) -**macroscopic changes** (enlarged spleen, two males) -**histopathological changes** in spleen (↑incidence of congestion (M, F), ↑extent of haemopoiesis (M), ↑extent of pigment (F)), in liver (sinusoidal cell pigment (M, F)), in kidneys (↑extent of eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium (M), ↑incidence of pigment (F), ↑extent of focal nephropathy (M, F), ↑ papillary interstitial eosinophilia (two males)), in thymus (thymic atrophy (M, F)) NOAEL (F): not established NOAEL (M): 50 ppm (corresponds to 3.61 mg/kg bw/day) LOAEL (F): 50 ppm (corresponds to 4.56 mg/kg bw/day) LOAEL (M): 200 ppm (corresponds to 13.89 mg/kg bw/day)	
Two generation reproduction study In-house method Rat Sprague-Dawley M, F 25/sex/group *Study was checked for compliance with OECD TG 416 (2001) and following deviations were noted:* *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*	K-1616 (Quinoclamine) Purity: 98.5% 0, 1, 25, 500 ppm Corresponding to: F0: 0, 0.07, 1.6, 30.9 mg/kg bw/day in males; 0, 0.08, 1.9 and 37.7 mg/kg bw/day in females F1: 0, 0.07, 1.7 and 37.0 mg/kg bw/day in males; 0, 0.08, 2.0 and 43.8 mg/kg bw/day in females The parents of both generations were fed the appropriate diets for at least nine weeks and then subjected to two subsequent mating trials. Fresh diets were prepared and presented weekly to the rats of all	<u>1 ppm:</u> <u>Parental:</u> -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 3%; P2 M: 7%; P2 F 4%) ↓ bw gain (P1 M: 4%, P2 M: 11%; P2 F: 4%) <u>Offspring:</u> -increased incidence of gray lung cysts in F2b offspring reared for 3 months (18 compared to 11 in control group) <u>25 ppm:</u> <u>Parental:</u> -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 1%; P2 M: 7%; P2 F 5%) ↓ bw gain (P1 M: 2%, P2 M: 11%; P2 F: 6%) <u>Offspring:</u> -increased incidence of gray lung cysts in F2b offspring reared for 3 months (29 compared to 11 in control group) <u>500 ppm:</u> Parental:	RAR Vol. 3, B.6.6.1/01 Anonymous 19 (1975) Report No.: 854-111 New data for the Annex I renewal: No

vi. Detailed testicular histopathology was not performed *vii. Postlactational ovary (primordial and growing follicles) histopathology was not performed* *viii. For the offspring, age at vaginal opening or PPS for the F1 and F2 was not determined* GLP: No	generations from initiation (P1) or weaning (F1b—>F2, F2b)	**-clinical signs** (F0/F1: hunched posture) ↓ **bw** (P1 M: 4%; P2 M: 10%; P2 F 10%) ↓ **bw gain** (P1 M: 7%, P2 M: 11%; P2 F: 9%) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) Offspring: -clinical signs (orange stained fur F2b offspring) ↓ **bw** during lactation (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) **-increased incidence of gray lung cysts** in F2b offspring reared for 3 months (39 compared to 11 in control group) NOAEL parental and offsprings: 25 ppm (1.6 mg/kg bw/day) NOAEL reproductive toxicity: 500 ppm (37 mg/kg bw/day)	
Oral 90-day study OECD 409 (1998) Dog Beagle M, F 4/sex/dose GLP: Yes	Quinoclamine (purity: 99%) Oral (capsules) 0, 3, 10 and 30 mg/kg bw/day 13 weeks	3 mg/kg bw/day: -clinical signs (coloured urine and faeces) (M, F) 10 mg/kg bw/dag: -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (F: 12% n.s.) ↓FC (M) **-changes in haematological parameters** (↓red blood cell count (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin concentration (M, F), ↑platelet count (F, n.s.), ↑platelet crit (F, n.s.), ↑total white blood cell count (F, n.s.)) **-changes in organ weights** (↑adjusted liver (F: 27%), ↑adjusted thyroid/parathyroid (M: 33%)) **-histopathological changes** in bone marrow (haemopoiesis (M, F)), liver (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F)), urinary bladder (cystitis (one female))	Vol. 3, B.6.3.2.2/01 Anonymous 20 (2002) Report No.: 0619/134 New data for the Annex I renewal: No

		30 mg/kg bw/day: -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (M: 31%, F: 35%) ↓FC (M, F) -**changes in haematological parameters** (↓red blood cell count (M, F), ↓haemoglobin (M: 18%, F: 19%), ↓packed cell volume (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin concentration (M, F), ↑mean cell volume (M, F), ↑platelet count (M, F), ↑platelet crit (M, F), ↑total white blood cell count (M, F)) -**changes in biochemistry** (↑mean total bilirubin (M, F)) -**changes in organ weights** (↑adjusted liver (M: 20%, F: 29%), ↑adjusted thyroid/parathyroid (M: 32%), ↑adjusted spleen (F: 56% n.s.)) -**macroscopic changes** in spleen (enlarged two females), liver (mootled, one female) and urinarybladder (red, one female) -**histopathological changes** in bone marrow (haemopoiesis characterised by greater cellularity (M, F)), spleen (haemopoiesis characterised by increased haemopoietic cells in the red pulp (M, F), congestion of the splenic red pulp (M, F)), liver (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F), bile duct hyperplasia (M, F)), kidney (pigment (M, F)), urinary bladder (transitional cell hyperplasia (M, F), arteritis (one male), cystitis (one female)) NOAEL (both sexes): 3 mg/kg bw/day LOAEL (both sexes): 10 mg/kg bw/day	
Oral 2-year study In house method Dog Beagle M, F 4/sex/dose GLP: Yes *The study is acceptable. It was checked for compliance with OECD TG 409 and the following deviations were noted:* *i. Study duration 2-years instead of 90 days.* *ii. Housing conditions is not presented in the study report* *iii. Ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, blood creatinine and serum protein were not included in*	Quinoclamine (purity: 98.5%) Oral (dietary) 0, 2, 10, 50, 250 and 1000 ppm (equivalent to 0, 0.06, 0.33, 1.42, 7.62 and 26.6 mg/kg bw/day in males, and 0, 0.06, 0.31, 1.39, 6.79 and 29.1 mg/kg bw/day in females) 2 year	2 ppm: No treatment-related effects 10 ppm: ↓bw (M: week 52: 5%, week 104: 12%; F: week 104: 19%) ↓bw gain (Week 0-52: M: 2.4% compared to 0.3% in controls, F: 3% compared to 1.7% in controls; Week 52-104: M: 0.3% compared to 1.2% in controls, F: 0.3% compared to 1.5% in controls) -haematological changes (↓erythrocytes (F: week 104) -macroscopical changes in urinary bladder (mucosa brown or tan in colour in 2 of 6 animals) 50 ppm: ↓bw (M: week 52: 3%, week 104: 8%; F: week 52: 2%, week 104: 5%) ↓bw gain (Week 0-52: M: 2.6% compared to 2.4% in controls, F: 2.3% compared to 1.7% in controls; Week 52-104: M: 0.1% compared to 1.2% in controls, F: 0.9% compared to 1.5% in controls)	Vol. 3, B.6.3.3.1/01 Anonymous 21 (1976) Report No: 854/110 New data for the Annex I renewal: No

the clinical biochemistry examination *iv. No ophthalmological examination performed* *v. The following tissues were not included in the histopathology investigation: peripheral nerve, uterus, eyes and spinal cord.*		-**changes in haematological parameters** ↓haematocrit (M, Week 76), ↓erythrocytes (M Week 76, F Week 104) -**macroscopical changes** in ovary (one small in size), urinary bladder (mucosa brown or tan in colour), spleen (dark in colour or margins dark)) -**histopathological changes** in lung (focal pneumonitis (one male)), liver (pigment in macrophages (one female), bile plugs in canaliculi (one female)), urinary bladder (pigment in mucosal cells (M,F)) 250 ppm: -**clinical signs** (brown-tined urine) ↓bw (week 104: M: 8%, F: 6%) ↓**bw gain or bw loss** (week 0-52: M: 1.9% compared to 2.4% in controls, F: 2% compared to 1.7% in controls, week 52-104: M: -0.1% compared to 1.2% in controls, F: 0.1% compared to 1.5% in controls) -**changes in haematological parameters** (↓haemoglobin (M: Weeks 26: 16%, 52: 17%, 76: 12%; F: 26: 16%, 76: 20%), ↓haematocrit (M: Weeks 26, 52, 76; F: Weeks 26, 76, 104), ↓erythrocytes (M: Weeks 26, 52, 76, 104; F: Weeks 76, 104): -**changes in biochemistry**: (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F),↑serum glutamic-oxaloacetic transaminase (M, F)) -**macroscopical changes** in urinary bladder (mucosal surface brown or yellow-gray), liver (brown in colour, rough surfaced, tough in consistency, firm), spleen (dark in colour or margins dark), kidneys (depressed areas on surface), ovary (cyst on one), lung (white foci on surface) -**histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), focal nonsupparative adrenalitis (one male)), lung (foci of foamy macrophages (M,F)), spleen (extramedullary haematopoesis (F), congestion (F)), liver (pigment in cytoplasm of hepatocytes and kuppfer cells (M,F), pigment in macrophages (F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (one female), sinusoidal distension (F)), kidney (tubular nephrosis (one female), urinary bladder (pigment in mucosal cells (M,F), pigment laden macrophages (F)) 1000 ppm: -**mortality** (one of each sex sacrificed in extremis during week 65) -**clinical signs** (brown-tined urine, orange stained hair around urogenital area, during the second year of study: pale appearing oral mucosal membranes, yellowish discoloration of the eyes and thinness in the female sacrificed in extremis, and unhealthy	

		appearance characterized by thinness and lethargy in the male sacrificed in extremis) ↓**bw** (week 52: M: 21%, F:26%; week 104: M: 23%, F: 33%) ↓**bw gain or bw loss** (Weeks 0-52: M: 0.3% compared to 2.4% in controls, F: -0.6% compared to 1.7% in controls; Weeks 52-104: M: 0.1% compared to 1.2% in controls, F: -0.6% compared to 1.5% in controls) **-changes in haematological parameters** (all post treatment intervals: ↓haemoglobin M: up to 26%, F: up to 47% ↓haematocrit (M, F), ↓erythrocytes (M, F): ↑platelet counts (F: Week 104) -**changes in biochemistry**: (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F), ↑bilirubin (F: Weeks 52, 78), ↑serum glutamineo-oxaloacetic transaminase (M, F)) -**changes in organ weights**: F: ↑rel lungs (92%) (F), ↑ rel spleen (77%) (F), ↑rel gonads (80%) (F), ↓rel gonads (55%) M, n.s), ↓rel prostate (45%), n.s)) -**macroscopical changes** in the liver (enlarged, lobes thickened and pale, rough surface and mottled, brown in colour, tough in consistency, firm), gall bladder (distended, walls thickened), kidneys (small, depressed areas on surface, contracted, polycystic-primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule), urinary bladder (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface), spleen (dark in colour or margins dark, enlarged), testes (small and soft), prostate (small at week 52), ovary (cyst on one), heart (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52), lung (raised yellow gray foci on all lobes, focal emphysematous appearing areas), cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in colour), mesenteric lymph nodes (dark in colour), small intestine (walls slightly thickened) -**histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), necrosis, one female), lung (foci of foamy macrophages (M,F), focal pneumonitis (M, F), cholesterol clefts (M,F), fibrosis (one female), edema (M,F), consolidation (one male)), spleen (extramedullary haematopoesis and congestion (M,F)), liver (pigment in cytoplasm of hepatocytes, kuppfer cells and macrophages (M,F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (M,F), sinusoidal distension (F)), kidney (tubular nephropathy with fibrosis and renal tubular regeneration (M,F)), urinary bladder	

		(pigment in mucosal cells (M,F), edema (one female), pigment laden macrophages (one female)), testis (aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis), ovary (lack of follicle development, follicular cysts (one female), mesenteric lymph nodes (edema, erythrophagocytosis, distension of medullary sinuses (F)), pancreas (edema (F)), gall bladder (hyperplasia (M,F), papillary infolding (M,F), cholelith (one female)), aorta (mineralisation in one female), small intestine (focal enteritis (one female), erosion (one female)) NOAEL (both sexes): 10 ppm (corresponds to 0.33 and 0.31 mg/kg bw/day in males and females, respectively) LOAEL (both sexes): 50 ppm (corresponds to 1.42 and 1.39 mg/kg bw/day in males and females, respectively)	
28-day dermal study OECD 410 Rat Crl:CD:(SD)IGSBR M, F 5/sex/dose GLP: Yes	Quinoclamine (purity: 99%) Vehicle: 2% methylcellulose (w/v) Dermal 0, 100, 300, 1000 mg/kg bw/day 28 days	100 mg/kg bw/day: -clinical signs (skin irritation) -macroscopic changes (sores at the treated site) (three females) 300 mg/kg bw/day: -clinical signs (skin irritation) -changes in biochemical parameters (↓aspartate aminotransferase (F), ↓mean alkaline phosphatase (F n.s.), ↑bilirubin (M n.s.), ↑glucose (M)) -macroscopic changes (sores at the treated site) (one female) 1000 mg/kg bw/day: -clinical signs (skin irritation) -changes in biochemical parameters (↓aspartate aminotransferase) (F n.s.), ↓mean alkaline phosphatase (F), ↑bilirubin (M), ↑glucose (M)) -changes in urinalysis (dark colour of the urine) (M, F) -macroscopical changes (sores at the treated skin site) (two males) **-histopathological changes** in skin (acanthosis/hyperkeratosis in the epidermis, subepidermal fibrosis, epidermatitis) and kidney (tubular degeneration/regeneration) (one female and one male), hydronephrosis (one female), pigment (one female)) NOAEL for systemic effects (M, F): 300 mg/kg bw/day LOAEL for systemic effects (M, F): 1000 mg/kg bw/day NOAEL local effects (M, F): not estimated	RAR Vol. 3, B.6.3.4.1/01 Anonymous 22 (2002) Report No.: 0619/133 New data for the Annex I renewal: No
Long-term toxicity and carcinogenicity	ACN technical (Quinoclamine)	4 ppm: -changes in urinalysis (yellow/brown or orange colour) (M, F)	RAR Vol. 3, B.6.5.1/01

Oral (dietary) No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group *The study is acceptable. It was checked for compliance with OECD TG 453 and following deviations were noted:* *i. Haematological examination was not carried out at 3 months (the guideline recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study)* *ii. Prothrombin time and activated partial thromboplastin time was not investigated* *iii. Urea was not investigated* *iv. Uterus and epididymides were not weighed* *v. Coagulating gland, ileum, lacrimal gland and seminal vesicle were not investigated for histopathology* GLP: No	Purity: 98.3% Carcinogenicity groups: 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females Chronic toxicology groups: 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females 104 weeks	52 ppm: **-changes in urinalysis** (yellow/brown or orange discoloration) (M, F) -changes in organ weights (Week 27: ↑ kidney (M: 8%)) **-histopathological changes** in urinary bladder (epithelial hyperplasia (M, F), kidneys (epithelial hyperplasis (M, F), ↑ renal focal calcification (F), ureter (epithelial hyperplasia (M, F), lungs (arterial calcification (M)) 676 ppm: -clinical signs (orange fur staining, ↓ incidence of mass bearing animals) (M, F) ↓ **bw gain** (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓ FC (M, F) **-changes in haematological parameters** (↓ packed blood cell volume (M week 27, 79; F week 53), ↓ haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓ red blood cell count (M week 27, 79; F: week 27, 53)) **-changes in biochemical parameters** (↑ blood urea nitrogen (M n.s., F n.s.), ↓ calcium (M: week 27, 79; F: n.s.), ↓ inorganic phosphorous (M: n.s, F: week 27, 53), ↓ lactate dehydrogenase (M: week 79, 103; F: week 103)) **-changes in organ weights** (Week 27: ↑ rel kidney (M: 15%), ↑ adrenals (F: 38%), Week 53: ↑ kidney (M: 10%), Week 79: ↑ heart (M: 18%, F: 28%), ↑ brain (F: 28%), ↑ spleen (F. 13%), ↑ kidney (F: 19%), Week 104: ↑ brain (F: 23%), ↑ thyroid (F:43%), ↑ (heart (F: 16%), ↑ adrenals (F: 9%), ↑ thymus (F: 50%)) **-changes in urinalysis** (yellow/brown or orange discoloration (M, F), diuretic animals (M)) **-macroscopical changes** in urinary bladder (orange discoloration of the urinary bladder serosa) (M, F) and skin (orange staining (M, F)) **-histopathological changes** in urinary bladder (benign transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), kidneys (epithelial hyperplasia (M, F), renal papillary degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑ renal focal calcification, ureter	Anonymous 23 (1991) AKJ/7/90 New data for the Annex I renewal: No

		(epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F), pancreas (↑ pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓ mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity)) NOAEL for systemic toxicity (M, F): 4 ppm (corresponds to 0.21 and 0.28 mg/kg bw/day in males and females, respectively) NOAEL for tumour incidence (M, F): 52 ppm (corresponds to 2.82 and 3.65 mg/kg bw/day in males and females, respectively)	
Carcinogenicity study Oral (dietary) No guideline claims in study report Mouse Crl:CD-1 (ICR)BR 50/sex/group *The study is acceptable. It was checked for compliance with OECD TG 451 (adopted 7 September 2009). Following deviations were noted:* *i. the duration of study was 20 months (according to the guideline the duration of the study will normally be 24 months for rodents. Shorter or longer study durations may be used but should be justified).* *ii. cervix, coagulating gland, Hardian gland and lacrimal gland were not included in the histopathological evaluation.* GLP: Yes	ACN technical (Quinoclamine) Purity: 98.57% 0, 3, 30 or 300 ppm (corresponding to averages of 0, 0.38, 3.82 and 40.2 mg/kg bw/day in males and 0, 0.44, 4.48 and 46.4 mg/kg bw/day in females) 80 weeks	3 ppm: -clinical signs (orange fur staining) (M, F) 30 ppm: ↑ **mortality** (M, F) -clinical signs (orange fur staining) (M, F) **-changes in organ weights** (↑ rel kidney, M: 14% n.s.) **-histopathological changes** in adrenal (adrenal spindle cell hyperplasia (F), brown athrophy (F)); stomach (hyperkeratosis and chronic inflammation (F)) 300 ppm: ↑ **mortality** (M, F) -clinical signs (orange fur staining) (M, F) ↓ **bw gain** (M: 33%, F: 30%) **-changes in organ weights** (↑ rel liver (F: 20%), ↑ rel kidney (M: 15% n.s., F: 24% n.s.), ↑ rel heart (F), ↑ brain (F)) **-histopathological changes** in adrenal (↑ adrenal spindle cell hyperplasia (M), brown athropy (F)), kidney (cortical scarring (M, F), hydronephrosis (M, F)), liver (chronic inflammation (F), brown pigmentation (F)), sciatic nerve (degeneration (F)), spleen (haemosiderosis (F), heart (generalised periarteritis (F), myocardial fibrosis (13 M, 2 F)), stomach (hyperkeratosis (M, F), epithelial hyperplasia (M), dilation of mucosal glands (M, F)), urinary bladder (epithelial hyperplasia (particularly F)), urether (dilation (M, F)), lymph nodes (histiocytosis (M, F), lympho reticular tissue malignant lymphoma (F)) NOAEL (M, F): 3 ppm (corresponding to 0.38 and 0.44 mg/kg bw/day for males and females, respectively)	RAR Vol. 3 B.6.5.2/01 Anonymous 24 (1993) New data for the Annex I renewal: No

		LOAEL (M, F): 30 ppm (corresponding to 3.82 and 4.48 mg/kg bw/day in males and females, respectively) NOAEL for tumour incidence (F): 30 ppm (4.48 mg/kg bw/day) NOAEL for tumour incidence (M): 300 ppm (40.2 mg/kg bw/day)	
Teratology study No guideline claimed in study Rat Crl:CD (SD) BR F 24/group GLP: Yes 24/group *The study is acceptable. It was checked for compliance with OECD TG 414 and following deviations were noted:* *i. Exposure time in study was once daily between days 7 and 17 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent) but also effects from preimplantation,* *when appropriate, through the entire period of gestation to the day before caesarean section)* *ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from* *mating to the day prior to scheduled kill)* *iii. The choice of vehicle was not justified in study report* GLP: Yes	ACN technical (Quinoclamine) Purity: 98.1% 0, 5, 20 and 75 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> **-macroscopic changes** (enlarged spleen, one dam) <u>75 mg/kg bw/day:</u> **- bw gain** (25% day 7-17) ↓ FC (Gestation Days 7-10: 25%, Gestation Days 10-13: 14%) **-macroscopic changes** (enlarged spleen, 4/24 dams) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> **-abnormalities** (innominate artery absent, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed) <u>75 mg/kg bw/day:</u> ↓ **foetal weight** (7%) **-abnormalities** (innominate artery absent, four foetuses; situs inversus, two foetuses; interrupt aortic arch, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite; sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned) NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	RAR Vol. 3 B.6.6.2.1/02 Anonymous 25 (1986) Report No.: AKJ/4/86 New data for the Annex I renewal: No
Teratology study No guideline claimed in study Rat Crl:CD (SD) IGSBR F 24/group	Quinoclamine Purity: 99.0% 0, 5, 20, 75 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 6-19	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 14 of gestation) ↓ **bw gain** (Days 7-8: 62%, Days 17-19: 21%) ↓ FC (Days 7-8: 14%, Days 9-12: 17%, Days 12-15: 10%, Days 15-17: 12%,	RAR Vol. 3, B.6.6.2.1/04 Anonymous 26 (2002) Report No.: 619/94-D6154

The study is acceptable. It was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted: *i. Exposure time in study was once daily between days 6 and 19 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent) but also effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section)* *ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *iii. The choice of vehicle was not justified in study report* GLP: Yes		Days 17-19: 12%) ↓ **mean gravid uterus weight** (15%) ↓ **mean litter weight** (13%) <u>75 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 10, nose rubbing) ↓ **bw gain** (Days 17-19: 41%) **-bw loss** (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) ↓ FC (Days 4-6: 9%, Days 6-7: 27%, Days 7-8: 44%, Days 8-9: 34%, Days 9-12: 30%, Days 12-15: 17%, Days 15-17: 13%, Days 17-19: 33%) ↓ **mean gravid uterus weight** (30%) ↑ **post-implantation loss** (11% compared to 5% in control, n.s.) ↑ **number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓ **mean litter size** (12 compared to 14.8 in control) ↓ **mean litter weight** (29%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↓**foetal weight** (7%) ↓**mean litter weight** (13%) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (12%) ↓**litter weight** 29%) ↑**post-implantation loss** (11% compared to 5% in control) ↑pre-implantation loss (17.4% compared to 8.6% in control but within current background data) ↑**number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter size** (12 compared to 14.8 in control) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) **-malformations** (subcutaneous oedema (one foetus), retro-oesophageal aortic arch (one foetus), kidney misshapen (one foetus), hydropnephrosis (three foetuses)) NOAEL maternal: 5 mg/kg bw/day NOAEL developmental: 5 mg/kg bw/day	New data for the Annex I renewal: No

Teratology study No guideline claimed in study Rabbit New Zealand White F 16/group *The study is acceptable. It was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted:* *i. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *ii. During the course of study relative humidity was within the range 54-76% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning)* *iii. The choice of vehicle was not justified in study report* GLP: Yes	ACN (Quinoclamine) Purity: 98.1% 0, 2.5, 7.5, 22.5 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 6-18	<u>Maternal effects:</u> <u>2.5 mg/kg bw/day:</u> No treatment-related effects <u>7.5 mg/kg bw/day:</u> No treatment-related effects <u>22.5 mg/kg bw/day:</u> ↓ bw gain (Day 6-9: 0 kg compared to 0.08 kg in control, Days 0-28: 5%) <u>Developmental effects:</u> <u>2.5 mg/kg bw/day:</u> No treatment related effects <u>7.5 mg/kg bw/day:</u> No treatment-related effects <u>22.5 mg/kg bw/day:</u> ↓ foetal weight (5% n.s.) ↑ **increased incidence of skeletal variants** (increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control)) **-malformations** (scoliosis, one animal, spina-bifida, three animals, anomalies of the aortic arch, two animals, sternebral fusions, three animals, hyperextension of limb or paw, one animal) NOAEL maternal toxicity: 22.5 mg/kg bw/day NOAEL developmental toxicity: 7.5 mg/kg bw/day	RAR Vol. 3, B.6.6.2.2/02 Anonymous 27 (1986) Report No.: AKJ/3/86 New data fpr the Annex I renewal: No
Teratology range finding study No guideline claimed in study Rabbit New Zealand White F 5/group GLP: Yes	ACN (Quinoclamine) Purity: 98.1% 0, 8, 20, 50, 80/8[a], 200/20[a], 500/50[a] Vehicle: 0.25% gum tragacanth Gestation Days 6-18	<u>Maternal effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↑ **post-implantation loss** (31.1 compared to 8.7 in control) <u>50 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓ bw (Day 10: 4%, Day 14: 5%) ↓ FC (Days 6-10: 49%, Days 10-14: 38%) ↑ **post-implantation loss** (61.0 compared to 8.7 in control) <u>80/8 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓ bw (Day 7: 4%, Day 8: 3%, Day 10: 4%) ↓ FC (n.s.) ↑ **post-implantation loss** (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> -clinical signs (coloured urine)	RAR Vol. 3, B.6.6.2.2/01 Anonymous 28 (1986) Report No: AKJ/1/86 New data for the Annex I renewal: No

		↓ bw (Day 7: 6%, Day 10: 6%) ↓ FC (Days 6-10: 36%) ↑ **post-implantation loss** (30.0 compared to 8.7 in control) <u>500/50 mg/kg bw/day:</u> **-mortality** (both animals died, one died on day 9 and the other on day 10 of pregnancy)[b] **-clinical signs** (lethargy, hunched posture, dark coloured urine) ↓ **bw** (Day 8: 12%) ↓ FC (Days 6-10: 80%) <u>Developmental effects:</u> <u>8 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> ↑ **post-implantation loss** (31.1 compared to 8.7 in control) **-malformations** (spina bifida, two animals, interrupted aortic arch major, one animal, hindlimb left malrotated, one animal) <u>50 mg/kg bw/day:</u> ↑ **post-implantation loss** (61.0 compared to 8.7 in control) **-malformations** (interrupted aortic arch major, one animal, kidney left agenesis, one animal) <u>80 mg/kg bw/day:</u> ↑ **post-implantation loss** (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> ↑ **post-implantation loss** (30.0 compared to 8.7 in control) *The study is acceptable as a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL.*	
Teratology study OECD 414 Rabbit Crl.NZW/Kbl BR F 24/group *The study follows OECD TG 414 except for following deviations:*	Quinoclamine Purity: 99.0% 0, 5, 17.5, 30 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 7-28	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day</u> ↓ **bw change** (bw change Days 12-15: 67% of control) ↓ **mean litter size** (8.4 foetuses per female compared to 9.5 in control) <u>30 mg/kg bw/day:</u>	RAR Vol. 3, B.6.6.2.2/04 Anonymous 29 (2002) Report No.: 619/155-D6154 New data for the Annex I renewal: No

i. Dosing of animals started on Day 7 of gestation (the guideline recommends administration to start on Day 6 of gestation) *ii. During the course of study relative humidity was within the range 30-80% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning)* *iii. The choice of vehicle was not justified in study report* GLP: Yes		**-mortality** (one female killed on Day 18 of gestationc) ↓ bw (Days 4-29: 7%) ↓ **bw change** (Days 12-15: 0 kg compared to 0.12 kg in control, Days 4-29: 46% of control) ↓ FC (Days 7-28: 2.4%, Days 28-29: 4%) ↑ **post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑ **early intrauterine deaths** (1.0 compared to 0.2 in control) ↑ **late intrauterine deaths** (1.4 compared to 0.3 in control) ↓ **mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓ **litter weight** (24%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓ **mean litter size** (8.4 foetuses per female compared to 9.5 in control) **-malformations** (hydronephrosis, one animal, increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 5.6% compared to 2.3% in control) <u>30 mg/kg bw/day:</u> ↑ **post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑ **early intrauterine deaths** (1.0 compared to 0.2 in control) ↑ **late intrauterine deaths** (1.4 compared to 0.3 in control) ↓ **mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓ **litter weight** (24%) ↑ **specific foetal variations** (kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, asymmetric ossification of cervical vertebral centra) **- malformations** (hydronephrosis, 2 animals; increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 6.4% compared to 2.3% in control; misshapen nasal bone (8.0%, not present in historial ctr data at time for study); misaligned thoracic vertebral arch, one foetus, increased incidence of absent frontal, mean % foetus: 8.9% compared to 0.0% in control)	

		NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	

M: males
F: females
FC: food consumption
n.s.: not statistically significant
[a]In the male with macroscopically large kidneys there was also a slight increase in basophilic cortical tubules, some of which had increased mitoses compared with the occasional inactive basophilic tubules sometimes seen in controls
[b]The high dose animals were removed from the study on Day 5 due to body weight loss and poor clinical condition

Table 2.6.3.1-2. Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.3.1-3. Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Dermal embryo-foetal development study Rat In house method GLP: Yes	Quinoclamine Purity: 97.7% 5, 100, 600 mg/kg bw/day Vehicle: 1% Tween 80 Day 6 to 15 *post-coitum*	The study was performed to investigate the effects of the test article on the embryonic and fetal development of the rat when administered during the period of organogenesis. Three groups of twenty five sexually mature and mated female Sprague Dawley Crl:CD (SD)BR rats (8-12 weeks old) received Quinoclamine by dermal application at dose levels of 5, 100 and 600 mg/kg bw/day for 10 consecutive days from day 6 to 15 *post-coitum*, inclusive.	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> -clinical signs (coloured urine) -macroscopical changes (reddish discolouration of treated skin) <u>100 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) -macroscopical changes (reddish discolouration of treated skin) <u>600 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) ↓**bw loss** (Days 6-9: -0.41 g) ↓**bw gain** (Days 6-16: 31%) ↓FC -macroscopical changes (reddish discolouration of treated skin) No embryotoxicity or teratogenicity was noted in this study NOAEL maternal: 100 mg/kg bw/day NOAEL teratogenic effects: 600 mg/kg bw/day *The study is acceptable as supplementary data only. The test substance was administered dermally instead of orally. The*	RAR Vol. 3, B.6.8.2/01 Anonymous 30 (1996) Report No.: 1312-1416-001 New data for the Annex I renewal: No

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			choice of administration route was not justified.	

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Short-term oral toxicity was tested in rats and dogs, while short-term dermal toxicity was tested in rats. No repeated dose toxicity study performed with quinoclamine in animals by the inhalation route is available. A subchronic toxicity study conducted on quinoclamine by the inhalation route is not considered necessary since quinoclamine is not a volatile substance. It could however be noted that the acute toxicity by the inhalation route is not fully investigated.

Rat:

28-day oral toxicity study in the Crl:CD®(SD)IGSBR rat:

In the 28-day oral toxicity study in the Crl:CD®(SD)IGSBR rat, treatment was associated with reduced bodyweight gain noted in males at ≥500 ppm (≥44 mg/kg bw/day) and in females at 500 ppm (48 mg/kg bw/day) (n.s.) and 1000 ppm (90 mg/kg bw/day) (s.s), reduced food consumption noted in males at 1000 ppm (84 mg/kg bw/day) and in females at ≥500 ppm, changes in haematological parameters noted in both sexes at ≥500 ppm, changes in biochemical parameters (indicating liver toxicity) noted in males at ≥500 ppm and in females at 1000 ppm, changes in urine analysis parameters (M: red-, brown- or dark coloured urine, increases in amorphous debris, increased urine volume; F: red-, brown- or dark coloured urine, increases in amorphous debris) noted at ≥500 ppm, reduced thymus weights noted in both sexes at 500 ppm, macroscopically changes (large kidney, one male) and histopathological changes in the kidneys noted in males at ≥500 ppm (At 500 ppm: eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium; At 1000 ppm: eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium, minor papillitis in two males and in one of these animals basophilic cortical tubules).

Changes in haematological parameters consisted of following statistically significant changes: reduced haemoglobin noted in males at 500 (10%) and 1000 ppm (8%) and in females at 1000 ppm (13%), reduced red blood cell count noted in males at 500 (8%) and 1000 ppm (12%) and in females at 1000 ppm (13%), reduced packed cell volume noted in males at 500 ppm (9%) and in females at 1000 ppm (11%), increased reticulocytes noted in males at 500 (95%) and 1000 ppm (170%) and in females at 1000 ppm (238%), increased red cell distribution width noted in males at 500 (30%) and 1000 ppm (37%) and in females at 500 (13%) and 1000 ppm (47%), increased haemoglobin distribution width noted in males at 500 (35%) and 1000 ppm (20%) and in females at 500 (17%) and 1000 ppm (25%), increased mean platelet volume noted in males at 1000 ppm (15%), increased platelet distribution width noted in males at 1000 ppm (24%), increased prothrombin time noted in males at

500 (60%) and 1000 ppm (85%), increased activated partial thromboplastin time noted in males at 1000 ppm (36%), increased plateletcrit noted in males at 500 ppm (38%) and in females at 1000 ppm (32%), and increased platelet noted in females at 1000 ppm (34%).
The decreased haemoglobin (up to 13%), red coloured urine (which might indicate presence of haemoglobin in the urine), presence of amorphous debris in urine, might indicate that quinoclamine causes haemolytic anaemia at high dose levels.
The NOAEL for both sexes was set at 50 ppm (equivalent to 4.7 and 5.3 mg/kg bw/day in males and females, respectively) based on reduced bodyweight noted in males at 1000 ppm and in females at ≥500 ppm, changes in haematological parameters (indicating haemolytic anaemia) noted in both sexes at ≥500 ppm, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 1000 ppm, changes in urine parameters noted in both sexes at ≥500 ppm, reduced thymus weight noted in both sexes at ≥500 ppm, enlarged kidney noted in one male at 1000 ppm, and histopathological changes in the kidneys noted in males at ≥500 ppm (RAR Vol. 3, B.6.3.1.1/01).

<u>90-day oral toxicity study in the Sprague-Dawley rat:</u>
In the 90-day oral toxicity study in the Sprague-Dawley rat, treatment was associated with reduced bodyweight gain noted in males at 200 ppm (14 mg/kg bw/day) (6%) and in females at 1000 ppm (65 mg/kg bw/day) (7%), reduced food consumption and water consumption noted in males at 1000 ppm (62 mg/kg bw/day) and in females at ≥50 ppm (≥3 mg/kg bw/day), changes in biochemical parameters (reduced serum A/G ratios noted in males at ≥50 ppm (≥3 mg/kg bw/day) and in females only at the lowest dose (50 ppm), increased GOT (n.s.) noted in males at 1000 ppm), changes in organ weights noted in males at 200 ppm (increased relative right kidney, increased relative left adrenal, increased relative mandibular gland) and in both sexes at 1000 ppm (males: increased absolute and relative spleen, increased absolute and relative liver, increased absolute and relative kidney, increased relative left adrenal, increased relative mandibular gland; females: increased absolute and relative spleen, increased relative liver, increased relative kidney) and histopathological changes noted in the kidneys (increased incidence of hyaline droplets in kidney cortical tubules noted in males at ≥200 ppm), liver (bile duct proliferation noted in both sexes at 1000 ppm), spleen (increased incidence of hemosiderin deposition noted in both sexes at ≥200 ppm (≥62/65 mg/kg bw/day in males and females, respectively). Increase of haemosiderin in the reticuloendothelial system is usually associated with erythrocyte destruction resulting in abnormalities normally detected in haematological examinations. In this study the haematological examinations were limited.
The NOAEL in males was set at 50 ppm (3 mg/kg bw/day) based on effects on the kidneys (increased organ weight, histopathological changes) noted at ≥200 ppm (≥14 mg/kg bw/day), effects on the liver (reduced serum A/G, increased organ weight, histopathological changes) noted at 1000 ppm (62 mg/kg bw/day), and effects on the spleen noted at 200 ppm (histopathological changes) and 1000 ppm (increased organ weight, histopathological changes). The NOAEL in females was set at 50 ppm (3 mg/kg bw/day) based on effects on the spleen noted at 200 ppm (13 mg/kg bw/day) (histopathological changes) and 1000 ppm (65 mg/kg bw/day) (increased organ weight, histopathological changes), effects on the liver noted at 1000 ppm (histopathological changes, increased organ weight), and effects on the kidney noted at 1000 ppm (increased organ weight) (RAR Vol. 3, B.6.3.2.1/01).

90-day oral toxicity study in the Crl:CD(SD)IGSBR rat:

In the 90-day oral toxicity study in the Crl:CD (SD)IGSBR rat, treatment was associated with reduced bodyweight gain noted in males at 800 ppm (56.74 mg/kg bw/day) and in all treated females, reduced food consumption noted in males at 800 ppm and in females at ≥200 ppm (≥17.81 mg/kg bw/day), changes in haematological parameters noted in both sexes at ≥200 ppm (≥13.89/17.81 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in males at ≥50 ppm (≥3.61 mg/kg bw/day) (indicating liver toxicity), changes in urinalysis parameters (dark straw coloured urine) noted in both sexes at ≥200 ppm, changes in organ weights (At 800 ppm (56.74/74.81 mg/kg bw/day in males and females, respectively): increased spleen and liver weights in both sexes, increased brain weights in males; At 200 ppm: increased spleen weight in males, increased liver weight in females, reduced thymus weight in females), histopathological changes in the spleen noted in males at 800 ppm (increased incidence of congestion, increase in the extent of haemopoiesis) and in females at 200 ppm (increase in the extent of pigment) and 800 ppm (increased incidence of congestion, textent of pigment), histopathological changes in the kidneys noted in males at 200 ppm (increase in the extent of hyaline droplets) and 800 ppm (increase in the extent of hyaline droplets, focal nephropathy, papillary interstitial eosinophilia) and in females at 800 ppm (increased incidence of pigment, focal nephropathy), histopathological changes in the liver noted in both sexes at ≥200 ppm (sinusoidal cell pigment), and changes in thymus (minor thymus atrophy) noted in both sexes at ≥200 ppm.

Changes in haematological parameters consisted of following statistically significant changes: increased haemoglobin distribution width noted in both sexes at 200 (M: 20%, F: 32%) and 800 ppm (M: 14%, F: 24%), increased reticulocytes noted in both sexes at 200 (M: 18%, F: 82%) and 800 ppm (M: 42%, F: 135%), increased absolute reticulocytes noted in both sexes at 800 ppm (M: 59%; F: 95%) and in females at 200 (38%), reduced red blood cell count noted in both sexes at 200 (M: 5%; F: 4%) and 800 ppm (M: 13%; F: 15%), reduced packed cell volume noted in both sexes at 800 ppm (M: 11%; F: 11%) and in females at 200 ppm (5%), reduced haemoglobin noted in both sexes at 800 ppm (M: 10%; F: 11%) and in females at 200 ppm (4%), increased activated partial thromboplastin time noted in males at 200 (21%) and 800 ppm (22%), increased red cell distribution width noted in both sexes at 800 ppm (M: 16%; F: 11%), increased mean cell volume noted in females at 800 ppm (5%), reduced platelet distribution width noted in males at 800 ppm (10%), reduced mean cell haemoglobin noted in females at 800 ppm (6%).

No NOAEL was established for females since adverse effects on bodyweight gain were noted at the lowest dose level of 50 ppm (3.61/4.56 mg/kg bw/day in males and females, respectively) (bodyweight gain reduced 17%).

The NOAEL for males was set at 50 ppm (3.61 mg/kg bw/day) based on reduced bodyweight gain noted in males at 800 ppm (56.74 mg/kg bw/day), changes in haematological parameters noted in males at ≥200 ppm (≥13.89 mg/kg bw/day), changes in biochemical parameters (indicating liver toxicity) noted in males at ≥200 ppm, changes in urinalysis parameters (dark straw coloured urine) noted in males at ≥200 ppm, increased organ weights (spleen, liver) noted in males at ≥200 ppm, macroscopic changes in the spleen (enlarged spleen) noted in males at 800 ppm, and histopathological changes noted in males at 800 (spleen and thymus) and ≥200 ppm (kidney and liver) (RAR Vol. 3, B.6.3.2.1/02).

28-day dermal toxicity study in the Crl:CD®(SD)IGSBR rat:

In the 28-day dermal toxicity study in the Crl:CD®(SD)IGSBR rat, treatment was associated with clinical signs of skin irritation (erythema and desquamation) noted in both sexes at ≥100 mg/kg bw/day, changes in biochemical parameters noted in both sexes at ≥300 mg/kg bw/day (reduced aspartate aminotransferase and alkaline phosphatase noted in females and increased total bilirubin and glucose noted in males), dark coloured urine noted in both sexes at 1000 mg/kg bw/day, macroscopic changes (sores at the treated site) noted in females at ≥100 mg/kg bw/day and in males at 1000 mg/kg bw/day, and histopathological changes in the skin and kidney noted in both sexes at 1000 mg/kg bw/day. The histopathological changes in the skin consisted of increased incidence of epidermatitis noted in both sexes at 1000 mg/kg bw/day, while the histopathological changes in the kidney consisted of tubular degeneration/regeneration in the kidney cortex noted in one high dose female and in one high dose male, and hydronephrosis and pigment noted in one female.
NOAEL for systemic effects was set at 300 mg/kg bw/day based on histopathological changes in the kidneys noted in both sexes at 1000 mg/kg bw/day. No NOAEL was set for local effects due to skin irritation noted in all treated groups (RAR Vol. 3, B.6.3.4.1/01).

2-year combined chronic toxicity and carcinogenicity study in the rat:
In the 2-year feeding study in the Crl:CD(SD)BR rat, treatment was associated with clinical signs (orange fur staining and reduced incidence of mass bearing) noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), reduced bodyweight gain noted in females at 676 ppm (carcinogenicity evaluation: 27%; toxicology evaluation: 28%), reduced food consumption noted in both sexes at 676 ppm, changes in haematological parameters (reduced packed blood cell volume, haemoglobin concentration and red blood cell count) noted in both sexes at 676 ppm, changes in biochemical parameters noted at 676 ppm (elevated blood urea nitrogen levels noted in males (n.s) and females (n.s), reduced calcium noted in males (s.s.) and females (n.s.), reduced inorganic phosphorous noted in males (n.s.) and females (s.s.), reduced lactate dehydrogenase noted in both sexes), findings in urinalysis such as yellow/brown or orange discoloration noted in both sexes in all treated groups and diuretic males noted at 676 ppm, changes in organ weights (increased relative kidney weights noted in males at ≥52 ppm (≥2.82 mg/kg bw/day), and in females at 676 ppm; increased relative spleen weights noted in females at 676 ppm; increased relative thymus weight noted in females at 676 ppm; increased relative thyroid weight noted in females at 676 ppm; increased relative heart weight noted in females at 676 ppm; increased relative adrenal weights noted in females at 676 ppm, increased relative brain weights noted in females at 676 ppm), gross pathology findings in the urinary bladder (orange discoloration) and skin (orange staining) noted in both sexes at 676 ppm, and histopathological changes noted in the urinary bladder (both sexes, ≥52 ppm corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively), kidneys (both sexes at ≥52 ppm), ureter (both sexes at 676 ppm), urethra (both sexes at 676 ppm), adrenals (both sexes at 676 ppm), pancreas (both sexes at 676 ppm), parathyroid (males at 676 ppm), mammary gland (females at 676 ppm) and lungs (males at ≥52 ppm and females at 676 ppm).
Neoplastic changes were noted in the urinary bladder (benign transitional cell papilloma noted in males at ≥52 ppm and in females at 676 ppm) and adrenals (increased incidence of benign phaeochromocytoma noted in both sexes at 676 ppm). The benign transitional cell papilloma noted in one single male at 52 ppm was considered

of no clear relevance. No other differences in tumour incidence or type were seen which were considered to be dependent on the dose levels of ACN-technical.

Non-neoplastic changes in the urinary bladder consisted of: epithelial hyperplasia (both sexes at ≥52 ppm, corresponding to ≥2.82 and ≥3.65 mg/kg bw/day in males and females, respectively), polyp noted in one female at 676 ppm (38.3 mg/kg bw/day) and chronic inflammation noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively). Non-neoplastic changes in the kidneys consisted of: epithelial hyperplasia (both sexes at ≥52 ppm), renal papillary degeneration/necrosis (both sexes at 676 ppm), increased incidence of renal cortical scarring (both sexes at 676 ppm), pelvis polyp noted in one male at 676 ppm and increased incidence of renal focal calcification (in females at ≥52 ppm). Epithelial hyperplasia was also noted in the ureters (both sexes at ≥52 ppm) and urethra (both sexes at 676 ppm), and in the parathyroid (males at 676 ppm). Non-neoplastic changes in pancreas consisted of increased incidence of pancreatic acinar atrophy (both sexes at 676 ppm). The histopathological changes in the mammary gland consisted of decreased incidence of mammary acinar development and secretion (females at 676 ppm). Increased incidence of arterial calcification was noted in the lungs (males at ≥52 ppm, females at 676 ppm).

Group 1-4 (carcinogenicity groups):

Non-neoplastic changes:

The following non-neoplastic changes were considered to be related to the dietary administration of ACN-technical:

-Hyperplasia of the urinary epithelium in the urinary bladder, kidneys and ureters at the 676 ppm and 52 ppm dose levels, and in the urethra at the 676 ppm dose level only in both males and females

Non-neoplastic pathology-urinary bladder (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	49	47	47	49	48	49	50
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
Cystitis/inflammation	1	2	1	3	0	0	0	2
Haemorrhage	0	0	0	1	0	0	0	2
Terminal kill: number examined	27	27	20	30	26	29	30	38
Epithelial hyperplasia	0	0	0	28	1	1	0	35
Squamous metaplasia	0	0	0	0	0	0	0	2
Polyp	0	0	0	0	0	0	0	1
Cystitis/inflammation	0	0	0	0	0	0	0	2
Haemorrhage	0	0	0	0	0	0	0	2
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Epithelial hyperplasia	2	2	4	5	0	0	6	9
Squamous metaplasia	0	0	0	0	0	0	0	1
Polyp	0	0	0	0	0	0	0	0
Cystitis/inflammation	0	2	0	1	0	0	0	0
Haemorrhage	0	0	0	0	0	0	0	0
Died: number examined	6	4	6	11	4	3	0	2
Epithelial hyperplasia	1	0	1	8	0	0	0	2
Squamous metaplasia	0	0	0	0	0	0	0	0
Polyp	0	0	0	0	0	0	0	0

Cystitis/inflammation	1	0	1	2	0	0	0	0
Haemorrhage	0	0	0	1	0	0	0	0

Non-neoplastic pathology-kidneys (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	49	48	48	50	50	50	50
Epithelial 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
Papilla haemorrhage/haemorrhage	2	0	1	10	0	5	1	6
Paiplla focal calcification	0	0	0	3	0	0	0	0
Cortical scar	3	3	2	11	0	0	1	6
Focal calcification	3	2	6	5	24	24	32	36
Terminal kill: number examined	27	27	20	30	26	29	30	38
Epithelial hyperplasia	1	4	6	25	0	0	3	29
Papillary necrosis	0	1	0	6	0	0	0	2
Papillary focal necrosis	0	2	0	10	0	0	0	0
Papilla haemorrhage/haemorrhage	0	0	1	9	0	4	1	6
Papilla focal calcification	0	0	0	3	0	0	0	0
Cortical scar	3	3	1	8	0	0	0	5
Focal calcification	1	1	4	2	16	13	21	30
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Epithelial hyperplasia	1	1	4	6	1	0	7	4
Papillary necrosis	0	0	0	1	0	0	0	1
Papillary focal necrosis	0	0	1	1	0	0	0	0
Papilla haemorrhage/haemorrhage	1	0	0	0	0	0	0	0
Papilla focal calcification	0	0	0	0	0	0	0	0
Cortical scar	0	0	1	1	0	0	1	1
Focal calcification	2	1	1	2	5	8	10	4
Died: Number examined	6	4	7	12	5	5	1	2
Epithelial hyperplasia	0	0	2	8	1	0	0	1
Papillary necrosis	0	0	0	2	0	0	0	0
Papillary focal necrosis	0	0	0	1	0	0	0	0
Cortical scar	0	0	0	2	0	0	0	0
Focal calcification	0	0	1	1	3	3	1	2

Non-neoplastic pathology-ureters and urethra (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Ureters								
Total: number examined	47	21	26	38	48	20	21	47
Epithelial hyperplasia	0	1	2	19	0	0	5	21
Haemorrhage	0	0	0	1	0	0	0	1
Metaplasia/keratin	0	0	0	0	0	0	0	1
Terminal kill: number examined	26	3	0	26	26	1	2	35
Epithelial hyperplasia	0	0	0	13	0	0	1	15
Haemorrhage	0	0	0	0	0	0	0	1
Metaplasia/keratin	0	0	0	0	0	0	0	1
Killed in extremis: number examined	16	15	21	6	18	15	18	10
Epithelial hyperplasia	0	1	1	3	0	0	4	5
Haemmorrhage	0	0	0	0	0	0	0	0
Metaplasia/keratin	0	0	0	0	0	0	0	0
Died: Number examined	5	3	5	6	4	4	1	2
Epithelial hyperplasia	0	0	1	3	0	0	0	1
Hamorrhage	0	0	0	1	0	0	0	0
Metaplasia/keratin	0	0	0	0	0	0	0	0
Urethra								
Total: number examined	44	33	35	41	42	18	13	36
Epithelial hyperplasia	1	0	0	4	1	1	0	6
Terminal kill: number examined	23	16	11	27	21	0	1	27
Epithelial hyperplasia	0	0	0	1	1	0	0	2

Killed in extremis: number examined	15	13	18	6	16	13	11	7
Epithelial hyperplasia	1	0	0	3	0	1	0	4
Died: number examined	6	4	6	8	5	5	1	2
Epithelial hyperplasia	0	0	0	0	0	0	0	0

The polyp seen in the urinary bladder of one high dose terminal kill female probably developed as a response to the irritant toxic effect of the test compound on the urothelium, according to study author.

-Renal papillary necrosis and an increased incidence of renal cortical scarring in the kidneys at the 676 ppm dose level only in males and females. The severity of the papillary necrosis which was often accompanied by haemorrhage in this carcinogenicity study was greater than that seen at any stage of the toxicity study and this lesion was considered to have been the cause of death or predominant pathology in six terminal kill and three decedent high dose animals.

-An increased incidence and severity of pancreatic acinar atrophy at the 676 ppm dose level in males and females

Non-neoplastic pathology- pancreas (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	28	29	46	48	20	23	50
Acinar atrophy- minimal	14	12	12	15	10	3	8	19
Acinar atrophy-moderate	3	1	4	14	0	0	0	9
Acinar atrophy- marked	0	0	1	5	0	0	1	7
Acinar atrophy- total	17	13	17	34	10	3	9	35
Fatty infiltration	11	6	3	14	6	1	3	10
Chronic inflammation	3	0	0	2	2	1	2	7
Terminal kill: number examined	27	6	1	30	25	1	3	38
Acinar atrophy- minimal	10	0	0	9	4	0	2	15
Acinar atrophy- moderate	1	0	0	11	0	0	0	6
Acinar atrophy- marked	0	0	0	5	0	0	0	7
Acinar atrophy-total	11	0	0	25	4	0	2	28
Fatty infiltration	5	0	0	10	3	0	1	8
Chronic inflammation	2	0	0	0	0	0	0	5
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Acinar atrophy- minimal	3	12	11	2	4	3	6	3
Acinar atrophy- moderate	1	1	4	2	0	0	0	2
Acinar atrophy- marked	0	0	0	0	0	0	1	0
Acinar atrophy- total	4	13	15	4	4	3	7	5
Fatty infiltration	5	6	3	3	3	1	2	2
Chronic inflammation	0	0	0	0	2	1	2	2
Died: number examined	6	4	7	10	4	3	1	2
Acinar atrophy- minimal	1	0	1	4	2	0	0	1
Acinar atrophy – moderate	1	0	0	1	0	0	0	1
Acinar atrophy- marked	0	0	1	0	0	0	0	0
Acinar atrophy- total	2	0	2	5	2	0	0	2
Fatty infiltration	1	0	0	1	0	0	0	0
Chronic inflammation	1	0	0	2	0	0	0	0

-A decrease in the incidence of mammary acinar development and secretion in 676 ppm females only. This effect was probably related to the reduced food consumption and the lower bodyweight in these high dose animals, according to the study author.

Non-neoplastic pathology- Mammary gland (groups 1-4 females)

	Females			
	0	**4**	**52**	**676**
Mammary gland (cranial)				
Total: number examined	50	22	22	50
Acinar development	25	13	12	15
Secretion	15	7	7	7
Terminal kill: number examined	26	1	2	38
Acinar development	13	0	1	12
Secretion	8	0	0	4
Killed in extremis: number examined	19	16	19	10
Acinar development	8	8	10	2
Secretion	6	3	6	2
Died: number examined	5	5	1	2
Acinar development	4	5	1	1
Secretion	1	4	1	1
Mammary gland (caudal)				
Total: number examined	50	23	22	50
Acinar development	32	16	18	28
Secretion	21	11	13	15
Terminal kill: number examined	26	2	2	38
Acinar development	18	0	2	22
Secretion	11	0	2	10
Killed in extremis: number examined	19	16	19	10
Acinar development	9	11	16	5
Secretion	7	6	11	4
Died: number examined	5	5	1	2
Acinar development	5	5	0	1
Secretion	3	5	0	1

-A decrease in the incidence of skin and tail scab formation in 676 ppm males, only. These effects might be related to reduced food consumption and lower body weight gain according to the study author.

Non-neoplastic pathology-skin and tail lesions (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	50	50	50	50	50	50	50
Scab formation (all sites)	18	21	17	6	5	8	6	3
Tail abcesses	21	17	15	7	10	6	6	1
Terminal kill: examined	27	27	20	30	26	29	30	38
Scab formation (all sites)	7	15	7	5	3	6	1	2
Tail abcesses	11	9	4	4	8	3	2	1
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Scab formation (all sites)	9	5	10	0	2	1	4	1
Tail abcessesAcinar atrophy- minimal	9	6	9	2	1	3	4	0
Died: number examined	6	5	9	14	5	5	1	2
Scab formation (all sites9	2	1	0	1	0	1	1	0
Tail abcesses	1	2	2	1	1	0	0	0

-A decrease in the incidence of tail abscess formation in 676 ppm males and females. This effect might be related to reduced food consumption and lower body weight gain according to the study author.

-A small increase in the incidence of arterial calcification in the lungs of males and females from the 676 ppm group and males from the 52 ppm dose group, and renal focal calcification in females from the 676 ppm and

52 ppm dose groups. According to study author it is possible that these effects together with a related small increase in the incidence of parathyroid hyperplasia in terminal kill and decedent high dose males were indicative of alterations in blood calcium levels due to interference in the renal regulation of phosphorus and calcium ions. It is possible that the treatment-related kidney lesions could have affected these excretory mechanism.

Non-neoplastic pathology- lungs (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	50	49	48	50	50	50	50
Arterial calcification	6	7	16	18	7	8	9	12
Terminal kill: number examined	27	27	20	30	26	29	30	38
Arterial calcification	1	2	7	10	0	5	4	9
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Arterial calcification	3	4	8	2	6	3	5	2
Died: number examined	6	5	8	12	5	5	1	2
Arterial calcification	2	1	1	6	1	0	0	1

-A small increase in the incidence of parathyroid hyperplasia in both terminal kill and decedent high dose males

Non-neoplastic pathology- parathyroids (groups 1-4)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	42	20	22	30	45	14	20	42
Hyperplasia	0	2	0	5	0	0	0	1
Terminal kill: number examined	23	1	0	19	25	1	2	32
Hyperplasia	0	0	0	3	0	0	0	0
Killed in extremis: number examined	15	15	17	5	17	11	18	8
Hyperplasia	0	1	0	1	0	0	0	1
Died: number examined	4	4	5	6	3	2	0	2
Hyperplasia	0	1	0	1	0	0	0	0

<u>Group 5-8 (toxicology groups):</u>

<u>Non-neoplastic changes:</u>

<u>Week 26 to 78 interim kill:</u> Treatment related histopathological changes were initially confined to urinary bladders of high dose (676 ppm) animals (week 26) and comprised epithelial hyperplasia and chronic inflammatory changes.

Non-neoplastic pathology Week 26 interim kill: Urinary bladder (groups 5-8)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	10	9	10	10	10	10	10	10
Epithelial hyperplasia	0	0	1	5	0	1	2	5
Chronic inflammation	0	1	0	4	0	1	0	0
Cystitis with focal hyperplasia	0	0	0	0	1	0	0	0
Eosinophilic plug	0	2	2	1	0	0	0	0

As the study progressed similar lesions appeared in occasional intermediate dose animals and were more widespread, affecting the ureters (epithelial hyperplasia seen in 3/7 high dose males and 4/8 females), urethras (epithelial hyperplasia seen in 1/6 high dose males) and kidneys as well as urinary bladders at the week 78 interim

kill. At week 52 cortical scars in the kidneys were seen in two out of ten high dose males. Changes seen in the kidneys noted at 78 interim kill showed a clear treatment and dose-related incidence included minimal and focal hyperplasia of the renal papillary and pelvic epithelium, minimal degeneration or necrosis in the papillae, a necrosis was confined to the tip of the papilla. The polyp present in the renal pelvis of a high dose male consisted of fibro-fatty tissue covered by hyperplastic epithelium. The hyperplasia of the pelvic epithelium seen in one control male was associated with the presence of calculi (stones).

Terminal (104 week) kill:

Urinary bladder: Epithelial hyperplasia was seen only in treated animals and was characterised by a generalised increase in the number of layers of cells in the urinary epithelium. The normal epithelium consisted of two to three layers of cells, minimal hyperplasia by five to eight layers of cells and marked hyperplasia by more than eight layers of cells. In several animals two degrees of hyperplasia were seen with focal area of moderate hyperplasia associated with diffuse minimal hyperplasia. Squamous metaplasia of the urinary epithelium was associated with epithelial hyperplasia in two high dose females. Epithelial haemorrhage was noted in two male and one female high dose animals. Acute inflammation was seen in association with a bladder tumour in an intermediate dose male.

Non-neoplastic pathology Terminal (104 week) kill: Urinary bladder (groups 5-8)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	9	10	9	5	11	9	10	15
Epithelial hyperplasia- total	0	0	2	5	1	0	0	15
Squamous metaplasia	0	0	0	0	0	0	0	2
Haemorrhage	0	0	0	2	0	0	0	1
Cystitis/acute inflammation	0	0	1	0	0	0	0	0
Chronic inflammation	0	0	1	0	1	0	1	0
Eosinophilic plug	0	0	1	0	0	0	0	0

Kidneys: Varying degrees of simple epithelial hyperplasia involving the renal pelvis and papilla, squamous metaplasia of the pelvic epithelium and cortical scars showed a treatment related increase in high dose animals

Non-neoplastic pathology Terminal (104 week) kill: Kidneys (groups 5-8)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Number examined	9	10	9	5	11	9	10	15
Epithelial hyperplasia- papilla/pelvis	1	0	4	4	0	0	1	8
Squamous metaplasia	0	0	0	0	0	0	0	1
Papillary degeneration/necrosis	0	1	0	0	0	0	0	0
Cortical scars	1	1	2	1	0	1	0	3

Ureters: A clear increase in the incidence of epithelial hyperplasia was seen in high dose group animals. Squamous metaplasia was seen in one high dose female. Acute inflammation and haemorrhage were seen in two separate high dose females

Non-neoplastic pathology Terminal (104 week) kill: Ureters (groups 5-8)

	Males	**Females**

	0	4	52	676	0	4	52	676
Number examined	7	10	8	5	11	8	9	11
Epithelial hyperplasia	0	0	1	3	1	0	1	5
Squamous metaplasia	0	0	0	0	0	0	0	1
Acute inflammation	0	0	0	0	0	0	0	1
Haemorrhage	0	0	0	0	0	0	0	1

Urethra: There was an increased incidence of epithelial hyperplasia in high dose females

Non-neoplastic pathology Terminal (104 week) kill: Urethra (groups 5-8)

	Males				Females			
	0	4	52	676	0	4	52	676
Number examined	7	10	9	4	8	7	9	11
Epithelial hyperplasia	0	0	1	0	1	1	0	3

Pancreas: Varying degrees of acinar atrophy were seen in a proportion of animals from both control and treatment groups. Both the incidence and severity of this change were increased in both sexes of the high dose group compared with the controls

Non-neoplastic pathology Terminal (104 week) kill: Pancreas (groups 5-8)

	Males				Females			
	0	4	52	676	0	4	52	676
Killed in extremis-Number examined	9	10	12	11	7	8	7	3
Acinar atrophy-minimal	1	1	2	4	0	1	3	1
Acinar atrophy-moderate	1	1	2	2	0	0	0	1
Acinar atrophy-marked	0	0	0	1	0	0	0	0
Acinar atrophy-total	2	2	4	7	0	1	3	2
Died: number examined	3	3	5	7	4	3	4	2
Acinar atrophy-minimal	0	0	1	3	0	0	0	1
Acinar atrophy-moderate	0	0	0	0	0	0	0	0
Acinar atrophy-marked	0	0	0	0	0	0	0	0
Total	0	0	1	3	0	0	0	1

Mammary Glands: Varying degrees of acinar development and secretion were seen in the mammary tissue of a proportion of females from both control and treatment groups. There was a decrease in the incidence of these changes in high dose animals compared with controls which was most clearly seen in the cranial mammary gland

Non-neoplastic pathology Terminal (104 week) kill: Mammary Glands (groups 5-8)

	Females			
	0	4	52	676
Caudal mammary gland				
Number examined	11	8	10	15
Acinar development	7	6	7	7
Secretion	3	6	3	0
Cranial mammary gland				
Number examined	11	8	10	15
Acinar development	8	4	4	2
Secretion	5	4	3	1

NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm (38.3 mg/kg bw/day), changes in

haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm.
NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm (RAR Vol. 3, B.6.5.1/01)

Two generation reproductive toxicity study:
The study is no GLP study and considered limited due to several deviations from the OECD TG 416.
In the study, groups of 25 male and 25 female Sprague-Dawley rats received K-1616 (quinoclamine) in the diet at dose level up to 500 ppm (corresponding to 30.9 and 37.7 mg/kg bw/day in F0 males and females, respectively, and 37.0 and 43.8 mg/kg bw/day in F1 males and females, respectively) through two successive generations.
Treatment with the test substance did not affect mating performance or fertility of the male and female parental animals and no consistent differences from control values were noted in comparisons of parental food consumption, survival rates and parturition indices or postnatal and postweaning survival. In addition, evaluations of the data obtained from foetuses taken by caesarean section did not reveal any findings indication teratogenic effects of the test substance at any of these concentrations.
Differences from control group data noted at the high dose level (500 ppm) included lower growth period mean body weight values in the P1 (4% at week 13) and P2 (10% at week 9) generation males and P2 generation females (10% at week 9), reduced bodyweight gain in P1 (7%) and P2 (11%) generation males and P2 (9%) generation females, lower mean offspring weights at weaning in all filial generations (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively), an increase in the observations of hunched appearance during the growth periods of both parental generations, and an increased incidence of gray lung cysts and orange-stained fur noted in the F2b offspring at necropsy. Mean litter size in F2a and F2b generations were also reduced at this dose level.
Differences noted to a lesser degree at the mid dose level (25 ppm) included slightly lower mean body weight values in the P1 (1% at week 13) and P2 (7% at week 9) generation males and P2 (5% at week 9) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (2%) and P2 (11%) generation males and P2 (6%) generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of gray lung cysts in the F2b offspring at necropsy.
Differences noted to a lesser degree at the low dose level (1 ppm) included slightly lower mean body weight values in the P1 (3%) and P2 (7%) generation males and P2 (4%) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (4%) and P2 (11%) generation males and P2 (4%)

generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of lung cysts in the F2b offspring at necropsy.
Increased incidence of gray lung cysts was noted in the F2b offspring reared for three months (at 1 ppm: 8 compared to 11 in control group; at 25 ppm: 29 compared to 11 in control group; at 500 ppm: 39 compared to control group). The findings of gray lung cysts in the F2b offspring was dose related although the relevance of this finding is not clear. The finding was not observed in the offspring of the F1b generation reared for 5 weeks or in other available toxicological studies on quinoclamine. Thus, it seems to be a finding occurring in adult F2b animals including control animals. In the high dose group the incidence of gray cysts was 3.5 times higher when compared to controls, and therefore considered adverse. In the low and mid-dose groups the incidences were less marked (1.6 to 2.6 times higher when compared to controls) and not considered adverse in the absence of other effects in the offsprings at these dose levels.
The NOAEL for parental animals was set at 25 ppm (1.6 mg/kg bw/day) based on clinical signs (hunched posture) noted in P1 and P2 generation animals at 500 ppm (37 mg/kg bw/day), reduced body weight noted in P2 males and females at 500 ppm, and reduced bodyweight gain noted in P2 males at 500 ppm. The NOAEL for offsprings was set at 25 ppm (1.6 mg/kg bw/day) based on reduced body weights at weaning in all filial generations noted at 500 ppm (37 mg/kg bw/day) and gray lung cysts noted in P2 offspring reared for 3 months at 500 ppm. The NOAEL for reproductive toxicity was set at 500 ppm(37 mg/kg bw/day) (RAR Vol. 3. B.6.6.1/01).

Teratology study in the rat:
Female CD rats of Sprague-Dawley origin (CD(SD)BR) were administered quinoclamine orally by gavage at doses up to 75 mg/kg bw/day. Treatment was associated with reduced bodyweight gain (25%) and food consumption noted in dams at 75 mg/kg bw/day, changes in gross pathology (enlarged spleen) noted in dams at ≥20 mg/kg bw/day, reduced foetal weight (7%) noted at 75 mg/kg bw/day and increased incidence of aortic abnormalities and skeletal variations noted at ≥20 mg/kg bw/day. The increased incidence of aortic abnormalities included innominate artery absent noted at ≥20 mg/kg bw/day and situs inversus and interrupt aortic arch noted at 75 mg/kg bw/day. The increased incidence of skeletal variants included effects on skull (hyoid not ossified) and vertebrae (thoracic centre one or more bilobed) noted at ≥20 mg/kg bw/day and effects on sternebrae (5th and 6th not ossified; one or more bilobed, bipartite or misaligned) noted at 75 mg/kg bw/day. The NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain (25%) noted in dams at 75 mg/kg bw/day and changes in gross pathology (enlarged spleen) noted at ≥20 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight (7%) noted at 75 mg/kg bw/day and increased incidence of aortic abnormalities and skeletal variations noted at ≥20 mg/kg bw/day (RAR Vol. 3, B.6.6.2.1/02).

Teratology study in the rat:
Female Crl:CD(SD)IGSBR rats were administered quinoclamine orally by gavage at doses up to 75 mg/kg bw/day. Treatment was associated with maternal clinical signs noted in dams at 20 mg/kg bw/day (paddling of the forelimbs) and 75 mg/kg bw/day (paddling of the forelimbs and nose rubbing), reduced maternal bodyweight gain noted in dams at 20 mg/kg bw/day (Days 7-8: 62%, Days 17-19: 21%) and 75 mg/kg bw/day (Days 17-19: 41%),

maternal bodyweight loss noted in dams at 75 mg/kg bw/day (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g), reduced food consumption noted in dams at ≥20 mg/kg bw/day, reduced gravid uterus weight noted at ≥20 mg/kg bw/day, reduced number of early intrauterine deaths noted at 75 mg/kg bw/day, reduced litter weight noted at ≥20 mg/kg bw/day, increased post-implantations loss noted at 75 mg/kg bw/day, reduced mean litter size noted at 75 mg/kg bw/day, reduced foetal weight noted at 20 mg/kg bw/day (7%) and 75 mg/kg bw/day (12%), increased incidence of skeletal variations (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) noted at ≥20 mg/kg bw/day, and malformations noted at 75 mg/kg bw/day. The observed malformations noted at 75 mg/kg bw/day consisted of subcutaneous oedema (one animal), retro-oesophageal aortic arch (one animal), kidney misshapen (one animal), and hydropnephrosis (three animals). NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain noted in dams at ≥20 mg/kg bw/day, body weight loss noted in dams at 75 mg/kg bw/day, reduced mean gravid uterus weight noted in dams at ≥20 mg/kg bw/day, reduced mean litter weight noted at ≥20 mg/kg bw/day, increased number of pre- and post-implantation losses and early intrauterine deaths noted at 75 mg/kg bw/day, and reduced mean litter size noted at 75 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight noted at ≥20 mg/kg bw/day, increased number of post-implantation loss and early intrauterine deaths noted at 75 mg/kg bw/day, reduced mean litter size noted at 75 mg/kg bw/day, increased incidence of skeletal variations noted at ≥20 mg/kg bw/day, and malformations noted at 75 mg/kg bw/day (RAR Vol. 3, B.6.6.2.1/04).

Terotology study in the rat (dermal exposure):

In the dermal embryo-foetal development study in the rat, treatment was associated with clinical signs (coloured urine noted at ≥5 mg/kg bw/day and encrusted skin noted at ≥100 mg/kg bw/day), reduced bodyweight growth noted at 600 mg/kg bw/day (bodyweight loss: -0.41 g, reduced bodyweight gain Days 6-16: 31%), reduced food consumption, and macroscopical changes (reddish discolouration of treated skin). No embryotoxicity or teratogenicity was noted (RAR Vol. 3, B.6.6.2.1/04).

Mouse:

80-week carcinogenicity study in the mouse:

In the 80-week carcinogenicity study in the Crl:CD-1(ICR)BR mouse, treatment was associated with clinical signs (orange fur staining) noted in both sexes at ≥3 ppm, increased mortality noted in both sexes at ≥30 ppm, reduced bodyweight gain (33% in males, 30% in females) noted at ≥300 ppm, changes in organ weight noted at ≥30 ppm (increased relative liver weight noted in females at 300 ppm, increased relative kidney weights (n.s.) noted in males at ≥30 ppm and in females at 300 ppm, increased relative heart and brain weights noted in females at 300 ppm), histopathological changes (At 300 ppm: increased incidence of malignant lymphoma noted in females, increased incidence of adrenal spindle cell hyperplasia noted in males, increased incidence of adrenal atrophy noted in females, kidney cortical scarring and hydronephrosis noted in both sexes, hepatic chronic inflammation and brown pigmentation noted in females, sciatic nerve degeneration noted in females, spleenic haemosiderosis noted in females, generalised periarteritis in females, myocardial fibrosis in particularly in males, hyperkeratosis in the stomach noted in both sexes, epithelial hyperplasia (males) and dilation of mucosal glands of the stomach (both sexes), epithelial hyperplasia of the urinary bladder (particularly in females), dilatation of the ureters in both

sexes and histiocytosis of lymph nodes in both sexes; At 30 ppm: adrenal spindle cell hyperplasia and adrenal atrophy noted in females, and hyperkeratosis and chronic inflammation in the stomach in females).

NOAEL was 3 ppm (0.38 and 0.44 mg/kg bw/day for males and females, respectively) based on increased mortality noted in both sexes at ≥30 ppm, reduced bodyweight gain (33% in males, 30% in females) noted at ≥300 ppm, increased relative liver weight noted in females at 300 ppm, increased relative kidney weights noted in males at ≥30 ppm and in females at 300 ppm and histopathological changes noted in the adrenal and stomach at ≥30 ppm and in the kidney, urether, urinary bladder, liver, sciatic nerve, spleen, heart and lymph nodes noted at 300 ppm, and increased incidence of malignant lymphoma noted in females at 300 ppm (RAR Vol. 3, B.6.5.2/01)

Dog:

28-day oral toxicity study in the dog:

In the 28-day oral toxicity study in the Beagle dog, treatment was associated with clinical signs of coloured urine noted in females at ≥10 mg/kg bw/day and in males at ≥30 mg/kg bw/day. At 100 mg/kg bw/day subdued behaviour (F) and vomiting (M, F) were noted in addition. Body weights and food consumption were reduced in these high dosed animals (on Day 4 the reduction in body weights were 13% in the male and 18% in the female). Due to body weight loss and poor clinical condition high dosed (100 mg/kg bw/day) animals were removed from study on Day 5. Changes in clinical chemistry were also noted in the high dosed animals (reduced plasma sodium, potassium and chloride concentrations, increased plasma urea, total bilirubin, creatinine and total cholesterol concentrations). Changes in organ weights were noted in both sexes at ≥30 mg/kg bw/day (At 30 mg/kg bw/day: increased spleen; At 100 mg/kg bw/day: increased spleen, kidney and liver). Macroscopic changes in the urinary bladder (abnormal urinary bladder contents) was noted in the high dosed female (100 mg/kg bw/day), and histopathological changes in the kidney and urinary bladder were noted in both sexes at ≥30 mg/kg bw/day. Histopathological changes in the kidney consisted of tubular nephropathy and transitional cell hyperplasia (both sexes at ≥30 mg/kg bw/day), while the histopathological changes in the urinary bladder consisted of transitional cell hyperplasia (both sexes at ≥30 mg/kg bw/day), arteritis (both sexes at 100 mg/kg bw/day) and epithelial necrosis (male at 100 mg/kg bw/day). The study was acceptable as a range finding study but not for NOAEL/LOAEL setting (RAR Vol. 3, B.6.3.1.2/01).

90-day oral toxicity study in the dog:

In the 90-day oral toxicity study in the Beagle dog, treatment was associated with clinical signs (coloured urine and faeces) noted in both sexes at ≥3 mg/kg bw/day, reduced bodyweight gain noted in females at ≥10 mg/kg bw/day (12% n.s.) and in both sexes at 30 mg/kg bw/day (males: 31%; females: 35%), reduced food consumption noted in males at ≥10 mg/kg bw/day and in females at 30 mg/kg bw/day, changes in haematological parameters noted in both sexes at ≥10 mg/kg bw/day, changes in biochemical parameters (increased mean total bilirubin) noted in both sexes at 30 mg/kg bw/day, changes in organ weights (increased adjusted liver weight noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day, increased adjusted thyroid/parathyroid noted in males at ≥10 mg/kg bw/day, increased adjusted spleen weight noted in females at 30 mg/kg bw/day), gross pathology changes (enlarged spleen noted in two females at 30 mg/kg bw/day, mottled liver noted in one female at

30 mg/kg bw/day, red urinary bladder noted in one female at 30 mg/kg bw/day), histopathological changes in bone marrow (both sexes at ≥10 mg/kg bw/day), liver (both sexes at ≥10 mg/kg bw/day), urinary bladder (in females at ≥10 mg/kg bw/day, in males at 30 mg/kg bw/day), spleen (in both sexes at 30 mg/kg bw/day), kidney (in both sexes at 30 mg/kg bw/day).

The haematological changes consisted of following changes: reduced red blood cell count noted in both sexes at 10 (M: 10%; F: 9%) and 30 mg/kg bw/day (19%), reduced haemoglobin noted in both sexes at 30 mg/kg bw/day (M: 18%; F: 19%), reduced mean cell volume noted in both sexes at 30 mg/kg bw/day (14%), increased reticulocytes noted in both sexes at 10 (M: 100%; F: 83%) and 30 mg/kg bw/day (M: 187%; F: 175%), increased Ret/ABS noted in both sexes at 30 mg/kg bw/day (M: 118%; F: 125%) and in males at 10 mg/kg bw/day (73%), reduced mean cell haemoglobin concentration noted in both sexes at 10 mg/kg bw/day (M: 3%; F: 2%) and 30 mg/kg bw/day (5%), increased plateletes noted in both sexes at 30 mg/kg bw/day (M: 42%; F: 36%), increased plateletcrit noted in both sexes at 30 mg/kg bw/day (M: 50%; F: 54%), increased total white blood cell noted in both sexes at 30 mg/kg bw/day (M: 38%; F: 27%).

The histopathological changes consisted of findings in haemopoietic tissues and in the liver and urinary system. There was increased haemopoiesis in the bone marrow and spleen together with the increase in iron-containing pigment in the liver, this was indicative for low grade haemolytic anaemia and correlated with the findings of reduced red blood cell count and haemoglobin concentration and increased reticulocyte count observed in the intermediate and high dose groups. The increased white blood cell count may be related to the general increase in haemopoiesis noted in the bone marrow. In the kidney pigment, characterised by yellow-brown cytoplasmic droplets, was seen. In addition, some high dose animals had bile duct hyperplasia in the liver and transitional cell hyperplasia in the urinary bladder. One high dose animal also had minor arteritis in the urinary bladder and one had cystitis.

NOAEL for both sexes was set at 3 mg/kg bw/day based on reduced bodyweight gain noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day, changes in haematological parameters (indicating haemolytic anaemia) noted in both sexes at ≥10 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 30 mg/kg bw/day, increased liver weight (noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day), increased thyroid/parathyroid weight noted in males at ≥10 mg/kg bw/day, increased spleen weight noted in females at 30 mg/kg bw/day), gross pathology changes noted in females at 30 mg/kg bw/day (enlarged spleen, mottled liver and red bladder) and histopathological changes noted in the bone marrow (both sexes at ≥10 mg/kg bw/day), liver (both sexes at ≥10 mg/kg bw/day), urinary bladder (noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day), kidney (noted in both sexes at 30 mg/kg bw/day) and spleen (noted in both sexes at 30 mg/kg bw/day) (RAR Vol. 3, B.6.3.2.2/01).

<u>2-year oral toxicity study in the dog:</u>

In the 2-year oral toxicity study in the Beagle dog, treatment was associated with mortalities noted at 1000 ppm (26.6/29.1 mg/kg bw/day in males and females, respectively) (one male and one female), clinical signs noted at ≥250 ppm (≥7.62/6.79 mg/kg bw/day in males and females, respectively) (At 250 ppm: brown-tined urine; At 1000 ppm (26.6/29.1 mg/kg bw/day in males and females, respectively): brown-tined urine, orange stained hair around urogenital area and pale appearing of oral mucosal membranes, a yellowish discoloration of the eyes and

thinness were observed in the female sacrificed in extremis and a general unhealthy appearance characterized by thinness and lethargy was noted in the male sacrificed in extremis), reduced bodyweight growth noted in both sexes at ≥10 ppm (≥0.33/0.31 mg/kg bw/day in males and females, respectively), changes in haematological parameters (indicating anaemia) noted in females at ≥10 ppm and in males at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistical significant increased organ weight changes (increased lung, spleen, gonads) noted in females at 1000 ppm, changes in gross pathology noted at 10 ppm and above (≥10 ppm: urinary bladder; ≥50 ppm (lung, liver); ≥250 ppm (spleen, kidney, ovaries) and 1000 ppm (gall bladder, testes, prostate, heart, cartilage, trachea, ribs, tendons, bones, mesenteric lymph nodes, small intestine), and histopathological changes noted in both sexes at ≥50 ppm.

The haematological changes consisted of following changes: reduced erythrocytes noted in males at 50 (statisticaly significant week 76 only: 12%), 250 (Week 104: 22%) and 1000 ppm (Week 104: 25%), and in females at 10 (statistically significant week 104 only: 14%), 50 (statistically significant week 104 only: 15%), 250 (Week 104: 27%) and 1000 ppm (Week 104: 51%), reduced haematocrit noted in males at 50 (statistically significant week 76 only: 11%), 250 ppm (17% at Week 76) and 1000 ppm (Week 104: 20%), and in females at 250 ppm Week 104: 15%) and 1000 ppm (Week 104: 43%), reduced haemoglobin noted in males at 250 (Week 76: 12%) and 1000 ppm (Week 104: 24%), and in females at 250 (Week 76: 20%) and 1000 ppm (Week 104: 44%), and increased platelet counts noted in one of the males and in both females of group 1000 ppm at Week 104 (M: 51% n.s.; F: 72%)

The histopathological changes consisted of changes in the adrenals (increased vacuolation of cortical cells noted in both sexes at ≥250 ppm (7.62/6.79 mg/kg bw/day in males and females, respectively) and necrosis noted in one female at 1000 ppm (29.1 mg/kg bw/day), lungs (foci of foamy macrophages noted in both sexes at ≥250 ppm, pneumonitis and cholesterol clefts noted in both sexes at 1000 ppm (26.6/29.1 mg/kg bw/day), fibrosis noted in one female at 1000 ppm, edema noted in both sexes at 1000 ppm and consolidation noted in one male at 1000 ppm), spleen (extramedullary haematopoiesis and congestion) noted in females at ≥250 ppm and in males at 1000 ppm, liver (At 50 ppm (0.33/0.31 mg/kg bw/day in males and females, respectively): pigment in groups of macrophages (one female), bile plugs in canaliculi (one female); At 250 ppm: pigment in groups of macrophages (females), pigment in kuppfer cells (both sexes), pigment in cytoplasm of hepatocytes (both sexes), bile plugs in canaliculi (one female), bile duct proliferation (both sexes), periportal fibrosis (both sexes), sinusoidal distension (females); At 1000 ppm: pigment in groups of macrophages (both sexes), pigment in kuppfer cells (both sexes), pigment in cytoplasm of hepatocytes (both sexes), bile plugs in canaliculi (both sexes), bile duct proliferation (both sexes), periportal fibrosis (both sexes), sinusoidal distension (females)), urinary bladder (pigment in mucosal cells noted in both sexes at ≥50 ppm, pigment laden macrophages noted in females at ≥250 ppm and edema noted in one female at 1000 ppm), gall bladder (At 1000 ppm: hyperplasia (both sexes), papillary infolding (both sexes) and choleliths (one female)), kidney (tubular nephrosis noted in one females at 250 ppm and in both sexes at 1000 ppm, healed areas of nephrosis (both sexes) noted at 1000 ppm, dilated renal tubules noted in males at 1000 ppm, cystic tubules (both sexes) noted at 1000 ppm, papillitis noted in one female at 1000 ppm), mesenteric lymph nodes (oedema, erythrophagocytosis and distension of medullary sinuses noted in females at 1000 ppm), pancreas (oedema noted in females at 1000 ppm), testis (focal nonsuppurative orchitis, testicular atrophy and

aspermatogenesis) noted in males at 1000 ppm, and ovaries (lack of cyclic activity, follicular cysts in one animal) noted at 1000 ppm.
In the heart section of one high dose female mineralisation in the coronary arteries as well as in the media and intima of the ank new the base of the heart was noted and considered possibly related to the severe nephropathy. Also this dog had mineralisation of the alveolar wall in the lung as well as a severe dermatitis, ulceration of the oesophagus and erosion in the small intestine.
The reduced bodyweight gain noted in males and females at 10 and 50 ppm were not considered adverse since the body weight gains of the dogs treated at these dose levels were greater than, or comparable to control gains during the first year of the study. Furthermore, the changes in haemotological parameters (reduced erythrocytes) noted in females at 10 ppm were not considered adverse in the absence of other effects on blood at this dose level. Gross pathology changes in urinary bladder (brown mucosa or tan in colour) was not considered adverse in the absence of histopathological findings noted in the urinary bladder at this dose level.
NOAEL for both sexes was set at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) based on mortalities noted in both sexes at 1000 ppm, reduced bodyweight gain noted in both sexes at ≥250 ppm, changes in haematological parameters (indicating anaemia) noted in both sexes at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistically significant changes in relative organ weights (lung, spleen and gonads) noted in females at 1000 ppm, changes in gross pathology noted at 50 ppm (urinary bladder, spleen, ovary) and 250 ppm (urinary bladder, spleen, liver, ovary, kidneys) and 1000 ppm (urinary bladder, spleen, ovary, liver, gall bladder, kidneys, testes, ovary, heart, lung, mesenteric lymph nodes) and histopathological changes noted in the liver (in females at ≥50 ppm; in males at ≥250 ppm), urinary bladder (in both sexes at ≥50 ppm), adrenals (in both sexes at ≥250 ppm), lungs (in both sexes at ≥250 ppm), spleen (in males at 1000 ppm; in females at ≥250 ppm), kidneys (in both sexes at 1000 ppm), mesenteric lymph node (in females at 1000 ppm), gall bladder (in both sexes at 1000 ppm), pancreas (in females at 1000 ppm), aorta (one female at 1000 ppm), testis (1000 ppm), and ovaries (1000 ppm) (RAR Vol. 3, B.6.3.3.1/01)

Rabbit:

Teratology study in the rabbit:

Female New Zealand White rabbits were dosed by the oral route with suspensions of ACN Technical (quinoclamine) at doses up to 22.5 mg/kg bw/day. Treatment was associated with maternal reduced bodyweight gain (5%) noted at 22.5 mg/kg bw/day, reduced foetal weight noted at 22.5 mg/kg bw/day (5%. n.s.), increased incidence of skeletal variants (increased number of caudal centra ≤15) noted at 22.5 mg/kg bw/day, and malformations noted at 22.5 mg/kg bw/day. The malformations noted at 22.5 mg/kg bw/day included scoliosis (one animal), spina-bifida (three animals), anomalies of the aortic arch (two animals), sternebral fusions (three animals) and hyperextension of limb or paw (one animal). NOAEL for maternal toxicity was 22.5 mg/kg bw/day (highest dose level). NOAEL for developmental toxicity was 7.5 mg/kg bw/day based on increased foetal variations (increased number of caudal centra ≤15) noted at 22.5 mg/kg bw/day, and malformations noted at

22.5 mg/kg bw/day (RAR Vol. 3, B.6.6.2.2/02).

In the range finding study to the above study, dose levels up to 500 mg/kg bw/day were initially tested. Because of severe toxicity elicited at the highest dose level, doses were reduced after one dose to 8, 20 or 50 mg/kg bw/day. Because mating was staggered over two days, half of the animals in each group received one dose at the initial high dose level and on subsequent days were dosed at the lower level. The other half of the animals in each group received the lowere dose levels throughout the dosing period. Treatment was associated with maternal mortalities noted at 500/50 mg/kg bw/day (both animals died on GD 8), clinical signs noted at ≥50 mg/kg bw/day (at 50 mg/kg bw/day: coloured urine, at 80/8 mg/kg bw/day: coloured urine, at 200/20 mg/kg bw/day: coloured urine, at 500/50 mg/kg bw/day: dark coloured urine, lethargy, hunched posture), reduced maternal bodyweight gain noted at ≥50 mg/kg bw/day (at 50 mg/kg bw/day: 4-5%, at 80/8 mg/kg bw/day: 4%, at 200/20 mg/kg bw/day: 6%, at 500/50 mg/kg bw/day: 12%), reduced food consumption noted at ≥50 mg/kg bw/day, increased incidence of post-implantation loss noted at ≥20 mg/kg bw/day, and malformations noted at ≥20 mg/kg bw/day. The malformations noted at 20 mg/kg bw/day consisted of spina bifida (two animals), interrupted aortic arch major (one animal) and hindlimb left malrotated (one animal). At 50 mg/kg bw/day interrupted aortic arch major (one animal) and kidney left agenesis (one animal) were noted (RAR Vol. 3, B.6.6.2.2/01)

Teratology study in the rabbit:

Female Crl.NZW/Kbl BR rabbits were administered quinoclamine orally by gavage at doses up to 30 mg/kg bw/day. Treatment was associated with mortality noted in one dam at 30 mg/kg bw/day (animal kille on day 18 of gestation), reduced maternal bodyweight/bodyweight change noted at 17.5 mg/kg bw/day (bodyweight change Days 12-15: 67% of control) and 30 mg/kg bw/day (reduced body weight Days 4-29: 7%, bodyweight change Days 4-29: 46% of control), reduced maternal food consumption noted at 30 mg/kg bw/day, reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, reduced litter weight noted at 30 mg/kg bw/day, increased specific foetal variations noted at 30 mg/kg bw/day and foetal malformations noted at 17.5 mg/kg bw/day (hydronephrosis, abnormal terminal caudal verebrae) and 30 mg/kg bw/day (hydronephrosis, abnormal terminal caudal vertebrae, misshapen nasal bone, misaligned thoracic vertebral arch, absent frontal). The increased incidence of specific foetal variations consisted of: kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, and asymmetric ossification of cervical vertebral centra. NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight growth noted at 17.5 mg/kg bw/day (bodyweight change Days 12-15: 67% of control) and 30 mg/kg bw/day (bodyweight change Days 4-29: 46% of control), reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, and reduced litter weight noted at 30 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, reduced litter weight noted at 30 mg/kg bw/day, increased incidence of specific foetal variations noted at 30 mg/kg bw/day, and malformations noted at ≥17.5 mg/kg bw/day (RAR Vol. 3, B.6.6.2.2/04).

Table 2.6.3.1.1-1. Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Anonymous 15 (2002) Report No.: 619/148 28-day oral toxicity study in the rat	500 ppm (44 mg/kg bw/day) (haemolytic anaemia)	28 days	30<C≤300 mg/kg bw/day	STOT-RE Category 2
Anonymous 22 (2002) Report No.: 0619/133 28-day dermal toxicity study in the rat	1000 mg/kg bw/day (tubular degeneration/regeneration in the kidney cortex, hydronephrosis)	28 days	60<C≤600 mg/kg bw/day	-
Anonymous 21 (1976) Report No.: 854/110 2-year feeding study in the dog	250 ppm (6.79 mg/kg bw/day) (haemolytic anaemia, tubular nephrosis)	2 years	2.5<C≤12.5 mg/kg bw/day	STOT-RE Category 2
Anonymous 23 (1991) Report No.: AKJ/7/90 104 week feeding study in the rat	50 ppm (3.65 mg/kg bw/day) (renal calcification)	104 weeks	2.5<C≤12.5 mg/kg bw/day	STOT-RE Category 2

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Regulation EC No 1272/2008 (CLP), Annex 1: 3.9.2.7.3, states for STOT RE:

"All available evidence, and relevance to human health, shall be taken into consideration in the classification process, including but not limited to the following toxic effects in humans and/or animals:

(a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell):

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver);

(g) evidence of appreciable cell death (including degeneration and reduced cell number) in vital organs incapable of regeneration.

According to the CLP Guidance (Table 3.9.2), a substance should be classified in Category 1 when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in table below:

Route of Exposure	Units	Guidance Values Ranges: (dose/concentration)
Oral (rat)	mg/kg bw/day	C≤10
Dermal (rat or rabbit)	mg/kg bw/day	C≤20
Inhalation (rat) gas	ppm V/6h/day	C≤50
Inhalation (rat) vapour	mg/litre/6h/day	C≤0.2
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	C≤0.2

According to the CLP Guidance (Table 3.9.3), a substance should be classified in Category 2 when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in table below:

Route of Exposure	Units	Guidance Values Ranges: (dose/concentration)
Oral (rat)	mg/kg bw/day	10<C≤100
Dermal (rat or rabbit)	mg/kg bw/day	20<C≤200
Inhalation (rat) gas	ppm V/6h/day	50<C≤250
Inhalation (rat) vapour	mg/litre/6h/day	0.2<C≤1.0
Inhalation (rat) dust/mist/fume	mg/litre/6h/day	0.02<C≤0.2

According to Annex 1 3.9.2.9.8, the guidance values in tables above is increased by a factor of three for a 28-day study.

The CLP Guidance also states the following for STOT RE (in 3.9.1):
"Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs."

Rat:

28-day oral toxicity study in the rat (RAR Vol. 3, B.6.3.1.1/01)

In this study, rats (Crl:CD®(SD)IGSBR) were administered quinoclamine in the diet for 4-weeks at doses up to 1000 ppm (84 and 90 mg/kg bw/day in males and females, respectively). The kidneys, liver and thymus were the apparent target organs in the study. Furthermore, changes in haematological parameters (decreased haemoglobin) in combination with red-,brown-or dark coloured urine and presence of amorphous debris in urine, indicate that quinoclamine causes haemolytic anaemia. Findings in the kidneys consisted of large kidney noted in one male at 1000 ppm (84 mg/kg bw/day) and histopathological changes noted in males at 500 ppm (44 mg/kg bw/day) (increased incidence of eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium) and 1000 ppm (84 mg/kg bw/day) (eosinophilic hyaline droplets in the cytoplasm of the proximal tubular epithelium, minor papillitis characterized by hyperbasophilia of the collecting duct epithelium, interstitial polymorph accumulation and hyperplasia of the urothelium overlying the renal papilla). Effects on the liver consisted of changes in biochemical parameters noted in males at ≥500 ppm (≥44 mg/kg bw/day) (decreased alanine aminotransferase) and in females at 1000 ppm (90 mg/kg bw/day) (increased bilirubin). Effects on the thymus consisted of increased adjusted organ weights noted in both sexes at ≥500 ppm (44/48 mg/kg bw/day in males and

females, respectively). Changes in haematological parameters were noted in both sexes at ≥500 ppm. The magnitude of reduced haemoglobin was up to 13% at the dose level of 1000 ppm. Changes in urine analysis parameters (red-,brown-or dark coloured urine and presence of amorphous debris in urine) were noted in both sexes at ≥500 ppm. Increased urine volume was noted in addition in males at 1000 ppm. The changes in blood indicating haemolytic anaemia (reduced haemoglobin at ≥10% in combination with red-,brown- or dark coloured urine and presence of amorphous debris in urine) were noted within the critical range of doses for Cat 2 classification (i.e. 30<C≤300 mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days). These effects support a classification of quinoclamine as STOT RE 2 (H373).

90-day oral toxicity study in the Sprague-Dawley rat (RAR Vol. 3, B.6.3.2.1/01)

In this study, rats (Sprague-Dawley) were administered quinoclamine in the diet for 13 weeks at doses up to 1000 ppm (62 and 65 mg/kg bw/day in males and females, respectively). The kidneys, liver and spleen were the apparent target organs in the study. The effects on the kidney consisted of increased kidney weights noted in males at 200 ppm (14 mg/kg bw/day) (relative weight of right kidney: 7%) and in both sexes at 1000 ppm (Males: absolute weight: 20%, relative weight: 11%; Females: relative weight: 17%). Furthermore, histopathological changes in the kidney were noted in males at 1000 ppm (62 mg/kg bw/day) (hyaline droplets in the cortical epithelium). The effects on the liver consisted of changes in biochemical parameters noted in males at ≥50 ppm (≥3 mg/kg bw/day) (decreased albumin/globulin ratio), increased liver weights noted in both sexes at 1000 ppm (Males: absolute weight: 12%, relative weight: 16%; Females: relative weight: 19%) and histopathological changes noted in both sexes at 1000 ppm (bile duct proliferation). The effects on the spleen consisted of increased spleen weights noted in both sexes at 1000 ppm (Males: absolute weight: 58%, relative weight: 63%; Females: absolute weight: 18%, relative weight: 35%) and histopathological changes noted in both sexes at 1000 ppm (increased hemosiderin deposition). Effects on the spleen (hemosiderin deposition) were noted within the critical range of doses for Cat 2 classification (i.e. 10<C≤100 mg/kg bw/day) but there were no other changes in this study indicating significant haemolytic anaemia (e.g. a reduction in Hb at ≥10%). The study was considered limited and only accepted as supportive data.

90-day oral toxicity study in the Crl:CD(SD)IGSBR rat (RAR Vol. 3, B.6.3.2.1/02)

In this study, rats (Crl:CD(SD)IGSBR) were administered quinoclamine in the diet for 13 weeks at doses up to 800 ppm (56.74 and 74.81 mg/kg bw/day in males and females, respectively). The kidneys, liver, spleen and thymus were the apparent target organs in the study. Furthermore, changes in haematological parameters (decreased haemoglobin ≥10%) in combination with dark straw coloured urine were noted at 800 ppm. The effects on the kidneys consisted of histopathological changes noted in both sexes at 800 ppm (increased extent of eosinoplic hyaline droplets in the cytoplasm of the proximal tubular epithelium in males, increased incidence of pigment in females, increased extent of focal nephropathy in males and females and papillary interstitial eosinophilia noted in males). The effects on the liver consisted of changes in biochemical parameters noted in males at 800 ppm (increased mean aspartate aminotransferase), increased liver weights noted in both sexes at 800 ppm (Males: relative weight: 10%; Females: relative weight: 16%) and histopathological changes noted in both sexes at ≥200 ppm (13.89 and 17.81 mg/kg bw/day in males and females, respectively) (sinusoidal cell

pigment). The effects in the spleen consisted of increased spleen weight noted in males at 200 ppm (relative weight: 22%) and in both sexes at 800 ppm (Males: relative weight: 44%; Females: relative weight: 22%), enlarged spleen noted in males at 800 ppm and histopathological changes noted in females at 200 ppm (increased extent of pigment) and in both sexes at 800 ppm (increased incidence of congestion in males and females, increased extent of haemopoiesis in males, and increased extent of pigment in females). The effects on the thymus consisted of reduced thymus weight noted in females at 200 ppm (relative weigth 40%) and 800 ppm (relative weight: 48%) and histopathological changes (minor thymic atrophy) noted in both sexes at 800 ppm.

The findings of reduced haemoglobin (>10%) in combination with dark straw coloured urine and effects on the spleen (increased extent of pigment and haemopoiesis) indicate haemolytic anaemia. This effect were considered relevant for human health and noted within the critical range of doses for STOT RE classification in Category 2 (i.e. 10<C≤100 mg/kg bw/day). This effect support a classification of quinoclamine as STOT RE 2 (H373).

Long-term toxicity and carcinogenicity in the rat (RAR Vol. 3, B.6.5.1/01)

In this study, Crl: CD(SD) BR rats were administered quinoclamine orally via the diet for 104 weeks at doses up to 676 ppm (37.6 and 49.4 mg/kg bw/day in males and females, respectively). The kidneys, urinary bladder, lungs, ureter, urethra, parathyroid, pancreas, spleen, thymus, thyroid, heart, adrenal and mammary gland were the apparent target organs in the study. Furthermore, changes in haematological parameters (decreased haemoglobin <10%, red blood cell volume and red blood cell count) were noted at the highest dose level (676 ppm).
Effects on the spleen, thyroid, thymus and heart consisted of organ weight changes noted at 676 ppm.
The effects on the kidneys consisted of increased relative kidney weights noted in males at ≥52 ppm (≥2.82 mg/kg bw/day) and in females at 676 ppm (49.4 mg/kg bw/day) and histopathological changes noted in both sexes at ≥52 ppm (≥2.82 and ≥3.65 mg/kg bw/day in males and females, respectively) (epithelial hyperplasia in both sexes at ≥52 ppm, increased incidence of renal calcification in females at ≥52 ppm, renal papillary degeneration/necrosis in both sexes at 676 ppm, increased incidence of renal cortical scarring in both sexes at 676 ppm, pelvis polyp noted in one male at 676 ppm).
Effects on the lungs consisted of histopathological changes (arterial calcification) noted in both sexes at 676 ppm and in males at 52 ppm. This effect and the renal calcification in females from the 676 ppm and 52 ppm together with a related small increase in the incidence of parathyroid hyperplasia in terminal kill and decedent high dose males were indicative of alterations in blood calcium levels due to interference in the renal regulation of phosphorus and calcium ions. It is possible that the treatment-related kidney lesions could have affected these excretory mechanism according to study author.
The effects on the urinary bladder consisted of histopathological changes noted in both sexes at ≥52 ppm (≥2.82 and 3.65 mg/kg bw/day) (epithelial hyperplasia noted in both sexes at ≥52 ppm, benign transitional cell papilloma noted in both sexes at 676 ppm and in males at 52 ppm, polyp noted in one female at 676 ppm and chronic inflammation noted in both sexes at 676 ppm). The benign transitional cell papilloma noted in one single male at 52 ppm was considered of no clear relevance. The polyp seen in the urinary bladder of one high dose terminal kill female probably developed as a response to the irritant toxic effect of the test compound on the urothelium, according to study author.

The effects on the adrenal consisted of increased weight (9%) noted in females at 676 ppm and histopathological changes (benign phaeochromocytoma) noted in both sexes at 676 ppm.
The effects on the ureter and urethra consisted of histopathological changes (epithelial hyperplasia) noted in both sexes at 676 ppm.
Effects on the mammary gland consisted of histopathological changes (reduced mammary acinar development and secretion) noted in females at 676 ppm. This effect was probably related to the reduced food consumption and the lower bodyweight in these high dose animals, according to the study author.

The effect of neoplastic changes in the adrenal (benign phaeochromocytoma) and urinary bladder (benign transitional cell papilloma) was not considered relevant for STOT-RE classification, but for carcinogenicity (section 2.6.5). No adverse reduction in haemoglobin was noted in this study. Effects on the kidneys (increased incidence of calcification) were noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. 2.5<C≤12.5 mg/kg bw/day) (Haber´s rule considered for exposure durations of 104 weeks).

Two generation reproduction toxicity study in the rat (RAR Vol. 3, B.6.6.1/01)

In this study, Sprague Dawley rats were administered quinoclamine in the diet at dose level up to 500 ppm (30.9 mg/kg bw/day). The reproductive performance was not affected by quinoclamine treatment. Systemic toxicity for both the parental and the offspring generation comprised a reduction in body weights and bodyweight gains, and reduced litter size was noted at the dose level of 500 ppm. Furthermore, increased incidence of gray lung cysts were noted in F2b generation offspring reared for 3 months. The findings of gray lung cysts in the F2b offspring was dose related although the relevance of this finding is not clear. The finding was not observed in the offspring of the F1b generation reared for 5 weeks or in other available toxicological studies on quinoclamine. The relevance of the effects noted in this study were too unclear for STOT-RE classification.

Rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/02)

In this study, female CD rats of Sprague-Dawley origin (CD(SD)BR) were administered quinoclamine orally by gavage during Gestation days 7-17 at doses up to 75 mg/kg bw/day. Adverse maternal findings were noted at 20 mg/kg bw/day (enlarged spleen in one dam) and 75 mg/kg bw/day (enlarged spleen in four dams and reduced bodyweight gain). Developmental effects such as abnormalities (aortic arch malformations) and increased incidence of skeletal variants were noted at ≥20 mg/kg bw/day. These developmental effects were not considered relevant for STOT-RE classification, but for reproductive toxicity (section 2.6.6). In the absence of data for haematology and histopathology, the findings of enlarged spleen were not considered severe enough for classification as STOT-RE.

Rat developmental toxicity study (RAR Vol. 3, B.6.6.2.1/04)

In this study, female Crl:CD(SD)IGSBR rats were administered quinoclamine orally by gavage during Gestation days 6-19 at doses up to 75 mg/kg bw/day. Adverse maternal findings were noted at 20 mg/kg bw/day (reduced

bodyweight gain) and 75 mg/kg bw/day (reduced body weight gain/bodyweight loss). Developmental effects such as reduced foetal weight and increased incidence of skeletal variants were noted at ≥20 mg/kg bw/day, and malformations were noted at 75 mg/kg bw/day. These effects were not considered relevant for STOT-RE classification, but for reproductive toxicity (section 2.6.6). Nor were the maternal effects noted in the study considered of concern for a classification as STOT-RE since the effects were not severe enough for classification.

28-day dermal toxicity study in the rat (RAR Vol. 3, B.6.3.4.1/01)

In this study, Crl:CD:(SD)IGSBR rats were administered quinoclamine by the dermal route for 28 days at doses up to 1000 mg/kg bw/day. The kidney was the apparent target organ for systemic toxicity. The finding in the kidney consisted of histopathological changes noted at 1000 mg/kg bw/day (tubular degeneration/regeneration in the kidney cortex noted in one animal of each sex and hydronephrosis and pigment noted in one female). The effects noted in the study were not considered of concern for a classification as STOT-RE since no adverse effects were noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. $60 < C \leq 600$ mg/kg bw/day) (Haber´s rule considered for exposure duration of 28 days).

Mouse:

80-week carcinogenicity study in the mouse (RAR Vol 3, B.6.5.2/01)

In this study, Crl:CD-1 (ICR)BR mice were administered quinoclamine orally via the diet for 80 weeks at doses up to 300 ppm (40.2 and 46.4 mg/kg bw/day in males and females, respectively). The kidney, adrenal, urinary bladder, urether, spleen, stomach, liver, heart, sciatic nerve, lymph nodes and lympho reticular tissue were the apparent target organs. Furthermore, increased mortalities were noted in both sexes at ≥30 ppm (≥3.82 and ≥4.48 mg/kg bw/day in males and females, respectively). It could however be noted that the mortality in the study was exceptionally low and only at the highest dose level (300 ppm) did mortality approach the levels expected in comparison to historical control data (-50%). The findings in the liver consisted of increased liver weight and histopathological changes noted in females at 300 ppm (46.4 mg/kg bw/day) (chronic inflammation and brown pigmentation). In the adrenal, histopathological changes were noted in females at 30 ppm (4.48 mg/kg bw/day) (adrenal spindle cell hyperplasia and brown athrophy) and in both sexes at 300 ppm (increased incidence of adrenal spindle cell hyperplasia noted in males and brown athrophy noted in females). The findings in the heart consisted of increased relative organ weight and histopathological changes (generalised periarteritis and myocardial fibrosis) noted in females at 300 ppm, and histopathological changes of myocardial fibrosis noted in males at the same dose level. In addition at 300 ppm, histopathological changes were noted in the kidneys (cortical scarring and hydronephrosis. both sexes), spleen (increased incidence of adrenal spindle cell hyperplasia and brown athropy, males only), urether (dilation, both sexes), urinary bladder (epithelial hyperplasia, particularly in females), stomach (hyperkeratois, both sexes), lymph nodes (histiocytosis, both sexes) and sciatic nerve (degeneration, females). The effects noted in the study were not considered of concern for a classification as STOT-RE. No effects of significant toxicity were noted within the critical range of doses for Cat 2 classification (i.e. $1.5 < C \leq 15$ mg/kg bw/day) (Haber´s rule considered for exposure duration of 80 weeks).

The effect of neoplastic changes (malignant lymphoma) was not considered relevant for STOT-RE classification, but for carcinogenicity (section 2.6.5).

Rabbit:

Rabbit developmental toxicity study (RAR Vol. 3, B.6.6.2.2/02)

In this study, female New Zealand rabbits were administered quinoclamine orally by gavage during Gestation Days 6-18 at doses up to 22.5 mg/kg bw/day. No adverse maternal effects were noted. Developmental effects such as increased incidence of skeletal variants were noted at 22.5 mg/kg bw/day. These effects were not considered relevant for STOT-RE classification, but for reproductive toxicity (section 2.6.6).

In the range finding study to the study above (RAR Vol. 3, B.6.6.2.2/01), female New Zealand White rabbits (5/group) were administered Quinoclamine orally by gavage during Gestation Days 6-18. Dose levels up to 500 mg/kg bw/day were initially tested. Because of severe toxicity elicited at the highest dose level, doses were reduced after one dose to 8, 20 or 50 mg/kg bw/day. Because mating was staggered over two days, half of the animals in each group received one dose at the initial high dose level and on subsequent days were dosed at the lower level. The other half of the animals in each group received the lowere dose levels throughout the dosing period. Maternal mortalities were noted in both animals exposed at 500/50 mg/kg bw/day (one animal died on Day 9 and the other on Day 10 of pregnancy) but no deaths were noted at 50 mg/kg bw/day. At necropsy these animals showed pale liver, abnormal spleen and dark intestinal contents. The mortalities noted in the study occurred after 3 to 4 days following administration, and could be considered as an acute effect rather than an effect of repeated administration.

Rabbit developmental toxicity study (RAR Vol. 3, B.6.6.2.2/04)

In this study, female Crl.NZW/Kbl BR rabbits were administered quinoclamine orally by gavage during Gestation days 7-28 at doses up to 30 mg/kg bw/day. Adverse maternal findings were noted at 17.5 mg/kg bw/day (reduced bodyweight change) and 30 mg/kg bw/day (mortality, one female killed on Day 18 of gestation, reduced bodyweight change). Developmental effects such as increased incidence of specific foetal variations and malformations were noted at 22.5 mg/kg bw/day. The developmental effects were not considered relevant for STOT-RE classification, but for reproductive toxicity (section 2.6.6).

Mortality was noted in this study in one dam (killed on Day 18 of gestation). This animal showed severe inappetence and body weight loss and clinical observation of red discharge from the urogenital region. Necropsy examination did not reveal any macroscopic abnormalities. Except for this animal, there were no adverse effects of treatment on clinical observations or necropsy findings. The single case of mortality noted in this study was considered of no clear relevance for classification.

Dog:

28-day oral toxicity study in the dog (RAR Vol. 3, B.6.3.1.2/01)

In this study, Beagle dogs were administered Quinoclamine by oral capsules for 28 days at dose levels of 3, 10, 30 and 100 mg/kg bw/day. The high dose animals were removed from the study on Day 5 due to body weight loss and poor clinical condition. The kidneys, urinary bladder, spleen, and liver were the apparent target organs.
Increased organ weights (absolute and relative) were noted in both sexes for the spleen (at ≥30 mg/kg bw/day) and for the liver (at 100 mg/kg bw/day). Findings in the kidneys consisted of increased absolute and relative kidney weights and histopathological changes (tubular nephropathy and transitional cell hyperplasia) noted in both sexes at 100 mg/kg bw/day. Findings in the urinary bladder consisted of histopathological changes noted at 100 mg/kg bw/day (transitional cell hyperplasia and arteritis noted in both sexes and epithelial necrosis noted in males).
The findings of tubular nephropathy in the kidney and epithelial necrosis in the urinary bladder were noted at a dose level expecting to cause lethality. These effects were not observed at the dose level of 30 mg/kg bw/day or in the 90-day oral toxicity study in the dog using doses up to 30 mg/kg bw/day. The relevance of the effects noted at 100 mg/kg bw/day were not considered clear for classification.

90-day oral toxicity study in the dog (RAR Vol. 3, B.6.3.2.2/01)

In this study, Beagle dogs were administered Quinoclamine by oral capsules for 90 days at doses up to 30 mg/kg bw/day. The bone marrow, spleen, liver, kidneys, urinary bladder and thyroid/parathyroid were the apparent target organs. Furthermore, changes in haematological parameters were noted in both sexes at ≥10 mg/kg bw/day (including changes such as reduced haemoglobin (>10%) noted at 30 mg/kg bw/day, reduced red blood cell count noted at ≥10 mg/kg bw/day and increased reticulocyte count noted at ≥10 mg/kg bw/day).
Increased adjusted thyroid/parathyroid organ weights were noted for males at ≥10 mg/kg bw/day. The histopathological findings noted in the bone marrow consisted of haemopoiesis characterised by greater cellularity noted in both sexes at ≥10 mg/kg bw/day. The findings in the spleen consisted of macroscopical changes (enlarged spleen) noted in two females at 30 mg/kg bw/day and histopathological changes (haemopoiesis characterised by increased haemopoietic cells in the red pulp and congestion of the splenic red pulp) noted in both sexes at 30 mg/kg bw/day. The findings in the liver consisted of increased adjusted liver weights noted in females at 10 mg/kg bw/day and in both sexes at 30 mg/kg bw/day. Furthermore, histopathological changes were noted in the liver in both sexes at ≥10 mg/kg bw/day (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment noted at ≥10 mg/kg bw/day and bile duct hyperplasia noted at 30 mg/kg bw/day). The findings in the urinary bladder consisted of histopathological changes noted at 10 mg/kg bw/day (cystitis in one female) and at 30 mg/kg bw/day (transitional cell hyperplasia noted in both sexes, arteritis in one male, and cystitis in one female). At 30 mg/kg bw/day, histopathological changes were also found in the kidneys (pigment noted in both sexes).
In this study, there was increased haemopoiesis in the bone marrow and spleen together with the increase in iron-containing pigment in the liver, this was indicative for low grade haemolytic anaemia and correlated with the findings of reduced red blood cell count and haemoglobin concentration (>10%) and increased reticulocyte count

observed in the intermediate (10 mg/kg bw/day) and high dose groups (30 mg/kg bw/day). These findings were considered of significant toxicity for STOT-RE classification. The effects were considered severe and relevant for human health and noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. 10<C≤100 mg/kg bw/day).

2-year oral toxicity study in the dog (RAR Vol. 3, B.6.3.3.1/01)

In this study, Beagle dogs were administered Quinoclamine in the diet for two years at doses up to 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively). The kidneys, spleen, liver, gall bladder, urinary bladder, lung, testes, prostate, ovary, adrenal gland, heart, mesenteric lymph nodes, pancreas and small intestine were the apparent target organs. Furthermore, changes in haematological parameters were noted in females at ≥10 ppm (≥0.31 mg/kg bw/day) and in males at ≥50 ppm (≥1.42 mg/kg bw/day). Changes in haematological parameters included changes such as reduced haemoglobin noted in both sexes at 250 ppm (7.62 and 6.79 mg/kg bw/day in males and females, respectively) (>10%) and 1000 ppm (>20%), reduced erythrocytes noted in females at ≥10 ppm (0.31 mg/kg bw/day) and in males at ≥50 ppm (1.42 mg/kg bw/day), and reduced haematocrit noted in males at 50 ppm (1.42 mg/kg bw/day) and in females at 250 ppm (6.79 mg/kg bw/day).

Treatment related mortalities were noted at 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (one animal of each sex sacrificed in extremis during week 65). Pale appearing oral mucosal membranes were observed in all high dose group animals, a yellowish discoloration of the eyes and thinness were observed in the 1000 ppm group female sacrificed in extremis, and a general unhealthy appearance characterized by thinness and lethargy was noted in the group 1000 ppm male sacrificed in extremis.

The findings in the kidneys consisted of histopathological changes noted at 250 ppm (6.79 mg/kg bw/day) (tubular nephrosis noted in one female) and 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (tubular nephropathy with fibrosis and renal tubular regeneration noted in both sexes). Furthermore, macroscopical changes in the kidneys were noted at 250 ppm (7.62 and 6.79 mg/kg bw/day in males and females, respectively) (depressed areas on surface) and 1000 ppm (small, depressed areas on surface, contracted, polycystic- primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule).

The histopathological findings in the spleen consisted of extramedullary haematopoiesis and congestion noted in females at ≥250 ppm (≥6.79 mg/kg bw/day) and in males at 1000 ppm (26.6 mg/kg bw/day). Furthermore, macroscopical changes in the spleen were noted at 50 ppm (1.42 and 1.39 mg/kg bw/day in males and females, respectively) (dark in colour or margins dark), 250 ppm (7.62 and 6.79 mg/kg bw/day in males and females, respectively) (dark in colour or margins dark) and 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (dark in colour or margins dark, enlarged), and increased relative spleen weight was noted in females at 1000 ppm (29.1 mg/kg bw/day).

The findings in the adrenal consisted of histopathological changes noted at 250 ppm (7.62 and 6.79 mg/kg bw/day in males and females, respectively) (increased vacuolation of cortical cells noted in both sexes, focal nonsupparative adrenalitis noted in one male) and 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (increased vacuolation of cortical cells noted in both sexes, and necrosis noted in one female).

The findings in the liver consisted of histopathological changes noted at 50 ppm (1.39 mg/kg bw/day) (pigment in macrophages noted in one female and bile plugs in canaliculi noted in one female), 250 ppm (7.62 and 6.79 mg/kg

bw/day in males and females, respectively) (pigment in cytoplasm of hepatocytes and Kuppfer cells noted in both sexes, pigment in macrophages noted in females, periportal fibrosis noted in both sexes, bile duct proliferation noted in both sexes, bile plugs in canaliculi noted in one female, sinusoidal distension noted in females) and 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (pigment in cytoplasm of hepatocytes, Kuppfer cells and macrophages noted in both sexes, periportal fibrosis noted in both sexes, bile duct proliferation noted in both sexes, bile plugs in canaliculi noted in both sexes and sinusoidal distension noted in females). Furthermore, macrosopical changes in the liver were noted at 250 ppm (brown in colour, rough surfaced, tough in consistency, firm) and 1000 ppm (enlarged lobes, thickened and pale, rough surfaced and motted, brown in colour, tough in consistency, firm). Changes in biochemical parameters also indicated liver toxicity (increased serum glutamic-pyruvic transaminase noted in both sexes at ≥250 ppm, increased alkaline phosphatase noted in both sexes at ≥250 ppm and increased serum glutamic-oxaloacetic transaminase noted in both sexes at ≥250 ppm, and increased bilirubin noted in females at 1000 ppm).

The findings in the gall bladder consisted of macroscopical changes noted at 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (distended, walls thickened) and histopathological changes noted at 1000 ppm (hyperplasia noted in both sexes, papillary infolding noted in both sexes, cholelith noted in one female).

The findings in the urinary bladder consisted of histopathological changes noted at 50 ppm (1.42 and 1.39 mg/kg bw/day in males and females, respectively) (pigment in mucosal cells noted in both sexes), 250 ppm (pigment in mucosal cells noted in both sexes, pigment laden macrophages noted in females) and 1000 ppm (26.6 and 29.1 mg/kg bw/day in males and females, respectively) (pigment in mucosal cells noted in both sexes, oedema noted in one female, pigment laden macrophages noted in one female). Furthermore, macroscopical changes were noted in the urinary bladder at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) (mucosa brown or tan in colour), 50 ppm (mucosa brown or tan in colour), 250 ppm (mucosal surface brown or yellow-gray) and 1000 ppm (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface).

The findings in the lungs consisted of histopathological changes noted at 50 ppm (1.42 mg/kg bw/day) (focal pneumonitis noted in one male) and 250 ppm (foci of foamy macrophages noted in both sexes) and 1000 ppm (foci of foamy macrophages noted in both sexes, focal pneumonitis noted in both sexes, cholesterol clefts noted in both sexes, fibrosis noted in one female, oedema noted in both sexes, consolidation noted in one male). Furthermore, macroscopical changes were noted in the lungs at 250 ppm (white foci on surface) and 1000 ppm (raised yellow gray foci on all lobes, focal emphysematous appearing areas).

The findings in the mesenteric lymph nodes consisted of histopathological changes (oedema, erythrophagocytosis, distension of medullary sinuses in females) and macroscopical changes (dark in colour) noted at 1000 ppm.

The findings in the pancreas consisted of histopathological changes (oedema) noted in females at 1000 ppm (29.1 mg/kg bw/day).

Findings in the heart were also noted, and consisted of histopathological changes (mineralisation noted in one female) and macroscopical changes (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52) noted at 1000 ppm. The findings in this female was considered possibly related to the severe nephropathy noted in this animal. Also this dog had mineralisation of the alveolar wall in the lung as well as a severe dermatitis, ulceration of the oesophagus and erosion in the small intestine.

In addition to the toxic changes described above, macroscopic changes were noted in the following organs: cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in colour) at 1000 ppm.

There were findings indicative of anaemia, characterized by decreased haemoglobin. At 250 ppm (7.62/6.79 mg/kg bw/day in males and females, respectively) haemoglobin was reduced >10%. At 1000 ppm (26.6/29.1 mg/kg bw/day in males and females, respectively) haemoglobin was reduced >20% and pale mucosal membranes were noted. Histomorphological changes of extramedullary haematopoesis and congestion were noted in the spleen at ≥250 ppm and pigmentation of urinary bladder was noted at ≥50 ppm. There were also clear evidence of marked organ dysfunction in the kidney. The marked organ dysfunction in the kidney consisted of tubular nephrosis noted in one female at 250 ppm (6.79 mg/kg bw/day) and tubular nephropathy with fibrosis and renal tubular regeneration noted in both sexes at 1000 ppm (26.6/29.1 mg/kg bw/day in males and females, respectively). These findings were considered of significant toxicity for STOT-RE classification. The effects were considered severe and relevant for human health and noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. 2.5<C≤12.5 mg/kg bw/day) (Haber´s rule considered for exposure duration of 2 years).

Overall conclusion- findings relevant for STOT-RE:

There were **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 quinoclomaine as STOT RE 1 (H372).

In the oral 28-day rat study (CD rat), changes in blood indicating haemolytic anaemia (reduced haemoglobin at ≥10% in combination with red-,brown- or dark coloured urine and presence of amorphous debris in urine) were noted at the dose level of 44 mg/kg bw/day. The effects were noted within the critical range of doses for STOT RE classification in Category 2 (i.e. 30<C≤300 mg/kg bw/day). The effects noted support a classification of quinoclamine as STOT RE 2 (H373).

In the 90-day oral toxicity study (CD rat), findings of reduced haemoglobin at (>10% in combination with dark straw colored urine, indicating haemolytic anaemia, were noted at the dose level of 56.74 mg/kg bw/day. The effects were considered relevant for human health and noted within the critical range of doses for STOT RE classification in Category 2 (i.e. 10<C≤100 mg/kg bw/day). The effects noted support a classification of quinoclamine as STOT RE 2 (H373).

In the 90-day oral toxicity study (Sprague Dawley rat), hemosiderin deposition was noted in the spleen at the dose level of 62 mg/kg bw/day. This effect indicate haemolytic anaemia, although there were no adverse reduction in haemoglobin noted in this study. The study was considered limited and accepted as supportive data only.

In the 28-day oral toxicity study in the dog, epithelial necrosis was noted in the urinary bladder of males at the dose level of 100 mg/kg bw/day after five days exposure. Body weight loss and poor clinical condition were also noted in these animals and all animals in the dose group were removed from the study on Day 5. Furthermore, effects on the kidneys (tubular nephropathy) were noted at this dose level after five days exposure. The findings of tubular nephropathy in the kidney and epithelial necrosis in the urinary bladder were noted at a dose level expecting to cause lethality. These effects were not observed at the dose level of 30 mg/kg bw/day or in the 90-day oral toxicity study in the dog using doses up to 30 mg/kg bw/day. The relevance of the effects noted at 100 mg/kg bw/day were considered equivocal for classification.

In the 90-day oral toxicity study in the dog, increased haemopoiesis in the bone marrow and spleen were noted together with the increase in iron-containing pigment in the liver, this was indicative for low grade haemolytic anaemia and correlated with the findings of reduced red blood cell count and haemoglobin concentration (>10%) and increased reticulocyte count observed in the intermediate (10 mg/kg bw/day) and high dose groups (30 mg/kg bw/day). The effects were considered severe and relevant for human health and noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. 10<C≤100 mg/kg bw/day).

In the 2-year feeding study in the dog, there were findings indicative of anaemia, characterized by decreased haemoglobin (>10% reduction) at the dose level of 6.79 mg/kg bw/day, pale mucosal membranes noted at 26.6 mg/kg bw/day and pigmentation of urinary bladder noted at 1.39 mg/kg bw/day. Histomorphological changes of extramedullary haematopoesis and congestion were also noted in the spleen at the dose level of 6.79 mg/kg bw/day. There were also clear evidence of marked organ dysfunction in the kidney. The marked organ dysfunction in the kidney consisted of tubular nephrosis noted in one female at 6.79 mg/kg bw/day and tubular nephropathy with fibrosis and renal tubular regeneration noted at a dose level of 26.6 mg/kg bw/day. The effects noted were considered severe, and relevant for human health, and noted within the critical range of doses for STOT RE 2 (H373) classification (i.e. 1.25<C≤12.5 mg/kg bw/day) (Haber´s rule considered for exposure duration of 2 years).

In the developmental toxicity study in the rabbit (exposure duration 21 days), a single case of maternal mortality (one dam killed on Day 18 of gestation). This animal showed severe inappetence and body weight loss and clinical observation of red discharge from the urogenital region. Necropsy examination did not reveal any macroscopic abnormalities. Except for this animal, there were no adverse effects of treatment on clinical observations or necropsy findings. The single case of mortality noted in this study was considered of no clear relevance for classification.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Classification in **STOT RE 2, H373** (“May cause damage to blood system and kidney through prolonged or repeated exposure”) is proposed 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). Since no information is available from the repeated dose studies where the inhalation route had been used no route could be specified.

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	<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	Oral (dietary) 90-day CD rat	10<C≤100 mg/kg bw/day	Cat. 2

point of view 10/sex/dose	↑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.			
B.6.3.2.1, Study 1 Anon., 1972 Supportive 5/sex/dose	<u>Highest Effective Dose:</u> 62/65 mg/kg bw/day (M/F) <u>Critical effects:</u> ↑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.	Oral (dietary) 90-day SD rat	10<C≤100 mg/kg bw/day	Cat. 2
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
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 to not indicate the route of exposure.

2.6.4 Summary of genotoxicity / germ cell mutagenicity

Table 2.6.4-1. Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial reverse mutation test (Ames test) OECD TG 471 GLP: Yes	Quinoclamine Purity: 99%	Tester strain(s): *Salmonella typhimurium* TA100 TA1535 TA1537 TA98 *Escherichia coli* WP2uvrA Concentrations: Experiment 1 (All strains): 0.1953-200 µg/plate (-S-9 and -S-9) Experiment 2: TA 98: 0.7813-25 µg/plate (-S-9) TA100, TA1537, WP2uvrA: 1.563-50 µg/plate (-S-9) TA 1535: 3.125-100 µg/plate (-S-9) TA98, TA100, TA1537: 1.563-50 µg/plate (+S-9) TA1535, WP2uvr: 3.125-100 µg/plate (+S-9) µg/plate (+S-9)	Quinoclamine did not induce increases in the number of revertant colonies of any strain at any dose that was both dose-related and reproducible. Quinoclamine was non-mutagenic. Evidence of toxicity was apparent in all strains at the highest one, two or three test doses in Experiment 1 (12.5, 50 and/or 200 µg/plate). In Experiment 2, toxic signs were observed at the highest one or two test doses in several of the test strains. The maximum test dose (100 µg/plate) used for Experiment 2 treatments of strain TA1537 in the presence of S-9 did not induce any indications of toxicity in this strain. However, it did resulted in a small increase in revertant numbers. A further treatment of TA1537 was therefore performed at a revised maximum test dose of 100 µg/plate. As a result no increase in revertant numbers was noted at the dose of 100 µg/plate.	RAR Vol. 3, B.6.4.1/01 Beevers (2002) New data for the Annex I renewal: No
Mammalian Chromosome Aberration Test OECD TG 473	Quinoclamine Purity: not stated in study report	Target cells: Human lymphocytes Concentrations: 1.125, 2.25, 4.5 and 9 µg/ml without S-9	Negative in absence of S-9 nix Positive in presence of S-9 nix at 9 and 18 µg/mL	RAR Vol. 3, B.6.4.1/02 Asquith (1987)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
GLP: Yes		and 2.25, 4.5, 9 and 18 µg/ml with S-9		New data for the Annex I renewal: No
Mammalian Cell Gene Mutation Test OECD TG 476 GLP: Yes	ACN Technical (Quinoclamine) Purity: 98.1%	Target cells: L5178 mouse lymphoma Concentrations: Mutation experiment 1: -S-9 mix: 0.15, 2.5, 10 µg/mL +S-9 mix: 0.4, 2, 8, 30 µg/mL Mutation experiment 2: -S-9 mix: 0.15, 0.0625, 2.5, 10 µg/mL +S-9 mix: 0.4, 2. 8. 30 µg/mL	ACN (Quinoclamine) did not cause mutations resistant to Ouabain in L5178Y cells in either the absence or presence of S-9.	RAR Vol. 3, B.6.4.1/03 Asquith (1989) New data for the Annex I renewal: No

Table 2.6.4-2. Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus test in mouse OECD TG 474 *Deviation: 1000 polychromatic erythrocytes were counted instead of 2000* GLP: Yes	ACN Technical (Quinoclamine) Purity: 98.1%	Target cells: Mouse bone marrow Mice of both sexes (LACA strain) Dose levels: 125, 250 and 500 mg/kg Single intraperitoneal injection	ACN (Quinoclamine) did not induce micronuclei in mouse bone marrow cells *The study is limited since bone marrow exposure was not shown in the study*	RAR Vol. 3, B.6.4.2/01 Anonymous 31 (1989) New data for the Annex I renewal: No
Unscheduled DNA Synthesis (UDS) in rat liver OECD TG 486	Quinoclamine Purity: 97.6%	Target cells: Rat liver Male Crl:CD®BR rats Dose levels: 800 or 2000 mg/kg	No induction of UDS in hepatocytes was noted in this study. *Negative results in the in vivo UDS test are not considered sufficient to overrule positive results in*	RAR Vol. 3, B.6.4.2/02 Anonymous 32 (1996) New data for the Annex I renewal: No

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
GLP: Yes		Single oral dose (gavage)	*either of the in vitro gene mutation test*	

Table 2.6.4-3. Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

The genotoxic potential of quinoclamine was investigated in three standard *in vitro* test systems (Ames test, mammalian chromosome aberration test and mammalian cell gene mutation test) and in two *in vivo* tests (*in vivo* mouse micronucleus test and *in vivo* UDS test). All these studies were conducted in accordance with the OECD Principles of Good Laboratory Practice (1981).

All tests were negative except the *in vitro* cytogenetic assay in human lymphocytes (*in vitro* chromosome aberration test) that was positive in the presence of metabolic activation. The in vivo micronucleus test in the mouse was considered limited, 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. Since no ADME data using the same route and the same species are available in the dossier, a **data gap** is identified for *in vivo* genotoxicity.

An *in vivo* UDS test is available. However, the negative results in the *in vivo* UDS test are not considered sufficient to overrule positive results in either of the *in vitro* gene mutation test (EFSA technical report (2016). Outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology, EFSA Supporting publication 2016:EN-1074).

No photomutagenicity study is available. However, quinoclamine has been shown to be negative in a standard *in vitro* phototoxicity study (Vol. 3, B.6.2.7/01). Thus, no photomutagenicity study is required.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

No comparison with the CLP criteria regarding genotoxicity/germ cell mutagenicity has been conducted since a data gap is identified for genotoxicity *in vivo* (see section 2.6.4.1).

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

No conclusion on classification and labelling for genotoxicity/germ cell mutagenicity could be drawn because the data were inconclusive. A data gap is identified for genotoxicity *in vivo*.

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

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 public 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 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

[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.

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.**

2.6.5 Summary of long term toxicity and carcinogenicity

Table 2.6.5-1. Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**

Long-term toxicity and carcinogenicity Oral (dietary) No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group *Study was checked for compliance with OECD TG 453 and following deviations were noted:* *i. Haematological examination was not carried out at 3 months (the guideline recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study)* *ii. Prothrombin time and activated partial thromboplastin time was not investigated* *iii. Urea was not investigated* *iv. Uterus and epididymides were not weighed* *v. Coagulating gland, ileum,*	ACN technical (Quinoclamine) Purity: 98.3% <u>Carcinogenicity groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females <u>Chronic toxicology groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females 104 weeks	<u>4 ppm:</u> -changes in urinalysis (yellow/brown or orange colour) (M, F)) <u>52 ppm:</u> **-changes in urinalysis** (yellow/brown or orange discoloration) (M, F) -changes in organ weights (Week 27: ↑kidney (M: 8%)) **-histopathological changes** in <u>urinary bladder</u> (epithelial hyperplasia (M, F), <u>kidneys</u> (epithelial hyperplasia (M, F), ↑ renal focal calcification (F), <u>ureter</u> (epithelial hyperplasia (M, F), <u>lungs</u> (arterial calcification (M)) <u>676 ppm:</u> -clinical signs (orange fur staining, ↓incidence of mass bearing animals) (M, F) ↓**bw gain** (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓FC (M, F) **-changes in haematological parameters** (↓packed blood cell volume (M week 27, 79; F week 53), ↓haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓red blood cell count (M week 27, 79; F: week 27, 53)) **-changes in biochemical parameters** (↑blood urea nitrogen (M n.s., F n.s.), ↓calcium (M: week 27, 79; F: n.s.), ↓inorganic phosphorous (M: n.s, F: week 27, 53), ↓lactate dehydrogenase (M: week 79, 103; F: week 103)) **-changes in organ weights** (<u>Week 27:</u> ↑rel kidney (M: 15%), ↑adrenals (F: 38%), <u>Week 53</u>: ↑kidney (M: 10%), <u>Week 79:</u> ↑heart (M: 18%, F: 28%), ↑brain (F: 28%), ↑spleen (F. 13%), ↑kidney (F: 19%), <u>Week 104:</u> ↑brain (F: 23%), ↑thyroid (F:43%), ↑(heart (F: 16%), ↑adrenals (F: 9%), ↑thymus (F: 50%)) **-changes in urinalysis** (yellow/brown or orange discoloration (M, F), diuretic animals (M)) **-macroscopical changes** in <u>urinary bladder</u> (orange discoloration of the urinary bladder serosa) (M, F) and <u>skin</u> (orange staining (M, F)) **-histopathological changes** in <u>urinary bladder</u> (benign transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), <u>kidneys</u> (epithelial hyperplasia (M, F), renal papillary degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑	RAR Vol. 3, B.6.5.1/01 Anonymous 23 (1991) Report No.: AKJ/7/90 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
lacrimal gland and seminal vesicle were not investigated for histopathology GLP: No		renal focal calcification, ureter (epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F), pancreas (↑pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity)) NOAEL for systemic toxicity (M, F): 4 ppm (corresponds to 0.21 and 0.28 mg/kg bw/day in males and females, respectively) NOAEL for tumour incidence (M, F): 52 ppm (corresponds to 2.82 and 3.65 mg/kg bw/day in males and females, respectively)	
Carcinogenicity study Oral (dietary) No guideline claims in study report Mouse Crl:CD-1 (ICR)BR 50/sex/group *The study was checked for compliance with OECD TG 451 (adopted 7 September 2009). Following deviations were noted:* *i. the duration of study was 20 months (according to the guideline the duration of the study will normally be 24 months for rodents. Shorter or longer study durations may be used but should be justified).* *ii. cervix, coagulating gland, Hardian gland and lacrimal gland were not included in the*	ACN technical (Quinoclamine) Purity: 98.57% 0, 3, 30 or 300 ppm (corresponding to averages of 0, 0.38, 3.82 and 40.2 mg/kg bw/day in males and 0, 0.44, 4.48 and 46.4 mg/kg bw/day in females) 80 weeks	3 ppm: -clinical signs (orange fur staining) (M, F) 30 ppm: ↑**mortality** (M, F) -clinical signs (orange fur staining) (M, F) **-changes in organ weights** (↑rel kidney, M: 14% n.s.) **-histopathological changes** in adrenal (adrenal spindle cell hyperplasia (F), brown athrophy (F)); Stomach (hyperkeratosis and chronic inflammation (F)) 300 ppm: ↑**mortality** (M, F) -clinical signs (orange fur staining) (M, F) ↓**bw gain** (M: 33%, F: 30%) **-changes in organ weights** (↑rel liver (F: 20%), ↑rel kidney (M: 15% n.s., F: 24% n.s.), ↑rel heart (F), ↑brain (F)) **-histopathological changes** in adrenal (↑adrenal spindle cell hyperplasia (M), ↑brown athropy (F)), kidney (cortical scarring (M, F), hydronephrosis (M, F)), liver (chronic inflammation (F), brown pigmentation (F)), sciatic nerve (degeneration (F)), spleen (haemosiderosis (F), heart (generalised periarteritis (F), myocardial fibrosis (13 M, 2 F)), stomach (hyperkeratosis (M, F), epithelial hyperplasia (M), dilation of mucosal glands (M, F)), urinary bladder (epithelial hyperplasia (particularly F)), urether (dilation (M, F)), lymph nodes (histiocytosis (M, F). lympho reticular tissues (malignant lymphoma (F)) NOAEL (M, F): 3 ppm (corresponding to 0.38 and 0.44 mg/kg bw/day for males and females, respectively) LOAEL (M, F): 30 ppm (corresponding to 3.82 and 4.48 mg/kg bw/day in males and females, respectively) NOAEL for tumour incidence (F): 30 ppm (4.48 mg/kg bw/day) NOAEL for tumour incidence (M): 300 ppm (40.2 mg/kg bw/day)	RAR Vol. 3, B.6.5.2/01 Anonymous 24 (1993) New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
histopathological evaluation. GLP: Yes			

Table 2.6.5-2. Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.5-3. Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

The data available to assess this endpoint include one combined chronic toxicity/carcinogenicity study in the rat and one carcinogenicity study in the mouse. The studies are thoroughly presented in Vol. 3 to the RAR. The mouse study was conducted in accordance with the OECD Principles of Good Laboratory Practice (1981) but not the rat study. Both studies are however acceptable.

Combined chronic toxicity/carcinogenicity study in the rat (RAR Vol. 3, B.6.5.1/01):

In this study, treatment was associated with clinical signs (orange fur staining and reduced incidence of mass bearing) noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), reduced bodyweight gain noted in females at 676 ppm (carcinogenicity evaluation: 27%; toxicology evaluation: 28%), reduced food consumption noted in both sexes at 676 ppm, changes in haematological parameters (reduced packed blood cell volume, haemoglobin concentration and red blood cell count) noted in both sexes at 676 ppm, changes in biochemical parameters noted at 676 ppm (elevated blood urea nitrogen levels noted in males (n.s) and females (n.s), reduced calcium noted in males (s.s.) and females (n.s.), reduced inorganic phosphorous noted in males (n.s.) and females (s.s.), reduced lactate dehydrogenase noted in both sexes), findings in urinalysis such as yellow/brown or orange discoloration noted in both sexes in all treated groups and diuretic males noted at

676 ppm, changes in organ weights (increased relative kidney weights noted in males at ≥52 ppm (≥2.82 mg/kg bw/day), and in females at 676 ppm; increased relative spleen weights noted in females at 676 ppm; increased relative thymus weight noted in females at 676 ppm; increased relative thyroid weight noted in females at 676 ppm; increased relative heart weight noted in females at 676 ppm; increased relative adrenal weights noted in females at 676 ppm, increased relative brain weights noted in females at 676 ppm), gross pathology findings in the urinary bladder (orange discoloration) and skin (orange staining) noted in both sexes at 676 ppm, and histopathological changes noted in the urinary bladder (both sexes, ≥52 ppm corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively), kidneys (both sexes at ≥52 ppm), ureter (both sexes at 676 ppm), urethra (both sexes at 676 ppm), adrenals (both sexes at 676 ppm), pancreas (both sexes at 676 ppm), parathyroid (males at 676 ppm), mammary gland (females at 676 ppm) and lungs (males at ≥52 ppm and females at 676 ppm).

Neoplastic changes were noted in the urinary bladder (benign transitional cell papilloma noted in males at ≥52 ppm and in females at 676 ppm) and adrenals (increased incidence of benign phaeochromocytoma noted in both sexes at 676 ppm). The benign transitional cell papilloma noted in one single male at 52 ppm was considered of no clear relevance. No other differences in tumour incidence or type were seen which were considered to be dependent on the dose levels of ACN-technical.

Non-neoplastic changes in the urinary bladder consisted of: epithelial hyperplasia (both sexes at ≥52 ppm, corresponding to ≥2.82 and ≥3.65 mg/kg bw/day in males and females, respectively), polyp noted in one female at 676 ppm (38.3 mg/kg bw/day) and chronic inflammation noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively). Non-neoplastic changes in the kidneys consisted of: epithelial hyperplasia (both sexes at ≥52 ppm), renal papillary degeneration/necrosis (both sexes at 676 ppm), increased incidence of renal cortical scarring (both sexes at 676 ppm), pelvis polyp noted in one male at 676 ppm and increased incidence of renal focal calcification (in females at ≥52 ppm). Epithelial hyperplasia was also noted in the ureters (both sexes at ≥52 ppm) and urethra (both sexes at 676 ppm), and in the parathyroid (males at 676 ppm). Non-neoplastic changes in pancreas consisted of increased incidence of pancreatic acinar atrophy (both sexes at 676 ppm). The histopathological changes in the mammary gland consisted of decreased incidence of mammary acinar development and secretion (females at 676 ppm). Increased incidence of arterial calcification was noted in the lungs (males at ≥52 ppm, females at 676 ppm).

NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm (38.3 mg/kg bw/day), changes in haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both

sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm.

NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm.

Relevance of neoplastic changes- benign transitional cell papilloma in urinary bladder:

Treatment related neoplastic changes in the carcinogenicity study were confined to the presence of benign transitional cell papillomas in the urinary bladder of 4/47 males and 6/50 females in high dose group (676 ppm) at week 104 (Table B.6.5.1-1). These benign tumours were considered by the study author not to have been the cause of death in any animal but a transitional cell papilloma together with renal papillary lesions were considered to be the predominant pathology in one high dose female which was killed in extremis. The tumours were characterised by discrete exophytic epithelial masses with branching papillary processes supported by a fibrovascular core. The majority of 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 according to study author (Table B.6.5.1-3). No epithelial cellular atypia was seen and there was no neoplastic invasion of subepithelial connective tissues or muscle. The tumour in one female (killed in extremis) diagnosed as a probable transitional cell papilloma was described at necropsy as a pedunculated mass within the bladder and at microscopy was seen to be a large necrotic mass in the bladder lumen with no evidence of neoplastic invasion of the bladder wall.

Transitional cell papillomas in association with epithelial hyperplasia in the urinary bladder were also found in the toxicity study at week 104 (one male and two females from the high dose groups, and a single intermediate dose male) (Table B.6.5.1-2). The significance of the occurrence of a single tumour in one male intermediate dose animal is not clear. Epithelial hyperplasia noted in the treated animals was characterised by a generalised increase in the number of layers of cells in the urinary epithelium. The normal epithelium consisted of two to three layers of cells, minimal hyperplasia by five to eight layers of cells and marked hyperplasia by more than eight layers of cells. In several animals two degrees of hyperplasia were seen with focal area of moderate hyperplasia associated with diffuse minimal hyperplasia. Squamous metaplasia of the urinary epithelium was associated with epithelial hyperplasia in two high dose females. Epithelial haemorrhage was noted in two male and one female high dose animals. Acute inflammation was seen in association with a bladder tumour in an intermediate dose male (Table B.6.5.1-4).

Table 2.6.5.1-1: Transitional cell papilloma of the urinary bladder (carcinogenicity evaluation)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	49	47	47	49	48	49	50
Transitional cell papilloma	0	0	0	4	0	0	0	6

Terminal kill: number examined	27	27	20	30	26	29	30	38
Transitional cell papilloma	0	0	0	3	0	0	0	5
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Transitional cell papilloma	0	0	0	0	0	0	0	1
Died: number examined	6	4	6	11	4	3	0	2
Transitional cell papilloma	0	0	0	1	0	0	0	0

Table 2.6.5.1-2: Neoplastic pathology Terminal (104 week): Urinary bladder (toxicity evaluation)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	9	10	9	5	11	9	10	15
Transitional cell papilloma	0	0	1	1	0	0	0	2

Table 2.6.5.1-3: Non-neoplastic pathology-urinary bladder (carcinogenicity evaluation)

	Males					**Females**			
	0	**4**	**52**	**676**		**0**	**4**	**52**	**676**
Total: number examined	50	49	47	47		49	48	49	50
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
Cystitis/inflammation	1	2	1	3		0	0	0	2
Haemorrhage	0	0	0	1		0	0	0	2
Terminal kill: number examined	27	27	20	30		26	29	30	38
Epithelial hyperplasia	0	0	0	28		1	1	0	35
Squamous metaplasia	0	0	0	0		0	0	0	2
Polyp	0	0	0	0		0	0	0	1
Cystitis/inflammation	0	0	0	0		0	0	0	2
Haemorrhage	0	0	0	0		0	0	0	2
Killed in extremis: number examined	17	18	21	6		19	16	19	10
Epithelial hyperplasia	2	2	4	5		0	0	6	9
Squamous metaplasia	0	0	0	0		0	0	0	1
Polyp	0	0	0	0		0	0	0	0
Cystitis/inflammation	0	2	0	1		0	0	0	0
Haemorrhage	0	0	0	0		0	0	0	0
Died: number examined	6	4	6	11		4	3	0	2
Epithelial hyperplasia	1	0	1	8		0	0	0	2
Squamous metaplasia	0	0	0	0		0	0	0	0
Polyp	0	0	0	0		0	0	0	0
Cystitis/inflammation	1	0	1	2		0	0	0	0
Haemorrhage	0	0	0	1		0	0	0	0

Table 2.6.5.1-4: Non-neoplastic pathology-urinary bladder (toxicity evaluation week 104)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	9	10	9	5	11	9	10	15
Epithelial hyperplasia- total	0	0	2	5	1	0	0	15
Squamous metaplasia	0	0	0	0	0	0	0	2
Haemorrhage	0	0	0	2	0	0	0	1
Cystitis/acute inflammation	0	0	1	0	0	0	0	0
Chronic inflammation	0	0	1	0	1	0	1	0
Eosinophilic plug	0	0	1	0	0	0	0	0

RMS conclusion:

The findings of benign cell papillomas in urinary bladder shows that when fed at a dose level of 676 ppm the test article or its metabolites can cause the development of benign epithelial tumours of the urinary bladder. There is however no evidence in this study that the feeding of the test compound at dose levels of up to 676 ppm for a period of two years induces the development of malignant tumours in the urinary bladder.

Relevance of neoplastic changes- benign phaeochromocytoma in adrenal:

There was an apparent increase in the incidence of benign phaeochromocytomas of the adrenal medulla in high dose animals noted in the carcinogenicity study (Table B.6.5.1-5). Although the incidences were outside the ranges for benign phaeochromocytoma from historical control data in study report (Table B.6.5.1-6), they were considered not to be of biological significance by the study author, and were shown not to be of clear statistical significance. No increased incidence of benign phaeochromocytomas of the adrenal medulla was noted in the toxicity study.

Table 2.6.5.1-5: Phaeochromocytoma of the adrenal medulla (carcinogenicity evaluation)

	Males				**Females**			
	0	**4**	**52**	**676**	**0**	**4**	**52**	**676**
Total: number examined	50	24	28	47	50	29	27	50
Benign phaeochromocytoma	8	4	2	14	1	0	0	4
Malign phaeochromocytoma	2	0	0	1	0	0	0	0
Medullary hyperplasia	7	4	3	7	3	3	0	6
Terminal kill: number examined	27	2	0	30	26	8	7	38
Benign phaeochromocytoma	5	1	0	10	1	0	0	4
Malign phaeochromocytoma	2	0	0	1	0	0	0	0
Medullary hyperplasia	4	0	0	5	2	0	0	4
Killed in extremis: number examined	17	18	21	6	19	16	19	10
Benign phaeochromocytoma	2	1	2	2	0	0	0	0
Malign phaeochromocytoma	0	0	0	0	0	0	0	0
Medullary hyperplasia	3	4	2	2	1	2	0	2
Died: number examined	6	4	7	11	5	5	1	2
Benign phaeochromocytoma	1	2	0	2	0	0	0	0
Malign phaeochromocytoma	0	0	0	0	0	0	0	0
Medullary hyperplasia	0	0	1	0	0	1	0	0

The trend was statistically significant ($p<0.05$) for combined sexes

Table 2.6.5.1-6: Historical control data (in study report)

Tumour: background incidence data from carcinogenicity studies conducted at the laboratory in question Total number of animals examined: 349			
	Cumulative incidence	Cumulative percentage incidence	Individual percentage incidence range
Males			
Benign phaeochromocytoma	42	12.0	6.0-16.0
Malign phaeochromocytoma	1	0.3	0.0-2.0
Females			
Benign phaeochromocytoma	8	2.3	0.0-4.0
Malign phaeochormocytoma	1	0.3	0.0-2.0

RMS conclusion:

Increased incidence of benign phaeochromocytomas of the adrenal medulla in high dose animals was noted in the carcinogenicity study. The trend was statistically significant ($p<0.05$) for combined sexes. No increased incidence of benign phaeochromocytoma was noted in the toxicity study but it could be noted that few animals were examined at interim sacrifice time. The incidence of malignant phaeochromocytoma in adrenals was not increased in the study.

Carcinogenicity study in the mouse (RAR Vol. 3, B.6.5.2-01):

In this study, treatment was associated with clinical signs (orange fur staining) noted in both sexes at ≥3 ppm, increased mortality noted in both sexes at ≥30 ppm, reduced bodyweight gain (33% in males, 30% in females) noted at ≥300 ppm, changes in organ weight noted at ≥30 ppm (increased relative liver weight noted in females at 300 ppm, increased relative kidney weights (n.s.) noted in males at ≥30 ppm and in females at 300 ppm, increased relative heart and brain weights noted in females at 300 ppm), histopathological changes (At 300 ppm: increased incidence of malignant lymphoma noted in females, increased incidence of adrenal spindle cell hyperplasia noted in males, increased incidence of adrenal atrophy noted in females, kidney cortical scarring and hydronephrosis noted in both sexes, hepatic chronic inflammation and brown pigmentation noted in females, sciatic nerve degeneration noted in females, spleenic haemosiderosis noted in females, generalised periarteritis in females, myocardial fibrosis in particularly in males, hyperkeratosis in the stomach noted in both sexes, epithelial hyperplasia (males) and dilation of mucosal glands of the stomach (both sexes), epithelial hyperplasia of the urinary bladder (particularly in females), dilatation of the ureters in both sexes and histiocytosis of lymph nodes in both sexes; At 30 ppm: adrenal spindle cell hyperplasia and adrenal atrophy noted in females, and hyperkeratosis and chronic inflammation in the stomach in females).

The mortality in the study was exceptionally low, especially in controls. Only at the highest dose level (300 ppm) did mortality approach the levels expected in comparison to historical control data (-50%) (Table below)

Mortalities (%) divided by died and killed in extremis by the end of week 80

ACN technical (ppm)	Male		Female	
	Died	Killed in extremis	Died	Killed in extremis
0	6/50 (12%)	2/50 (4%)	5/50 (10%)	4/50 (8%)
3	12/50 (24%)	6/50 (12%)	7/50 (14%)	5/50 (10%)
30	15/50 (30%)	4/50 (8%)	11/50 (22%)	3/50 (6%)
300	17/50 (34%)	8/50 (16%)	19/50 (38%)	2/50 (4%)

NOAEL was 3 ppm (0.38 and 0.44 mg/kg bw/day for males and females, respectively) based on increased mortality noted in both sexes at ≥30 ppm, reduced bodyweight gain (33% in males, 30% in females) noted at ≥300 ppm, increased relative liver weight noted in females at 300 ppm, increased relative kidney weights noted in males at ≥30 ppm and in females at 300 ppm and histopathological changes noted in the adrenal and stomach at ≥30 ppm and in the kidney, urether, urinary bladder, liver, sciatic nerve, spleen, heart and lymph nodes noted at 300 ppm, and malignant lymphoma noted in females at 300 ppm.

Discussion- tumour incidence:

There were weakly statistically significant ($p<0.05$) positive trends for adrenal spindle cell tumour or hyperplasia (in fact virtually all hyperplasia in males but not in females), malignant lymphoma in females, but not males and histiocytic sarcoma (but only when both sexes were combined). Tumours identified in study are presented in Table 2.6.5.1-7.

Table 2.6.5.1-7: Tumours identified

Site	Tumour	Males				Females			
		Control	3 ppm	30 ppm	300 ppm	Control	3 ppm	30 ppm	300 ppm
Adenoma cortical	Cortical hyperplasia	3	1	0	2	0	0	0	0
Adrenal cortex	Benign tumour	3	0	1	2	0	0	1	0
	Tumour or hyperplasia	5	0	1	4	0	0	1	0
Adrenal spindle cell	Hyperplasia	11	3	4	18*	33	36	41*	30
	Spindle cell adenoma	0	0	0	0	1	0	0	0
Adrenal medulla	Hyperplasia	1	1	0	2	0	0	0	0
Harderian gland	Adenoma	3	1	1	0	1	0	0	0
Liver hepatocellular	Malignant tumour	4	3	4	4	1	0	0	0
	Haemangiosarcoma	0	1	0	0	0	0	0	0
	Benign tumour	9	7	7	5	0	1	0	0
	Adenoma	5	8	6	5	0	0	0	0
	Hyperplasia	2	2	0	2	0	1	0	0
Lung pulmonary	Carcinoma	7	4	7	1	2	2	1	1
	Haemangiosarcoma	0	0	0	1	0	1	0	0
	Hyperplasia	4	5	2	7	8	3	5	3
	Adenoma	10	11	7	8	1	7	2	2
Pancreas islet cell	Hyperplasia	3	1	0	0	2	0	0	0
Pituitary	Tumour or hyperplasia	0	0	1	0	1	0	0	1
	Benign tumour	0	0	1	0	0	0	0	1
Testis interstitial-cell	Cell adenoma	2	0	0	3	-	-	-	-
	Tumour or hyperplasia	5	0	1	2	-	-	-	-
Thyroid gland	Follicular adenoma	0	3	1	2	1	0	1	0
	Follicular hyperplasia	0	1	0	0	1	0	0	0
Skin/subcutaneous tissue	Lipoma	1	0	0	0	2	1	1	1
	Fibrosarcoma	0	1	0	0	5	4	2	8
Skin/subcutis epithelial	Fibrosarcoma	3	0	1	0	0	2	0	0
	Carcinoma	0	0	1	0	0	0	0	0
	Epidermal hyperplasia	18	14	18	15	0	0	0	0
Smooth muscle	Benign or malignant tumour	0	0	0	1	2	1	2	1
Fibrous tissue	Malignant tumour	3	1	1	0	0	1	1	0
Skin epidermal	Hyperplasia	0	5	2	9	6	2	21	15
	Fibrosarcoma	3	0	2	0	0	0	0	0
	Squamous carcinoma Squamous papilloma	0	0	0	1	0	1	0	0
Kidneys	Osseus metaplasia	0	0	0	1	0	0	0	0
Lympho reticular tissues	Malignant lymphoma	1	2	3	0	3	11	7	12
	Histiocytic sarcoma	0	0	0	1	0	1	1	2

*Significance at $p<0.05$, two-tailed probability values based on the chi squared test

Discussion- Malignant lymphoma:

Malignant lymphoma were seen in 1 male and 3 females in the control. In females there was a marginally significant ($p<0.05$) positive trend due mainly to a higher incidence at 300 ppm (12 cases) (Table 2.6.5.1-7) than in the controls (3 cases). The dose-response relationship was not smooth and no evidence of an effect was seen in males. In the study report, historical control data for Crl:CD-1(ICR)BR (VAF PLUS) mice are reported showing that the occurrence of malignant lymphoma in background data of females ranged from 2 to 11 cases (Table 2.6.5.1-9). In document "Reporting Table No. 2(8) (2007)" applicant has presented additional historical background data for CD-1 mouse for the laboratory in question (studies conducted between years 1991 to 1994), showing that the occurrence of malignant lymphoma in females ranged from 0-38%. According to the applicant the trend in the current study seems likely to be due to chance finding. It is stated by the study author, that a possible explanation for the increased variability might be a "cage effect". The meaning of "cage effect" was however not specified by the study author, and a substance related effect could not be excluded.

Comment by Co-RMS: It is proposed that the positive trend is not ignored and a substance related effect cannot be excluded. Therefore, the increased trend for malignant lymphoma in mice supports the proposed classification as carcinogenic in Cat. 2 (H351).

Table 2.6.5.1-8: Historical control data-lympho reticular tissues. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) male mice from carcinogenicity studies (in study report)

Number examined	50	50	50	50	50*	50	50	50*
Study duration in weeks	80	80	80	80	80	96	80	80
Malignant lymphoma	3	1	3	0	0	0	2	1
Monocytic leukaemia	1	0	0	0	0	0	0	0
Histiocytic sarcoma	0	0	1	0	1	0	0	0

* All were dietary and group housed, except those marked * which were singly housed, dermal studies

Table 2.6.5.1-9: Historical control data-lympho reticular tissues. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) female mice from carcinogenicity studies (in study report)

Number examined	50	50	50	50	50	50*	50	50	50*
Study duration in weeks	80	80	80	80	80	96	80	80	80
Malignant lymphoma	6	3	2	3	2	8	3	7	11
Monocytic leukaemia	1	1	1	1	0	0	0	0	0
Histiocytic sarcoma	0	0	3	3	1	6	3	2	3

* All were dietary and group housed, except those marked * which were singly housed, dermal studies

Table 2.6.5.1-10: Historical control data-haemopoietic tissue. Neoplastic historical control information in male CD-1 mouse for the laboratory in question (1991-1994) (data provided by applicant after preparation of DAR)

Study No.*	1	3	4	5	6	2	7		8	9	10	11		
End Date	1991	1992	1992	1992	1992	1993	1994		1994	1994	1994	1994		
Animals/cage	5	1	1	5	5	4	5		5	5	5	5		
No. examined	50	60	60	50	50	50	55		55	25	25	50	Total 530	Range of percentages
M-histiocytic sarcoma														
Incidence	0	2	1	0	0	0	0		2	0	0	0	5	
Percentage	0.0%	3.3%	1.7%	0.0%	0.0%	0.0%	0.0%		3.6%	0.0%	0.0%	0.0%	0.9%	0.0-3.6%
M-lymphoma														
Incidence	1	2	3	3	3	1	3		2	1	0	7	26	
Percentage	2.0%	3.3%	5.0%	6.0%	6.0%	2.0%	5.5%		3.6%	4.0%	0.0%	14.0%	4.9%	0.0-14.0%

* Dose route: Dietary (study no. 1, 2, 6 and 11), gavage (study no. 3, 4, 7, 8, 9, 10), subcut (study no. 5)

Table 2.6.5.1-11: Historical control data-haemopoietic tissue. Neoplastic historical control information in female CD-1 mouse for the laboratory in question (1991-1994) (data provided by applicant after preparation of DAR)

Study No.*	1	3	4	5	6	2	7	8	9	10	11		
End Date	1991	1992	1992	1992	1992	1993	1994	1994	1994	1994	1994		
Animals/cage	5	1	1	5	5	4	5	5	5	5	5		
No. examined	50	60	60	50	50	50	55	55	25	25	50	Total 530	Range of percentages
M-histiocytic sarcoma													
Incidence	0	3	3	2	0	1	1	0	1	0	4	15	
Percentage	0.0 %	5.0%	5.0%	4.0%	0.0%	2.0%	1.8 %	0.0 %	4.0%	0.0%	8.0%	2.8%	0.0-8.0%
M-lymphoma													
Incidence	3	6	6	12	6	19	4	0	3	3	9	71	
Percentage	6.0 %	10.0 %	10.0 %	24.0 %	12.0 %	38.0 %	7.3 %	0.0 %	12.0 %	12.0 %	18.0 %	13.4 %	0.0-38.0%

* Dose route: Dietary (study no. 1, 2, 6 and 11), gavage (study no. 3, 4, 7, 8, 9, 10), subcut (study no. 5)

Discussion- Histiocytic sarcoma:

No occurrence of histiocytic sarcoma was reported for the control. When treatment results for the sexes were combined, the trend, with 3 cases at 300 ppm (Table 2.6.5.1-7) was marginally significantly positive ($p<0.05$). The histiocytic sarcoma incidence was within background for both historical control data sets. In the study report, historical control data for Crl:CD-1(ICR)BR (VAF PLUS) mice are reported showing that the occurrence of histiocytic sarcoma in background data of mice ranges from 0 to 1 case in males, and 0-6 cases in females (Table 2.6.5.1-9 to 10). In document "Reporting Table No. 2(8) (2007)" applicant has presented additional historical background data for CD-1 mouse for the laboratory in question (studies conducted between years 1991 to 1994), showing that the occurrence of histiocytic sarcoma in mice ranged from 0-3.6% in males, and 0-8% in females. The occurrence of histiocytic sarcoma in background data suggests this is a chance finding due to an unusually low incidence on the controls in this study.

Discussion- Adrenal cortex/adrenal spindle cells (with and without control):

A spindle-cell adenoma was seen in one female (control), with spindle-cell hyperplasia (usually graded as minimal) seen in a further 11 males (control) and 33 female (control) (Table 2.6.5.1-7). In females, no dose-relationship was seen, but incidence of spindle-cell hyperplasia at 30 ppm was marginally significantly elevated ($p<0.05$) compared to the controls. In males, there was a significant increase ($p<0.05$) at 300 ppm. Cortical adenomas were seen in 6 males and 1 female (Table 2.6.5.1-7), however, no increased incidence was noted in treated groups compared to the control group, thus this incidence was unrelated to treatment. As a conclusion, there was no evidence of tumour formation in the adrenal.

Comment: No historical control data are available for adrenal spindle cell hyperplasia.

RMS conclusion:

The incidence of 3 histiocytic sarcoma at 300 ppm (showing a statistical significant positive trend but only when both sexes were combined) was within background for both historical control data sets. The occurrence of histiocytic sarcoma in background data suggests this is a chance finding due to an unusually low incidence on the controls in this study.

The adrenal spindle cell hyperplasia was significantly elevated for males at the top dose and at 30 ppm in females, thus there were no clear dose response relationship. Furthermore, there were no significant increases in benign or malign adrenal tumours.

The incidence of malignant lymphoma in females of the high dose group showed a significantly increased trend and was slightly outside historical control data in study report. Althought the incidence of malignant lymphoma did not show a smooth dose-response pattern, a treatment related effect could not be excluded.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 2.6.5.2-1. Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat	Urinary bladder benign transitional cell papilloma	Yes (two tumour types in one species)	No	No	Both sexes	No	Oral (dietary)	No data
Rat	Adrenal benign phaeochomocytoma	Yes (two tumour types in one species)	No	No	Both sexes	No	Oral (dietary)	No data

According to Regulation 1272/2008 (CLP) substances are classified for carcinogenicity in Category 1 (known or presumed human carcinogens) on the basis of epidemiological and/or animal data. Category 1 is subcategorised into 1A if the substance is *"known to have carcinogenic potential for humans, classification is largely based on human evidence" and 1B if "presumed to have carcinogenic potential for humans classification is largely based on animal evidence."*

As there is no human data available for quinoclamine that may be relevant for carcinogenicity, criteria for category 1A are not fulfilled.

For classification in category 1B evidence may be derived from *"[…]animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen) […] In addition on a case–by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."*
Sufficient evidence from animal studies is explained as *"a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. […]"*

Quinoclamine does not fulfil these criteria. Benign transitional cell papillomas in urinary bladder and phaeochromocytoma in adrenals were only found in one species (rat) and in one study. The incidence of malignant lymphoma was noted in one species only (mouse) and was slightly outside the historical control data in study report but was within historical control data submitted by the applicant. The effect showed no smooth dose response and was difficult to rule out.

The effects noted in the rat and mouse were not considered of a convincing evidence for a classification in category 1B.
The placing of substance in Category 2 (suspected human carcinogens) *"is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (2) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."*
Limited evidence from animal studies is explained as *"data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues organs"*

The significance of tumours observed in the chronic toxicity/carcinogenicity study in the rat, i.e. benign transitional cell papillomas in urinary bladder and phaeochromocytoma in adrenals, and the malignant lymphoma noted in female mice is discussed below based on considerations included in the CLP guidance:

(a) tumour type and background incidence;

Tumour type:
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 mouse (Crl:CD-1 (ICR) BR strain)

Background incidence:
Historical control data for benign transitional cell papilloma and benign phaeochromocytoma in adrenals in the rat are available in study report (time period for studies not specified) (Table 2.6.5.2-2)

Table 2.6.5.2-2: Historical control data for benign transitional cell papilloma in urinary bladder and benign phaeochromocytoma in adrenals (rat)

Tumour: background incidence data from carcinogenicity studies conducted at the laboratory in question Total number of animals examined: 349			
	Cumulative incidence	Cumulative percentage incidence	Individual percentage incidence range
Males			
Benign transitional cell papillomas in urinary bladder	1	0.3	0.0-2.0
Benign phaeochromocytoma in adrenals	42	12.0	6.0-16.0
Malign phaeochromocytoma in adrenals	1	0.3	0.0-2.0
Females			
Benign transitional cell papillomas in urinary bladder	-[1]	-[1]	-[1]
Benign phaeochromocytoma in adrenals	8	2.3	0.0-4.0
Malign phaeochromocytoma in adrenals	1	0.3	0.0-2.0

[1]No data

The incidence of benign transitional cell papillomas in urinary bladder noted in the rat was 4/47 (9%) in males and 6/50 (12%) in females. The frequencies observed in the study for males are outside of the background incidence of 2.0 (Table 2.6.5.2-2). Thus, the tumour can be considered to result from treatment with quinoclamine. No historical control data for this tumour type was available for females.

The incidence of benign phaeochromocytoma in adrenals noted in the rat was 14/47 (30%) in males and 4/50 (8%) in females. The frequencies observed in the study for males and females are outside of the background incidence of 16% and 4% for males and females, respectively (Table 2.6.5.2-2). Thus, the tumour can be considered to result from treatment with quinclamine.

Historical control data for malignant lymphoma in the mouse (Table 2.6.5.2-3)

Table 2.6.5.2-3: Historical control data-lympho reticular tissues. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) male mice from carcinogenicity studies (in study report, time spans for studies not specified)

Number examined	50	50	50	50	50*	50	50	50*
Study duration in weeks	80	80	80	80	80	96	80	80
Malignant lymphoma	3	1	3	0	0	0	2	1
Monocytic leukaemia	1	0	0	0	0	0	0	0
Histiocytic sarcoma	0	0	1	0	1	0	0	0

Table 2.6.5.2-4: Historical control data-lympho reticular tissues. Tumour incidence in untreated Crl:CD-1(ICR)BR (VAF PLUS) female mice from carcinogenicity studies (in study report, time spans for studies not specified)

Number examined	50	50	50	50	50	50*	50	50	50*
Study duration in weeks	80	80	80	80	80	96	80	80	80
Malignant lymphoma	6	3	2	3	2	8	3	7	11
Monocytic leukaemia	1	1	1	1	0	0	0	0	0
Histiocytic sarcoma	0	0	3	3	1	6	3	2	3

Table 2.6.5.2-5: Historical control data-haemopoietic tissue. Neoplastic historical control information in male CD-1 mouse for the laboratory in question (1991-1994) (data provided by applicant after preparation of DAR)

Study No.*	1	3	4	5	6	2	7		8	9	10	11		
End Date	1991	1992	1992	1992	1992	1993	1994		1994	1994	1994	1994		
Animals/cage	5	1	1	5	5	4	5		5	5	5	5		
No. examined	50	60	60	50	50	50	55		55	25	25	50	Total 530	Range of percentages
M-histiocytic sarcoma														
Incidence	0	2	1	0	0	0	0		2	0	0	0	5	
Percentage	0.0%	3.3%	1.7%	0.0%	0.0%	0.0%	0.0%		3.6%	0.0%	0.0%	0.0%	0.9%	0.0-3.6%
M-lymphoma														
Incidence	1	2	3	3	3	1	3		2	1	0	7	26	
Percentage	2.0%	3.3%	5.0%	6.0%	6.0%	2.0%	5.5%		3.6%	4.0%	0.0%	14.0%	4.9%	0.0-14.0%

Table 2.6.5.2-6: Historical control data-haemopoietic tissue. Neoplastic historical control information in female CD-1 mouse for the laboratory in question (1991-1994) (data provided by applicant after preparation of DAR)

Study No.*	1	3	4	5	6	2	7	8	9	10	11		
End Date	1991 (Diet)	1992 (Gavage)	1992 (Gavage)	1992 (Subcut)	1992 (Diet)	1993 ((diet)	1994 (Gavage)	1994 (Gavage)	1994 (Gavage)	1994 (Gavage)	1994 (Diet)		
Animals/cage	5	1	1	5	5	4	5	5	5	5	5		
No. examined	50	60	60	50	50	50	55	55	25	25	50	Total 530	Range of percentages
M-histiocytic sarcoma													
Incidence	0	3	3	2	0	1	1	0	1	0	4	15	
Percentage	0.0%	5.0%	5.0%	4.0%	0.0%	2.0%	1.8%	0.0%	4.0%	0.0%	8.0%	2.8%	0.0-8.0%
M-lymphoma													
Incidence	3	6	6	12	6	19	4	0	3	3	9	71	
Percentage	6.0%	10.0%	10.0%	24.0%	12.0%	38.0%	7.3%	0.0%	12.0%	12.0%	18.0%	13.4%	0.0-38.0%

The incidence of malignant lymphoma noted in the female mouse was 12/50 (24%). The frequencies were slightly outside of the historical control data in the study report of 11/50 (22%) but within historical control data provided by the applicant (up to 38%) (Table 2.6.5.2-3 to 6). Historical control data indicate high variability.

(b) multi-site responses;

Increased incidence of tumours at multiple sites were noted in the rat (benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals). In the mouse one site reponse was noted (malignant lymphoma in females)

(c) progression of lesions to malignancy;

Both types of tumour noted in the rat are considered benign. The type of tumour noted in the mouse (malignant lymphoma) is malign

Urinary bladder:
The benign transitional cell papillomas in urinary bladder in rats were characterised by discrete exophytic epithelial masses with branching papillary processes supported by a fibrovascular core. The majority of 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 noted and there was no neoplastic invasion of subepithelial connective tissues or muscle. Thus, a progression into malignancy was not observed.

Adrenals:
The incidence of malignant phaeochromocytoma in adrenals in rats was not increased in the study. Thus, a progression into malignancy was not observed.

(d) reduced tumour latency;

Urinary bladder:
The incidence of benign transitional cell papillomas in urinary bladder was noted in rats of both sexes at terminal sacrifice (Week 104). The tumours were not noted in the toxicology evaluation at interim kills (Weeks 26, 52, 78). Thus, there is no indication for reduced tumour latency.

Adrenals:
The incidence of benign phaeochromocytoma in adrenals in the rat was noted in the carcinogenicity study only. No increased incidence of benign phaeochromocytoma in adrenals was noted in the toxicology study at interim sacrifice (Weeks 26, 52, 78, 104). Thus, there is no indication for reduced tumour latency.

Lympho reticular tissues:
The incidence of malignant lymphoma in female mice was investigated at study termination (80 weeks).

(e) whether responses are in single or both sexes;

Benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals occurred in rats in both sexes. Malignant lymphoma occurred in mice in one sex (females).

(f) whether responses are in a single species or several species;

Benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals were observed in rats only. Malignant lymphoma was observed in the mice only.

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

No information available.

(h) routes of exposure;

Information restricted to studies performed using oral administration (via diet).

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

No information on human toxicokinetics available.

(j) the possibility of a confounding effect of excessive toxicity at test doses;

There was no adverse effect on mortality in the rat study. A slightly improved survival was recorded for female rats treated at 676 ppm (Table 2.6.5.2-3). In the mouse study the survival was adversely affected at 30 and 300 ppm (Table 2.6.5.2-4)

Table 2.6.5.2-3: Mortalities in the carcinogenicity (1-4) and toxicology (5-8) groups after 104 weeks [%]

ACN (ppm)	Male		Female	
	Group 1-4	Group 5-8	Group 1-4	Group 5-8
0	44	24	48	22
4	46	26	42	22
52	60	34	40	24
676	40	36	24	10

Table B.6.5.2/01-01: Mortalities (%) divided by died and killed in extremis by the end of week 80

ACN technical (ppm)	**Male**		**Female**	
	Died	**Killed in extremis**	**Died**	**Killed in extremis**
0	6/50 (12%)	2/50 (4%)	5/50 (10%)	4/50 (8%)
3	12/50 (24%)	6/50 (12%)	7/50 (14%)	5/50 (10%)
30	15/50 (30%)	4/50 (8%)	11/50 (22%)	3/50 (6%)
300	17/50 (34%)	8/50 (16%)	19/50 (38%)	2/50 (4%)

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

No data available.

RMS conclusion:

Quinoclamine induces benign tumours at multiple sites in Crl:CD(SD)BR rats of both sexes. The type of tumours consisted of benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals. In addition, malignant lymphoma was noted in female mice of the Crl: CD-1 (ICR) BR strain but not in males.

The incidences of the tumours noted in the rat (benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals) were clearly outside the historical control data, while the indicidence of the tumour noted in the mouse (malignant lymphoma) was just slightly outside the historical control data for the study report.

For the adrenal pheochromocytoma, high spontaneous tumour incidences are reported in male F344 rats and Sprague-Dawley rats (Regulation 1272/2008 (CLP) Part 3, section 3.6.2.3.2). However, although a high spontaneous tumour incidence is reported for some strain of rats, an effect caused by quinoclamine in Crl:CD(SD)BR rats could not be excluded. The incidence in the study was clearly above the historical control data.

With regard to the malignant lymphoma in the mouse, this tumour was noted in one species only and in one sex (females). The incidence (24%) was slightly above the historical control data for the study report (22%) but within historical control data by the applicant (0-38%). Thus, the historical control data showed high variability. However, the effect showed a statistically significant trend, which could not be dismissed, also taking into consideration that the control group in the study takes precedence over historical control data. On the other hand the tumour in the mouse was not a multiple response.

RMS proposes a classification of quinoclamine as carcinogenic in category 2 based on benign tumours (benign transitional cell papillomas in urinary bladder and benign phaeochromocytoma in adrenals) noted in the rat and malignant lymphoma noted in the mouse. The incidence of malignant lymphoma noted in the mouse was not considered of a convincing evidence for a classification in category 1B (no smooth dose response was shown, historical control data showed high variability, no multiple response).

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Classification of quinoclamine as carcinogenic in Category 2 (H351 “Suspected human carcinogens”) is proposed.

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 Acinar atrophy[1]	 50 3	 28 1	 29 5	 46 **19**	 48 0	 20 0	 23 1	 50 **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 Transitional cell papilloma	 50 0	 49 0	 47 0	 47 **4**	 49 0	 48 0	 49 0	 50 **6**
Adrenal medulla: Total: number examined Pheochromocytomas	 50 8	 24 4	 28 2	 47 14	 50 1	 29 0	 27 0	 50 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

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[2]. 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:

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

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

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[3]. 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%.

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

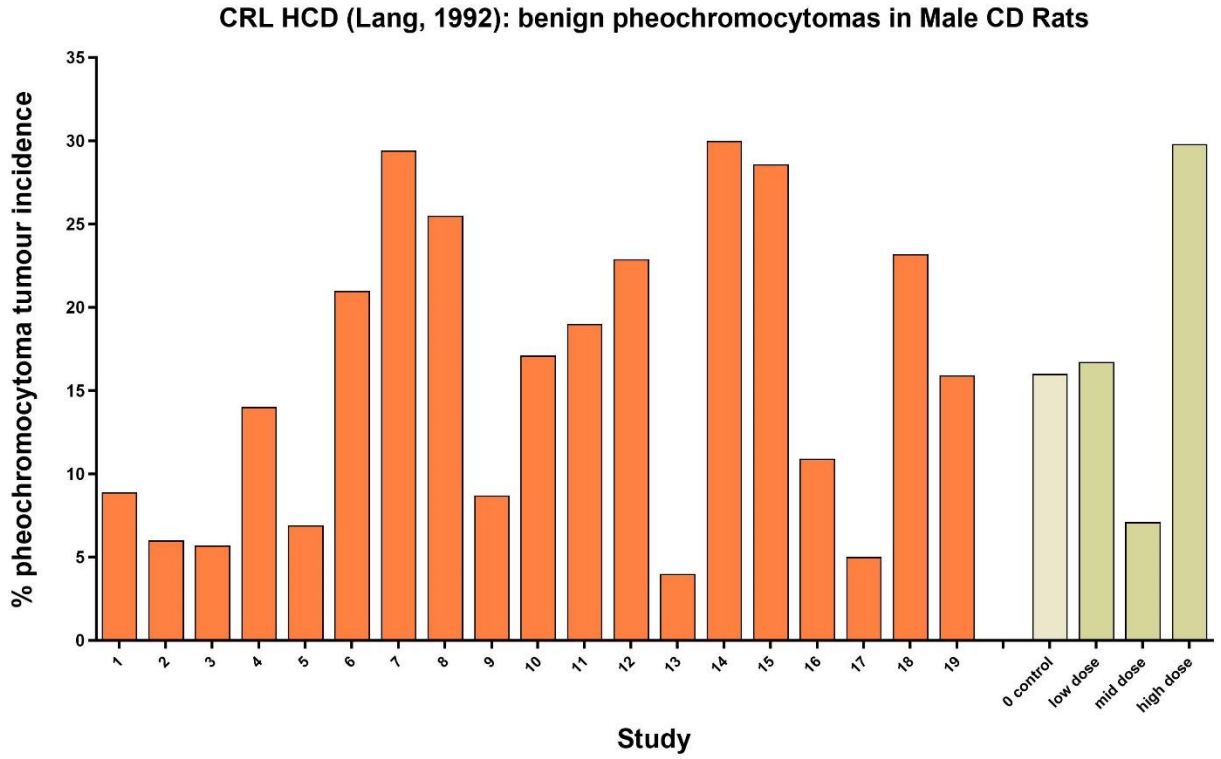


Figure: CRL study historical control data taken from Lang, 1992 which reported the incidence of spontaneous benign pheochromocytomas of the adrenal medulla in Crl:CD (SD) BR Rats from several control groups. This graph illustrates the high background and variable incidence of this tumour type in males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

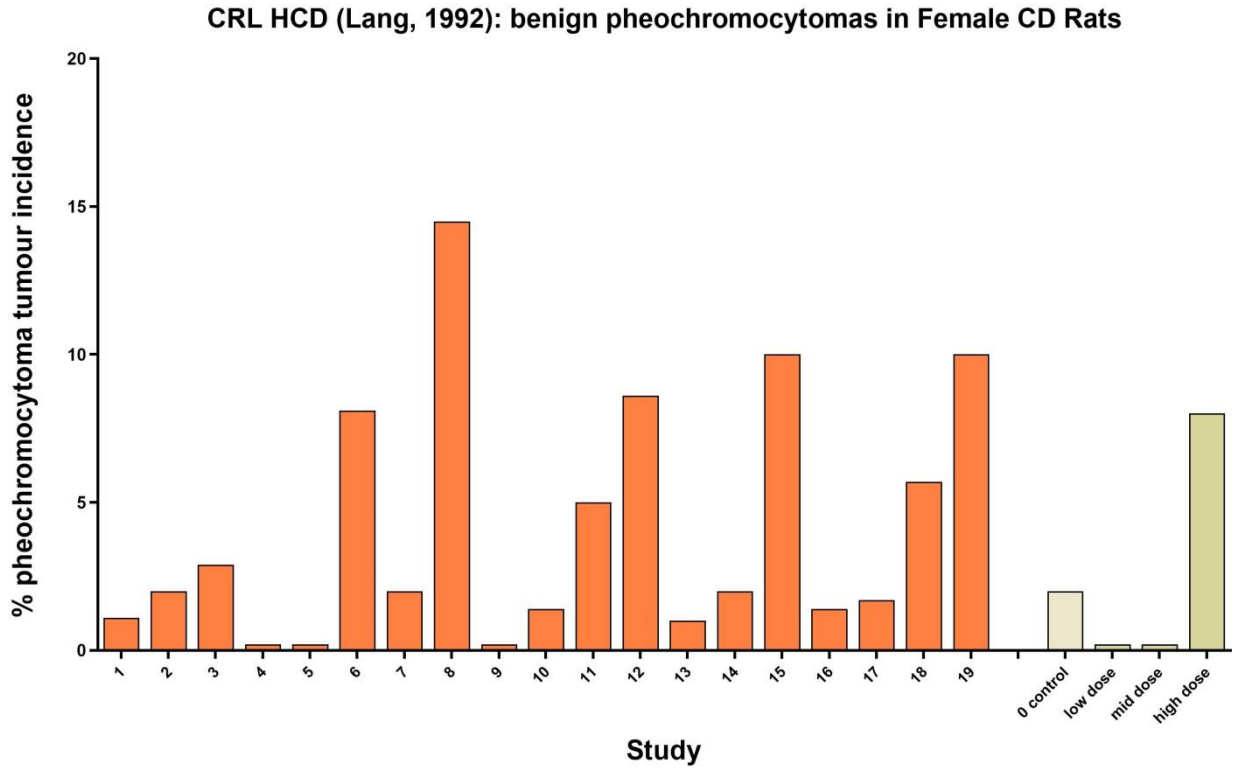


Figure: CRL study historical control data taken from Lang, 1992 which reported the incidence of spontaneous benign pheochromocytomas of the adrenal medulla in Crl:CD (SD) BR Rats from several control groups. This graph illustrates the high background and variable incidence of this tumour type in females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

2.6.6 Summary of reproductive toxicity

2.6.6.1 Adverse effects on sexual function and fertility – generational studies

Table 2.6.6.1-1. Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
Two generation reproduction study In-house method Rat Sprague-Dawley M, F 25/sex/group *Study was checked for compliance with OECD TG 416 (2001) and following deviations were noted:* *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*	K-1616 (Quinoclamine) Purity: 98.5% 0, 1, 25, 500 ppm Corresponding to: F0: 0, 0.07, 1.6, 30.9 mg/kg bw/day in males; 0, 0.08, 1.9 and 37.7 mg/kg bw/day in females F1: 0, 0.07, 1.7 and 37.0 mg/kg bw/day in males; 0, 0.08, 2.0 and 43.8 mg/kg bw/day in females The parents of both generations were fed the appropriate diets for at least nine weeks and then subjected to two subsequent mating trials. Fresh diets were prepared and presented weekly to the rats of all generations from initiation (P1) or weaning (F1b—>F2, F2b)	1 ppm: Parental: -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 3%; P2 M: 7%; P2 F 4%) ↓ bw gain (P1 M: 4%, P2 M: 11%; P2 F: 4%) Offspring: -increased incidence of gray lung cysts in F2b offspring reared for 3 months (18 compared to 11 in control group) 25 ppm: Parental: -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 1%; P2 M: 7%; P2 F 5%) ↓ bw gain (P1 M: 2%, P2 M: 11%; P2 F: 6%) Offspring: -increased incidence of gray lung cysts in F2b offspring reared for 3 months (29 compared to 11 in control group) 500 ppm: Parental: -**clinical signs** (F0/F1: hunched posture) ↓ **bw** (P1 M: 4%; P2 M: **10%**; P2 F **10%**) ↓ **bw gain** (P1 M: 7%, P2 M: **11%**; P2 F: 9%) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) Offspring: -clinical signs (orange stained fur F2b offspring) ↓ **bw** during lactation (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group)	RAR Vol. 3, B.6.6.1/01 Anonymous 19 (1975) Report No.: 854-111 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL (for sexual function and fertility, parents)** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
vii. Postlactational ovary (primordial and growing follicles) histopathology was not performed *viii. For the offspring, age at vaginal opening or PPS for the F1 and F2 was not determined* GLP: No		**-increased incidence of gray lung cysts** in F2b offspring reared for 3 months (39 compared to 11 in control group) NOAEL parental and offsprings: 25 ppm (1.6 mg/kg bw/day) NOAEL reproductive toxicity: 500 ppm (37 mg/kg bw/day)	

Table 2.6.6.1-2. Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.6.1-3. Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

Two generation reproductive toxicity study (RAR Vol. 3, B.6.6.2/01)

The study is no GLP study and considered limited due to several deviations from the OECD TG 416.

In the study, groups of 25 male and 25 female Sprague-Dawley rats received K-1616 (quinoclamine) in the diet at dose level up to 500 ppm (corresponding to 30.9 and 37.7 mg/kg bw/day in F0 males and females, respectively, and 37.0 and 43.8 mg/kg bw/day in F1 males and females, respectively) through two successive generations. Treatment with the test substance did not affect mating performance or fertility of the male and female parental animals and no consistent differences from control values were noted in comparisons of parental food consumption, survival rates and parturition indices or postnatal and postweaning survival. In addition, evaluations of the data obtained from foetuses taken by caesarean section did not reveal any findings indication teratogenic effects of the test substance at any of these concentrations.

Differences from control group data noted at the high dose level (500 ppm) included lower growth period mean body weight values in the P1 (4% at week 13) and P2 (10% at week 9) generation males and P2 generation females (10% at week 9), reduced bodyweight gain in P1 (7%) and P2 (11%) generation males and P2 (9%) generation females, lower mean offspring weights at weaning in all filial generations (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively), an increase in the observations of hunched appearance during the growth periods of both parental generations, and an increased incidence of gray lung cysts and orange-stained fur noted in the F2b offspring at necropsy. Mean litter size in F2a and F2b generations were also reduced at this dose level.

Differences noted to a lesser degree at the mid dose level (25 ppm) included slightly lower mean body weight values in the P1 (1% at week 13) and P2 (7% at week 9) generation males and P2 (5% at week 9) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (2%) and P2 (11%) generation males and P2 (6%) generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of gray lung cysts in the F2b offspring at necropsy.

Differences noted to a lesser degree at the low dose level (1 ppm) included slightly lower mean body weight values in the P1 (3%) and P2 (7%) generation males and P2 (4%) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (4%) and P2 (11%) generation males and P2 (4%) generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of lung cysts in the F2b offspring at necropsy.

Increased incidence of gray lung cysts was noted in the F2b offspring reared for three months (at 1 ppm: 18 compared to 11 in control group; at 25 ppm: 29 compared to 11 in control group; at 500 ppm: 39 compared to control group). The findings of gray lung cysts in the F2b offspring was dose related although the relevance of this finding is not clear. The finding was not observed in the offspring of the F1b generation reared for 5 weeks or in other available toxicological studies on quinoclamine. Thus, it seems to be a finding occurring in adult F2b animals including control animals. In the high dose group the incidence of gray cysts was 3.5 times higher when compared to controls, and therefore considered adverse. In the low and mid-dose groups the incidences were less marked (1.6 to 2.6 times higher when compared to controls) and not considered adverse in the absence of other effects in the offsprings at these dose levels.
The NOAEL for parental animals was set at 25 ppm (1.6 mg/kg bw/day) based on clinical signs (hunched posture) noted in P1 and P2 generation animals at 500 ppm (37 mg/kg bw/day), reduced body weight noted in P2 males and females at 500 ppm, and reduced bodyweight gain noted in P2 males at 500 ppm.

The NOAEL for offsprings was set at 25 ppm (1.6 mg/kg bw/day) based on reduced body weights at weaning in all filial generations noted at 500 ppm (37 mg/kg bw/day) and gray lung cysts noted in P2 offspring reared for 3 months at 500 ppm.

The NOAEL for reproductive toxicity was set at 500 ppm (37 mg/kg bw/day).

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

According to CLP Guidance Annex 1: 3.7.2.4.3, *"Classification is not necessarily the outcome in the case…when there is only a small reduction in foetal/pup weight…"*

Two- generation reproductive toxicity study:

Administration of quinoclamine at dietary concentrations of up to 500 ppm (30.9 mg/kg bw/day) did not have any effect on mating performance or fertility. Parental adverse findings were noted at 500 ppm and included clinical signs (hunched posture, F0/F1), reduced bodyweight (P1 males: 4%, P2 animals of both sexes: 10%) and reduced bodyweight gain (P1 males: 7%, P2 males: 11%, P2 females: 9%). Reduced litter size was noted in the second generation at 500 ppm (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group), and reduced body weights were noted in the offspring during lactation (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively). Furthermore, increased incidence of gray cysts in the lungs were noted in F2b offspring reared for 3 months (at 1 ppm: 18 compared to 11 in control group; at 25 ppm: 29 compared to 11 in control group; at 500 ppm: 39 compared to 11 in control group).

The observed effects in offspring noted at the highest dose level (500 ppm) (reduced bodyweight and reduced litter size) were not considered of concern for a classification for fertility effects, taken into account that the effects were seen in association with maternal toxicity (hunched posture and reduced body growth).

2.6.6.2 Adverse effects on development

Table 2.6.6.2-1. Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Teratology range finding study No guideline claimed in study Rat Crl:CD (SD) BR	ACN technical (Quinoclamine) Purity: 98.1% 0, 8, 50, 80, 200, 500 mg/kg bw/day	<u>Maternal effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>50 mg/kg bw/day:</u> -clinical signs (staining around eye) <u>80 mg/kg bw/day:</u> -clinical signs (stained urine, stained fur around head)	RAR Vol. 3, B.6.6.2.1/01 Anonymous 33 (1986) Anonymous 33 (1989) (addendum) Report No.: AKJ/2/86

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
F 5/group GLP: Yes	Vehicle: 0.25% gum tragacanth Gestation Days 7-17	**- bw loss/↓bw gain** (day 7-10: -3.5 g, day 10-13: 14% (n.s)) ↓FC (Pregnancy Days 7-10: 27%, Pregnancy Days 10-13: 20%, Pregancy Days: 13-17: 17%) **-macroscopic changes** (enlarged spleen in one female) <u>200 mg/kg bw/day:</u> **-mortality** (one animal died, two animals were killed in extremis) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss/↓bw gain** (day 7-10: -19.8 g, day 10-13:-1.5 g, day 13-17: 42% (n.s.)) ↓FC (Pregnancy Days 7-10: 52%, Pregnancy Days 10-13: 43%, Pregancy Days: 13-17: 29%) **-macroscopic changes** (enlarged spleen and adrenals, erosion of the stomach mucosa) ↑ **post-implantation loss** (24.5% compared to 2.4% in controls) <u>500 mg/kg bw/day:</u> **-mortality** (one animal died on day 10 of pregnancy, the remaining four animals were killed in extremis on days 10 or 11 of pregnancy) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss** (-34 g, day 7-10) **↓FC** <u>Developmental effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>50 mg/kg bw/day:</u> No treatment-related effects <u>80 mg/kg bw/day:</u> ↓mean foetal weight (8% n.s.) <u>200 mg/kg bw/day:</u> ↑ **postimplantation loss** (24.5% compared to 2.4% in controls) **↓mean foetal weight** (27%) *The study is acceptable as a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL.*	Report No.: AKJ/2A/89 (addendum) New data for the Annex I renewal: No
Teratology study No guideline claimed in study Rat	ACN technical (Quinoclamine) Purity: 98.1%	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects 20 mg/kg bw/day:	RAR Vol. 3, B.6.6.2.1/02 Anonymous 25 (1986) Report No.: AKJ/4/86

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Crl:CD (SD) BR F 24/group *The study is acceptable. It was checked for compliance with OECD TG 414 and following deviations were noted:* *i. Exposure time in study was once daily between days 7 and 17 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent) but also effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section)* *ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *iii. The choice of vehicle was not justified in study report* GLP: Yes	0, 5, 20 and 75 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17	**-macroscopic changes** (enlarged spleen, one dam) <u>75 mg/kg bw/day:</u> **- bw gain** (25% day 7-17) ↓ FC (Gestation Days 7-10: 25%, Gestation Days 10-13: 14%) **-macroscopic changes** (enlarged spleen, 4/24 dams) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> **-abnormalities** (innominate artery absent, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (7%) **-abnormalities** (innominate artery absent, four foetuses; situs inversus, two foetuses; interrupt aortic arch, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite; sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned) NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	New data for the Annex I renewal: No
Teratology range finding study No guideline claimed in study Rat	Quinoclamine Purity: 99.0% 0, 10, 50, 100 mg/kg bw/day	<u>Maternal effects:</u> <u>10 mg/kg bw/day:</u> ↓**bw gain** (18%) (Day 6-20) <u>50 mg/kg bw/day:</u> ↓**bw gain** (27%) (Day 6-20)	RAR Vol. 3, B.6.6.2.1/03 Anonymous 34 (2002) Report No.: 619/123-D6154

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
Crl:CD (SD) IGSBR F 7/group GLP: Yes	Vehicle: 1% aqueous methylcellulose Gestation Days 6-19	↓FC (Days 4-20: 14%), Days 6-19: 14%, Days 19-20: 48%) ↑**postimplantation loss** (6.2% compared to 2.8% in controls, mainly due to three deaths in one litter) ↑**number of early intrauterine deaths** (mean number: 1.0 compared to 0.4 in control) ↓**mean litter weight** (2%) <u>100 mg/kg bw/day:</u> ↓**bw gain** (41%) (Day 6-20) ↓FC (Days 4-20: 21%), Days 6-19: 21%, Days 19-20: 30%) ↓**gravid uterus weight** (17%) ↑**postimplantation loss** (10.7% compared to 2.8% in controls) ↑**number of early intrauterine deaths** (mean number: 1.2 compared to 0.4 in control) ↓**mean litter weight** (16%) ↓**mean litter size** (12 compared to 12.6 in control) <u>Developmental effects:</u> <u>10 mg/kg bw/day:</u> ↓**mean foetal weight** (8%) <u>50 mg/kg bw/day:</u> ↓**mean foetal weight** (11%) ↑**postimplantation loss** (6.2% compared to 2.8% in controls, mainly due to three deaths in one litter) ↑**number of early intrauterine deaths** (mean number: 1.0 compared to 0.4 in control) ↓**mean litter weight** (2%) <u>100 mg/kg bw/day:</u> ↓**mean foetal weight** (12%) ↑**postimplantation loss** (10.7% compared to 2.8% in control) ↑**number of early intrauterine deaths** (mean number: 1.2 compared to 0.4 in control) ↓**mean litter weight** (16%) ↓**mean litter size** (12 compared to 12.6 in control) *The study is acceptable as a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL.*	New data for the Annex I renewal: No
Teratology study No guideline claimed in study Rat	Quinoclamine Purity: 99.0% 0, 5, 20, 75 mg/kg bw/day	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 14 of gestation)	RAR Vol. 3, B.6.6.2.1/04 Anonymous 26 (2002) Report No.: 619/94-D6154

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Crl:CD (SD) IGSBR F 24/group GLP: Yes *The study is acceptable. It was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted:* *i. Exposure time in study was once daily between days 6 and 19 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent) but also effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section)* *ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *iii. The choice of vehicle was not justified in study report*	Vehicle: 1% aqueous methylcellulose Gestation Days 6-19	↓**bw gain** (Days 7-8: 62%, Days 17-19: 21%) ↓FC (Days 7-8: 14%, Days 9-12: 17%, Days 12-15: 10%, Days 15-17: 12%, Days 17-19: 12%) ↓**mean gravid uterus weight** (15%) ↓**mean litter weight** (13%) <u>75 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 10, nose rubbing) ↓**bw gain** (Days 17-19: 41%) **-bw loss** (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) ↓FC (Days 4-6: 9%, Days 6-7: 27%, Days 7-8: 44%, Days 8-9: 34%, Days 9-12: 30%, Days 12-15: 17%, Days 15-17: 13%, Days 17-19: 33%) ↓**mean gravid uterus weight** (30%) ↑**post-implantation loss** (11% compared to 5% in control, n.s.) ↑ **number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter size** (12 compared to 14.8 in control) ↓**mean litter weight** (29%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↓**foetal weight** (7%) ↓**mean litter weight** (13%) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (12%) ↓**litter weight** (29%) ↑**post-implantation loss** (11% compared to 5% in control) ↑pre-implantation loss (17.4% compared to 8.6% in control but within current background data) ↑**number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter size** (12 compared to 14.8 in control) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) **-malformations** (subcutaneous oedema (one foetus), retro-oesophageal aortic arch (one foetus), kidney misshapen (one foetus), hydropnephrosis (three foetuses)) NOAEL maternal: 5 mg/kg bw/day	New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		NOAEL developmental: 5 mg/kg bw/day	
Teratology range finding study No guideline claimed in study Rabbit New Zealand White F 5/group GLP: Yes	ACN (Quinoclamine) Purity: 98.1% 0, 8, 20, 50, 80/8[a], 200/20[a], 500/50[a] Vehicle: 0.25% gum tragacanth Gestation Days 6-18	<u>Maternal effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↑**post-implantation loss** (31.1 compared to 8.7 in control) <u>50 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 10: 4%, Day 14: 5%) ↓FC (days 6-10) ↑**post-implantation loss** (61.0 compared to 8.7 in control) <u>80/8 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 4%, Day 8: 3%, Day 10: 4%) ↓FC (n.s.) ↑**post-implantation loss** (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 6%, Day 10: 6%) ↓FC (Day 6-10: 80%) ↑**post-implantation loss** (30.0 compared to 8.7 in control) <u>500/50 mg/kg bw/day:</u> **-mortality** (both animals died, one died on day 9 and the other on day 10 of pregnancy)[b] **-clinical signs** (lethargy, hunched posture, dark coloured urine) ↓**bw** (Day 8: 12%) ↓FC (Day 6-10: 80%) <u>Developmental effects:</u> <u>8 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> ↑**post-implantation loss** (31.1 compared to 8.7 in control) **-malformations** (spina bifida, two animals, interrupted aortic arch major, one animal, hindlimb left malrotated, one animal) <u>50 mg/kg bw/day:</u> ↑**post-implantation loss** (61.0 compared to 8.7 in control) **-malformations** (interrupted aortic arch major, one animal, kidney left agenesis, one animal) <u>80 mg/kg bw/day:</u> ↑**post-implantation loss** (25.0 compared to 8.7 in control)	RAR Vol. 3, B.6.6.2.2/01 Anonymous 28 (1986) Report No.: AKJ/1/86 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
		200/20 mg/kg bw/day: ↑**post-implantation loss** (30.0 compared to 8.7 in control) *The study is acceptable as a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL.*	
Teratology study No guideline claimed in study Rabbit New Zealand White F 16/group *The study is acceptable. It was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted:* *i. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *ii. During the course of study relative humidity was within the range 54-76% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning)* *iii. The choice of vehicle was not*	ACN (Quinoclamine) Purity: 98.1% 0, 2.5, 7.5, 22.5 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 6-18	Maternal effects: 2.5 mg/kg bw/day: No treatment-related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day: ↓ bw gain (Day 6-9: 0 kg compared to 0.08 kg in control, Days 0-28: 5%) Developmental effects: 2.5 mg/kg bw/day: No treatment related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day: ↓foetal weight (5% n.s.) ↑**increased incidence of skeletal variants** (increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control)) -**malformations** (scoliosis, one animal, spina-bifida, three animals, anomalies of the aortic arch, two animals, sternebral fusions, three animals, hyperextension of limb or paw, one animal) NOAEL maternal toxicity: 22.5 mg/kg bw/day NOAEL developmental toxicity: 7.5 mg/kg bw/day	RAR Vol. 3, B.6.6.2.2/02 Anonymous 27 (1986) Report No.: AKJ/3/86 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
justified in study report GLP: Yes			
Teratology range finding study No guideline claimed in study Rabbit Crl.NZW/Kbl BR F 7/group GLP: Yes	Quinoclamine Purity: 99.0% 0, 5, 17.5, 30 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 7-28	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓**bw change** (Days 7-28: 12% of controls) ↓FC <u>30 mg/kg bw/day:</u> **-abortion** (one animal, on Day 29) ↓**bw change** (Days 7-28: 10% of controls) ↓FC (Days 7-28: 2.4%, Days 28-29: 4%) ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>17.5 mg/kg bw/day:</u> No treatment related effects <u>30 mg/kg bw/day:</u> **-abortions** (one animal, on Day 29) ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%) ↓**mean foetal weight** (3%) *The study is acceptable as a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL*	RAR Vol. 3, B.6.6.2.2/03 Anonymous 35 (2002) Report No.: 619/122-D6154 New data for the Annex I renewal: No
Teratology study OECD 414 Rabbit Crl.NZW/Kbl BR F 24/group	Quinoclamine Purity: 99.0% 0, 5, 17.5, 30 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 7-28	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day</u> ↓**bw change** (bw change Days 12-15: 67% of control) ↓**mean litter size** (8.4 foetuses per female compared to 9.5 in control) <u>30 mg/kg bw/day:</u>	RAR Vol. 3, B.6.6.2.2/04 Anonymous 29 (2002) Report No.: 619/155-D6154 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
The study follows OECD TG 414 except for following deviations: *i. Dosing of animals started on Day 7 of gestation (the guideline recommends administration to start on Day 6 of gestation)* *ii. During the course of study relative humidity was within the range 30-80% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning)* *iii. The choice of vehicle was not justified in study report* GLP: Yes		**-mortality** (one female killed on Day 18 of gestation[c]) ↓bw (Days 4-29: 7%) ↓**bw change** (Days 12-15: 0 kg compared to 0.12 kg in control, Days 4-29: 46% of control) ↓FC ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control) ↓**mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓**mean litter size** (8.4 foetuses per female compared to 9.5 in control) **-malformations** (hydronephrosis, one animal, increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 5.6% compared to 2.3% in control) <u>30 mg/kg bw/day:</u> ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control) ↓**mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) ↑ **specific foetal variations** (kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, asymmetric ossification of cervical vertebral centra) **- malformations** (hydronephrosis, 2 animals; increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 6.4% compared to 2.3% in control; misshapen nasal bone (8.0%, not present in historial ctr data at time for study); misaligned thoracic vertebral arch, one foetus, increased incidence of absent frontal, mean % foetus: 8.9% compared to 0.0% in control) NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	

[a]: The original design of the study was to dose five animals in each group 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.

Because mating was staggered over two days, half of the animals in each group received one dose at the initial high dose level and on subsequent days were dosed at the lower level (abbr. "a"). The other half of the animals in each group received the lower dose level throughout the dosing period.
[b]: At necropsy these animals showed pale liver, abnormal spleen and dark intestinal contents.
[c]: One pregnant high dose group female was killed on Day 18 of gestation following severe inappetence and body weight loss and clinical observation of red discharge from the urogenitial region. Necropsy examination did not reveal any macroscopic abnormalities.

Table 2.6.6.2-2. Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.6.2-3. Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Dermal embryo-foetal development study Rat In house method GLP: Yes	Quinoclamine Purity: 97.7% 5, 100, 600 mg/kg bw/day Vehicle: 1% Tween 80 Day 6 to 15 *post-coitum*	The study was performed to investigate the effects of the test article on the embryonic and fetal development of the rat when administered during the period of organogenesis. Three groups of twenty five sexually mature and mated female Sprague Dawley Crl:CD (SD)BR rats (8-12 weeks old) received Quinoclamine by dermal application at dose levels of 5, 100 and 600 mg/kg bw/day for 10 consecutive days from day 6 to 15 *post-coitum*, inclusive.	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> -clinical signs (coloured urine) -macroscopical changes (reddish discolouration of treated skin) <u>100 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) -macroscopical changes (reddish discolouration of treated skin) <u>600 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) ↓**bw loss** (Days 6-9: -0.41 g) ↓**bw gain** (Days 6-16: 31%) ↓FC -macroscopical changes (reddish discolouration of treated skin) No embryotoxicity or teratogenicity was noted in this study NOAEL maternal: 100 mg/kg bw/day NOAEL teratogenic effects: 600 mg/kg bw/day *The study is acceptable as supplementary data.*	RAR Vol. 3, B.6.8.2/01 Anonymous 30 (1996) Report No.: 1312-1416-001 New data for the Annex I renewal: No

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

This section is presented by two teratology studies in the rat and two teratology studies in the rabbit. In addition the results of range finding studies (two studies for each species) to the main studies are given in the Table 2.6.6.2-1. Furthermore, a supplementary study is available conducted in the rat by the dermal route (Table 2.6.6.2-3). All studies were conducted in accordance with the OECD Principles of Good Laboratory Practice.

Short summary on provided information

Rat:

In the first teratology study in the rat, the highest applied dose was 75 mg/kg bw/day. Treatment was associated with reduced bodyweight gain (25%) and food consumption noted in dams at 75 mg/kg bw/day, changes in gross pathology (enlarged spleen) noted in dams at ≥20 mg/kg bw/day, reduced foetal weight (7%) noted at 75 mg/kg bw/day and increased incidence of aortic abnormalities and skeletal variations noted at ≥20 mg/kg bw/day. The increased incidence of aortic abnormalities included innominate artery absent noted at ≥20 mg/kg bw/day and situs inversus and interrupt aortic arch noted at 75 mg/kg bw/day. The increased incidence of skeletal variants included effects on skull (hyoid not ossified) and vertebrae (thoracic centre one or more bilobed) noted at ≥20 mg/kg bw/day and effects on sternebrae (5th and 6th not ossified; one or more bilobed, bipartite or misaligned) noted at 75 mg/kg bw/day. The NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain (25%) noted in dams at 75 mg/kg bw/day and changes in gross pathology (enlarged spleen) noted in dams at ≥20 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight (7%) noted at 75 mg/kg bw/day and increased incidence of aortic abnormalities and skeletal variations noted at ≥20 mg/kg bw/day (Anonymous 25, 1986, Report No.: AKJ/4/86).

In the range finding study to the above mentioned main study, dose levels up to 500 mg/kg bw/day were tested. Treatment was associated with maternal mortalities (at 500 mg/kg bw/day: one animal died, the remaining four animals were killed in extremis on days 10 or 11 of pregnancy; at 200 mg/kg bw/day: one animal died, two were killed in extremis), clinical signs noted in dams at ≥50 mg/kg bw/day (at 500 mg/kg bw/day: lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head; at 200 mg/kg bw/day: lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head; at 80 mg/kg bw/day: stained urine, stained fur around head; at 50 mg/kg bw/day: staining around eye), maternal bodyweight loss or reduced maternal bodyweight (at 500 mg/kg bw/day: -34 g (days 7-10); at 200 mg/kg bw/day: -19.8 g (days 7-10), -1.5 g (days 10-13), 42% (n.s.) (days 13-17), at 80 mg/kg bw/day: -3.5 g (days 7-10), 14% n.s. (days 10-13)), reduced food consumption in dams noted at ≥80 mg/kg bw/day, macroscopic changes in dams (At 200 mg/kg bw/day: enlarged spleen and adrenals, erosion of the stomach mucosa; At 80 mg/kg bw/day: enlarged spleen in one dam), increased postimplantation loss noted at 200 mg/kg bw/day, and reduced mean foetal weight noted at 200 mg/kg bw/day (27%) and 80 mg/kg bw/day (8% n.s.) (Anonymous 33, 1986, Report No.: AKJ/2/86).

In the second teratology study in the rat, the highest applied dose was 75 mg/kg bw/day. Treatment was associated with maternal clinical signs noted in dams at 20 mg/kg bw/day (paddling of the forelimbs) and 75 mg/kg bw/day (paddling of the forelimbs and nose rubbing), reduced maternal bodyweight gain noted in dams at 20 mg/kg bw/day (Days 7-8: 62%, Days 17-19: 21%) and 75 mg/kg bw/day (Days 17-19: 41%), maternal bodyweight loss noted in dams at 75 mg/kg bw/day (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g), reduced food consumption noted in dams at ≥20 mg/kg bw/day, reduced gravid uterus weight noted at ≥20 mg/kg bw/day, reduced number of early intrauterine deaths noted at 75 mg/kg bw/day, reduced litter weight noted at ≥20 mg/kg bw/day, increased post-implantations loss noted at 75 mg/kg bw/day, reduced mean litter size noted at 75 mg/kg bw/day, reduced foetal weight noted at 20 mg/kg bw/day (7%) and 75 mg/kg bw/day (12%), increased incidence of skeletal variations (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) noted at ≥20 mg/kg bw/day, and malformations noted at 75 mg/kg bw/day. The observed malformations noted at 75 mg/kg bw/day consisted of subcutaneous oedema (one animal), retro-oesophageal aortic arch (one animal), kidney misshapen (one animal), and hydropnephrosis (three animals). NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain noted in dams at ≥20 mg/kg bw/day, body weight loss noted in dams at 75 mg/kg bw/day, reduced mean gravid uterus weight noted in dams at ≥20 mg/kg bw/day, reduced mean litter weight noted at ≥20 mg/kg bw/day, increased number of pre- and post-implantation losses and early intrauterine deaths noted at 75 mg/kg bw/day, and reduced mean litter size noted at 75 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight noted at ≥20 mg/kg bw/day, increased number of post-implantation loss and early intrauterine deaths noted at 75 mg/kg bw/day, reduced mean litter size noted at 75 mg/kg bw/day, increased incidence of skeletal variations noted at ≥20 mg/kg bw/day, and malformations noted at 75 mg/kg bw/day (Anonymous 26, 2002, Report No: 619/94-D6154).

In the range finding study to the above mentioned main study, dose levels up to 100 mg/kg bw/day were tested. Treatment was associated 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, increased post-implantation loss noted at ≥50 mg/kg bw/day, increased number of intrauterine deaths noted at ≥50 mg/kg bw/day, reduced mean litter size noted at 100 mg/kg bw/day, reduced mean litter weight noted at ≥50 mg/kg bw/day, and reduced foetal weight noted at all dose levels (8%, 11%, 12% in foetus of 10, 50 and 100 mg/kg bw/day groups, respectively) (Anonymous 34, 2002, Report No.: 619/123-D6154).

In the supplementary study, rats received Quinoclamine by the dermal route at dose levels up to 600 mg/kg bw/day. Treatment was associated with maternal toxicity consisted of clinical signs (coloured urine noted at ≥5 mg/kg bw/day and encrusted skin noted at ≥100 mg/kg bw/day), reduced bodyweight growth noted at 600 mg/kg bw/day (bodyweight loss: -0.41 g, reduced bodyweight gain Days 6-16: 31%), reduced food consumption, and macroscopical changes (reddish discolouration of treated skin). No embryotoxicity or teratogenicity were noted in this study. The maternal NOAEL was 100 mg/kg bw/day, and the NOAEL for teratogenic effects was 600 mg/kg bw/day (Anonymous 30, 1996, Report No.: 1312-1416-001).

Rabbit:

In the first teratology study in the rabbit, the highest applied dose was 22.5 mg/kg bw/day. Treatment was associated with maternal reduced bodyweight gain (5%) noted at 22.5 mg/kg bw/day, reduced foetal weight noted at 22.5 mg/kg bw/day (5%. n.s.), increased incidence of skeletal variants (increased number of caudal centra ≤15) noted at 22.5 mg/kg bw/day, and malformations noted at 22.5 mg/kg bw/day. The malformations noted at 22.5 mg/kg bw/day included scoliosis (one animal), spina-bifida (three animals), anomalies of the aortic arch (two animals), sternebral fusions (three animals) and hyperextension of limb or paw (one animal). NOAEL for maternal toxicity was 22.5 mg/kg bw/day (highest dose level). NOAEL for developmental toxicity was 7.5 mg/kg bw/day based on increased foetal variations (increased number of caudal centra ≤15) noted at 22.5 mg/kg bw/day, and malformations noted at 22.5 mg/kg bw/day (Anonymous 27, 1986, Report No.: AKJ/3/86).

In the range finding study to the above mentioned main study, dose levels up to 500 mg/kg bw/day were initially tested. Because of severe toxicity elicited at the highest dose level, doses were reduced after one dose to 8, 20 or 50 mg/kg bw/day. Because mating was staggered over two days, half of the animals in each group received one dose at the initial high dose level and on subsequent days were dosed at the lower level. The other half of the animals in each group received the lowere dose levels throughout the dosing period. Treatment was associated with maternal mortalities noted at 500/50 mg/kg bw/day (both animals died), clinical signs noted at ≥50 mg/kg bw/day (at 50 mg/kg bw/day: coloured urine, at 80/8 mg/kg bw/day: coloured urine, at 200/20 mg/kg bw/day: coloured urine, at 500/50 mg/kg bw/day: dark coloured urine, lethargy, hunched posture), reduced maternal bodyweight gain noted at ≥50 mg/kg bw/day (at 50 mg/kg bw/day: 4-5%, at 80/8 mg/kg bw/day: 4%, at 200/20 mg/kg bw/day: 6%, at 500/50 mg/kg bw/day: 12%), reduced food consumption noted at ≥50 mg/kg bw/day, increased incidence of post-implantation loss noted at ≥20 mg/kg bw/day, and malformations noted at ≥20 mg/kg bw/day. The malformations noted at 20 mg/kg bw/day consisted of spina bifida (two animals), interrupted aortic arch major (one animal) and hindlimb left malrotated (one animal). At 50 mg/kg bw/day interrupted aortic arch major (one animal) and kidney left agenesis (one animal) were noted (Anonymous 28, 1986, Report No.: AKJ/1/86)

In the second teratology study in the rabbit, the highest applied dose was 30 mg/kg bw/day. Treatment was associated with mortality noted in one dam at 30 mg/kg bw/day, reduced maternal bodyweight/bodyweight change noted at 17.5 mg/kg bw/day (bodyweight change Days 12-15: 67% of control) and 30 mg/kg bw/day (reduced body weight Days 4-29: 7%, bodyweight change Days 4-29: 46% of control), reduced maternal food consumption noted at 30 mg/kg bw/day, reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, reduced litter weight noted at 30 mg/kg bw/day, increased specific foetal variations noted at 30 mg/kg bw/day and foetal malformations noted at 17.5 mg/kg bw/day (hydronephrosis, abnormal terminal caudal verebrae) and 30 mg/kg bw/day (hydronephrosis, abnormal terminal caudal vertebrae, misshapen nasal bone, misaligned thoracic vertebral arch, absent frontal). The increased incidence of specific foetal variations consisted of: kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, and asymmetric ossification of cervical vertebral centra. NOAEL

for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight growth noted at 17.5 mg/kg bw/day (bodyweight change Days 12-15: 67% of control) and 30 mg/kg bw/day (bodyweight change Days 4-29: 46% of control), reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, and reduced litter weight noted at 30 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, reduced litter weight noted at 30 mg/kg bw/day, increased incidence of specific foetal variations noted at 30 mg/kg bw/day, and malformations noted at ≥17.5 mg/kg bw/day (Anonymous 29, 2002, Report No.: 619/155-D6154).

In the range finding study to the above mentioned main study, dose levels up to 30 mg/kg bw/day were tested. Treatment was associated with abortion noted in one dam at 30 mg/kg bw/day, reduced maternal bodyweight gain noted in dams at 17.5 mg/kg bw/day (12%) and 30 mg/kg bw/day (10%), increased post-implantation loss and increased number of late intrauterine deaths noted at 30 mg/kg bw/day, reduced mean litter weight noted at 30 mg/kg bw/day, and reduced mean foetal weight (3%) noted at 30 mg/kg bw/day (Anonymous 35, 2002, Report No.: 619/122-D6154).

In addition, to the provided studies on developmental toxicity on Quinoclamine, the notifier has asked an independent expert to assess the teratogenicity of Quinoclamine to provide an independent opinion as to whether the Risk Phrase R63 is justified. The view of the expert was given in following document:

- Reporting Table No. 2 (10-14). Quinoclamine "Assessment of Reproductive Toxicity Studies" (2002)

The conclusion from this assessment is summarised below:

Extract:

*"**In the two rat studies**, oral administration of Quinoclamine (ACN Technical) at dosages of 5, 20 or 75 mg/kg/day during the period of organogenesis (and fetal growth in the Covance study) was associated with maternal toxicity (retarded body weight gain/ body weight loss and reduced food consumption) at 20 mg/kg/day (Covance study) and 75 mg/kg/day (both studies). This resulted in reduced fetal body weight and associated retarded fetal ossification at 20 mg/kg/day (Covance study) and 75 mg/kg/day (both studies). In the Covance study, where dosing started one day earlier (DG 6), there was also an increase in pre-and post-implantation loss at 75 mg/kg/day.*

Considering both rat studies, the type, incidence and distribution of abnormalities/ malformations observed did not indicate that Quinoclamine was teratogenic at the dosages investigated. On the basis of these results, the NOEL (No Observed Effect Level) for Quinoclamine was 5 mg/kg/day for both the dam and embryo/fetus.

***In the two rabbit studies**, oral administration of Quinoclamine (ACN Technical) at dosages of 2.5, 7.5 or 22.5 mg/kg/day (Toxicol study) and 5.0, 17.5 or 30.0 mg/kg/day (Covance study) during the period of organogenesis (and fetal growth in the Covance study) was associated with maternal toxicity (retarded body*

weight gain/ body weight loss, reduced food consumption) at dosages of ≥17.5 mg/kg/day. This resulted in an increased incidence of post-implantation loss at 17.5 and 30 mg/kg/day in the Covance study, reduced fetal and/or litter weight at dosages of ≥ 17.5 mg/kg/day (both studies) and associated retarded fetal ossification at 22.5 mg/kg/day in the Toxicol study.

Considering both rabbit studies, neither the type, incidence or distribution of abnormalities/malformations observed indicated that Quinoclamine showed a specific dysmorphogenic effect at dosages up to and including those that were maternally toxic. The NOEL (No Observed Effect Level) for Quinoclamine was considered to be 5.0/7.5 mg/kg/day for both maternal and fetal parameters, in the Covance and Toxicol studies, respectively.

***In conclusion**, for both the rat and the rabbit oral embryo-fetal development toxicity studies, there is evidence of maternal toxicity and secondary embryo-fetal toxicity, as indicated by increased pre- and/or post-implantation loss, reduced fetal body weights and retarded fetal ossification in both rats and rabbits. However, for both species, the type, incidence and distribution of abnormalities/malformations were considered not to indicate a teratogenic potential for Quinoclamine when administered to pregnant animals during the period of organogenesis (and fetal growth). This conclusion is in agreement with that in the Assessment for Teratogenicity with ACN written by Anonymous 36 in 1989 based on the rat and rabbit studies performed at Toxicol in 1986 and indicates that the Risk Phrase R63 is not justified."*

An assessment of reproduction toxicity conducted by the applicant (Anonymous 36, 1989) is reported in Vol. 3, section B.6.6.2.3. In this assessment malformations as found in the teratogenicity studies in rats and rabbits of Anonymous 25 (Report No.: AKJ/4/86, Report No.: AKJ73/86, Report No.: AKJ/1A/89) were viewed. Furthermore the
2-generation study by Anonymous 19 (Report No.: 854-111) were considered. The conclusion from this assessment is summarised below (for details see Vol. 3, B.6.6.2.3):

"Retardation of foetal development involving retardation of ossification was induced with quinoclamine at a dose level showing maternal toxicity in the rat and the rabbit, but no obvious evidence indicated that quinoclamine has a capability of developing a teratogenic effect."

Overall relevance of the provided information on adverse effects on development (RMS):

Relevance of aortic arch malformations:

In the rat study by Anonymous 25, 1986 (Report No.: AKJ/4/86) incidences of aortic arch malformations were noted at the dose levels of 20 mg/kg bw/day (innominate artery absent) and 75 mg/kg bw/day (innominate artery absent, situs inversus and interrupt aortic arch). The incidence of situs inversus (mean% foetus: 0.8%) was outside historical control data for the laboratory in question (mean % foetus: 0.4%) (Table 2.6.6.2.1-1) while the defects innominate artery absent and interrupted aortic arch were not presented in historical control data for the study

performing laboratory in 1985. Additional historical control data are available for other laboratories than the study-performing laboratory for time points several years later (Table 2.6.6.2.1-2 to 4). These historical control data show that the effects on aorta arch can occur in controls. The opinion of RMS is however that it is not accurate to compare the study performed in 1986 with control data produced by another than the study-performing laboratory. Furthermore, it is not accurate to consider historical control data several years later compared to the time point of the study. According to OECD TG No. 43 (guidance document on mammalian reproductive toxicity testing and assessment) ±2 years are recommended for historical control data as a reasonable amount of time prior to the study being interpreted in order to avoid genetic drift in the laboratory animal population.

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) a single incidence of "interrupted aortic arch" occurred in the low dose group (incidence: 0.3%). No data for interrupted aortic arch were presented in the historical control data submitted by applicant for the laboratory in question (consisted of 6 studies preceding the rat study by Anonymous 26, but study year not specified) (Table 2.6.6.2.1-5). The incidence of interrupted aortic arch was however considered as incidental in this study since it occurred at a low frequency in the low dose group only.

In the rabbit study by Anonymous 27 (Report No.: AKJ/3/86) aortic arch abnormality was noted in two high dose foetuses (1.7%) (Table 2.6.6.2.1-6). Historical control data for the laboratory in question in 1985, shows that the incidence of aortic arch abnormality was within historical control value (2.2%) (Table 2.6.6.2.1-7). Aortic arch abnormality (interrupted aortic arch) was also noted in the range finding study (Report No.: AKJ/1A/86) to the main study of Anonymous 27 at the dose level of 20 mg/kg bw/day (one foetus) and 50 mg/kg bw/day (one foetus) (Table 2.6.6.2.1-8).

Table 2.6.6.2.1-1: Incidences with aortic arch malformations - rat study of Anonymous 25, 1986 (Report No.: AKJ/4/86)

Symptoms	Malformations in Quinoclamine study Anonymous 25 1986				Range of group means % in background data of the laboratory in 1985
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	
Litters: Foetuses:	21 273	20 285	21 273	21 263	167 2171
Innominate artery absent	0	0	1 (0.4 %)	4 (1.8 %)	--
Interrupted aortic arch	0	0	0	1 (0.3 %)	--
Situs inversus	0	0	0	2 (0.8 %)	0 – 0.4 %

--= malformation not included in the background data report

Table 2.6.6.2.1-2: Historical control data- MARTA 1989-1992. Visceral anomalies in Crl:CD(SD)BR rats. Summary of all studies

Symptoms	Malformations in Quinoclamine study Anonymous 25 1986				Historical control data compiled by MARTA 1989-1992
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	
Litters: Foetuses:	21 273	20 285	21 273	21 263	4698 37868 Total studies: 223
Innominate artery absent	0	0	1 (0.4 %)	4 (1.8 %)	3.39% (max foetal incidence) 18.18% (max litter incidence)
Interrupted aortic arch	0	0	0	1 (0.3 %)	0.45% (max foetal incidence) 6.25% (max litter incidence)
Situs inversus	0	0	0	2 (0.8 %)	0.86 % (max foetal incidence) 4.56% (max litter incidence)

Table 2.6.6.2.1-3: Historical control data- MARTA/MTA 1992-1994. Visceral alterations in Crl:CD(SD)BR rats. Gestation days 20 and 21

Symptoms	Malformations in Quinoclamine study Anonymous 25 1986				Historical control data compiled by Marta/MTA 1992-1994
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	
Litters: Foetuses:	21 273	20 285	21 273	21 263	4.935 24.340 Total studies: 229
Innominate artery absent	0	0	1 (0.4 %)	4 (1.8 %)	1.40% (max foetal) 9.10% (max litter)
Interrupted aortic arch	0	0	0	1 (0.3 %)	0.00% (max foetal incidence) 0.00% (max litter incidence)
Situs inversus	0	0	0	2 (0.8 %)	2% (max foetal incidence) 10% (litter incidence)

Table 2.6.6.2.1-4: Background pregnancy and foetal abnormality data from developmental toxicity studies on the Sprague-Dawley rat (Crl: CD(SD) BR. Charles River (UK) Limited. June 2000 to October 2005

Symptoms	Malformations in Quinoclamine study Anonymous 25 1986				Historical control data (range of group means %) Charles River (UK) Limited 2000-2005
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	
Litters: Foetuses:	21 273	20 285	21 273	21 263	458 2931
Innominate artery absent	0	0	1 (0.4 %)	4 (1.8 %)	0.0-1.8%

Table 2.6.6.2.1-5: Incidences with aortic arch malformations - rat study of Anonymous 26 2002 (Report No.: 619/94-D6154)

Symptom	Malformations in Quinoclamine study				Incidences reported in 6 studies[1] preceding the present study					
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	1	2	3	4	5	6
External / visceral abnormalities										
Litters: Foetuses:	24 354	24 307	24 330	24 263	22 293	22 273	23 299	23 306	22 323	23 319
Interrupted aortic arch	--	1 (0.3 %)	--	--	--	--	--	--	--	--

Retro-oesophageal aortic arch	--	--	--	1 (0.4 %)	--	--	--	--	--	--

[1]Year of study not specified

Table 2.6.6.2.1-6: Incidences with aortic arch malformations - rabbit study of Anonymous 27, 1986 (Report No.: AKJ/3/86)

Symptom	**Quinoclamine dose level (mg/kg bw/day)**				
	0	**2.5**	**7.5**	**22.5**	
External/visceral abnormalities					
Aortic arch abnormality	1 (0.8%)	-	-	2 (1.7%)	

Table 2.6.6.2.1-7: Comparison of incidences with aortic arch abnormalities noted at 22.5 mg/kg bw/day in the rabbit study of Anonymous 27, 1986 (Report No.: AKJ/3/86) and historical control data of the laboratory in 1985

IFIS-No.	Symptoms acc. to IFIS (symptom as described in study)	Malformations in Quinoclamine study at 22.5 mg/kg bw/day	Range of group means % in background data of the laboratory in 1985 (mean %)
External / visceral abnormalities		Total no. foetuses examined*: 915	Total no. foetuses examined*: 708
	Aortic arch abnormality	2 (1.7 %)	0 – 2.2 % (0.5%)

Table 2.6.6.2.1-8: Incidences with aortic arch malformations - range finding rabbit study of Anonymous 28, 1986 (Report No.: AKJ/1/86)

	0 mg/kg bw/day	8 mg/kg bw/day	20 mg/kg bw/day	50 mg/kg bw/day	80/8 mg/kg bw/day	200/20 mg/kg bw/day
	Number of affected foetuses in the group (mean %)					
Interrupted aortic arch major			1 (8.4%)	1 (8.4%)		

In November 2006, Sweden proposed to classify Quinoclamine with Repr. Cat. 3, R63 at the meeting of the Technical Committee on Classification and labelling in Arona, 15-16 may 2007. The industry was invited to submit their arguments for absence of developmental effects during the Follow-Up procedure. An independent expert was asked by the applicant to provide comments. These comments are presented in following paper:

- "Comments to FU I. Follow-up to the meeting of the Technical Committee on Classification and Labelling in Arona, 15-16 May 2007"

The conclusion drawn by the independent expert regarding the aortic arch abnormalities was that these findings were not considered to be indicative of a teratogenic effect for the following reasons:

1. In the rat study of Anonymous 26, 2002 (Report No.: 619/94-D6154) at the same dosages as used in the rat study of Anonymous 25, 1986 (Report No.: AKJ/4/86) the single incidence of "interrupted aortic arch" occurred in the low dose group. This argues for a spontaneous occurrence of this finding rather than a treatment-related effect.

2. For the abnormality "interrupted aortic arch", there was both a lack of a relationship to dose and lack of reproducibility of the finding. Interrupted aortic arch was observed in one high dose foetus in the rat study of Anonymous 25, 1986 (Report No.: AKJ/4/86), and in one high dose foetus in the rabbit study of Anonymous 27 (Report No.: AKJ/3/86). This finding was observed in one low dose foetus in the rat study of Anonymous 26,2002

(Report No.: 619/94-D6154) and was not observed in any foetuses in the rabbit study of Anonymous 29 (Report No.: 619/155-D6154) in which higher dosages were used and the dosing period was extended.
3. "Innominate artery absent" is described as an abnormality in the rat study of Anonymous 25, 1986 (Report No.: AKJ/4/86) while it is described as a variation in the rat study of Anonymous 26, 2002 (Report No.: 619/94-D6154). With the exclusion of the variation, "innominate artery absent", the overall incidence of abnormalities in the rat study of Anonymous 25, 1986 (Report No.: AKJ/4/86) would be 1, 0, 0, 3 (in three litters) foetuses in the control, 5, 20 and
75 mg/kg bw/day groups, respectively. Assessed together with the findings from the rat study of Anonymous 26, 2002 (Report No.: 619/94-D6154) there is no indication of a dose-related increase in any particular abnormality or group of related abnormalities in the two rat studies.
4. Three of the foetuses with "innominate artery absent" occurred in one litter in the rat study of Anonymous 25 1986 (Report No.: AKJ/4/86). This dam showed clear evidence of maternal toxicity – body weight loss between DG 8-9 (-8g), reduced food consumption between DG 7-10 (16.7 g compared with a control mean of 21.7 g), increased post-implantation loss and an enlarged spleen at necropsy.

Comments (RMS):
The two main rat studies (Anonymous 25, 1986 and Anonymous 26, 2002) were comparable with the major differences being the length on the dosing period (DG 7-17 and DG 6-19 in the respective studies), and the vehicle (0.25% gum tragacanth and 1% aqueous methylcellulose in the respectively studies). The maternal effects, i.e. body weight reductions and reductions in food consumption were more marked in the rat study by Anonymous 26 (2002). This may be explained by a problem with allocation of the animals for this study. Prior to the start of the study there was a statistically significant reduction of mean body weight in all dose groups and therefore the animals located in the dose groups may have been younger than control animals (animals used in this study weighted between 189.8 and 314.4 g).
RMS agrees with the conclusion by the expert that the effects on aortic arch occurred at low incidences. On the other hand the effect of aortic arch was presented in several studies and in both species. Thus, this effect could suggests an adverse effect of treatment, although the dose response pattern was not clear. It has been argued by the expert that the observed incidences of the aortic arch abnormalities can be attributed either to spontaneously occurring abnormalities or to marked maternal toxicity in some of the dams. RMS agrees that the incidence of interrupted aortic arch noted in the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) could be considered incidental since it occurred at a low frequency in the low dose group only. However, RMS does not agree that the findings of aortic arch malformations could be explained by maternal toxicity. It could be noted that aortic arch malformations could be found at dose levels without marked maternal toxicity.

RMS conclusion: Findings of aortic arch malformations were noted in both species and could not be explained by maternal toxicity. In the rabbit study by Anonymous 25 (1986) interrupted aortic arch was noted in two high dose foetuses (1.7%) at the dose level of 22.5 mg/kg bw/day. The incidence of interrupted aortic arch was within historical control data (2.2%). Incidences of interrupted aortic arch were also noted in the range finding study to the rabbit study of Anonymous 25 (1986) at the dose level of 20 mg/kg bw/day (one foetus) and 50 mg/kg bw/day

(one foetus). In the rat study of Anonymous 25 (1986) interrupted aortic arch was noted at the dose level of 75 mg/kg bw/day (one foetus). In addition, the aortic malformations situs inversus (two foetuses) and innominate artery absent (four foetuses) were noted at this dose level. Innominate artery absent was also noted in this study at the dose level of 20 mg/kg bw/day (one foetus). The incidence of situs inversus (mean% foetus: 0.8%) was outside the historical control data for the laboratory in question in 1985 (mean % foetus: 0.4%), while the defects innominate artery absent and interrupted aortic arch were not presented in this historical control data. An effect of Quinoclamine could not be excluded, although the incidence of aortic arch malformations was low and the effect was not reproducible in the study of Anonymous 26 (2002).

Relevance of hydronephrosis (=severe increased renal pelvis cavitation) and misshapen kidney:
In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) findings of the malformation hydronephrosis were noted at dose levels of 17.5 (one animal) and 30 mg/kg bw/day (two animals) (Table 2.6.6.2.1-9). The incidence of hydronephrosis noted in this study was dose related. No findings of hydronephrosis were presented in the historical control data in six studies preceding the present study (Table 2.6.6.2.1-10).

Additional historical control data are available for the laboratory in question (Table 2.6.6.2.1-11). These historical control data reflect cumulative defect data and the actual incidences in separate studies are not given. Furthermore, the period for the conduct of the studies is unknown. The opinion of RMS is that it is not accurate to compare the study performed in 2002 with control data produced several years from the time point of the study. According to OECD TG No. 43 (guidance document on mammalian reproductive toxicity testing and assessment) ±2 years are recommended for historical control data as a reasonable amount of time prior to the study being interpreted in order to avoid genetic drift in the laboratory animal population.

There were no foetuses with hydronephrosis, misshapen kidney or slight increased renal pelvis cavitation in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) at doses up to 22.5 mg/kg bw/day (highest dose level used in this study). At the dose level of 50 mg/kg bw/day, one single case of kidney left agenesis was found in the range finding study to this study Anonymous 28, 1986 (Report No.: AKJ/1/86).

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) findings of hydronephrosis were noted at the highest dose level of 75 mg/kg bw/day (3 animals) (Table 2.6.6.2.1-12). The incidence of hydronephrosis (1.1%) was slightly outside the control range (1.0%) of the six studies preceding the present study (Table 2.6.6.2.1-13). Furthermore, a single case of misshapen kidney occurred at 75 mg/kg bw/day. No findings of misshapen kidney were presented in the historical control data.

There were no foetus with hydronephrosis, misshapen kidney or slight increased renal pelvis cavitation in the rat study by Anonymous 27, 1986 (Report No.: AKJ/3/86) at doses up to 75 mg/kg bw/day.

Table 2.6.6.2.1-9: Incidences with hydronephrosis - rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154)

	Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day
	Incidence (mean % foetuses) Number of litters affected			
Kidney, cavitation increased, severe (hydronephrosis)			1 (0.8) 1	2 (1.6) 2
Variation: increased renal pelvic cavitation	1 (0.3) 1	3 (1.6) 2	6 (4.4) 3	11 (8.8) 6*

* $p<0.05$

Table 2.6.6.2.1-10: Comparison of incidences with hydronephrosis in rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) and historical control data of the laboratory

External / visceral abnormalities										
Symptom as described in study **Symptom as described in background data	Malformations in Quinoclamine study				Incidences reported in 6 studies[1] preceding the present study					
	Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day	1	2	3	4	5	6
Litters: Foetuses:	21 200	18 176	19 160	16 124	20 181	21 188	21 193	21 183	20 186	20 193
Hydronephrosis	-	-	1	2	-	-	-	-	-	-

[1]Year of study not specified

Table 2.6.6.2.1-11: Hydronephrosis in rabbits - Cumulative defect data in the historical background data of the laboratory[1]

Symptom	Cumulative defect data in historical background data of laboratory[1]		
	Control group	Inactive group	Combined control & inactive group
Litters: Foetuses:	Number and %	Number and %	Number and %
Increased renal pelvis cavitation (bilateral)	5 (0.12%)	4 (0.05%)	9 (0.08%)
Increased renal pelvis cavition (unilateral)	3 (0.07%)	16 (0.21%)	19 (0.16%)

[1] Incidences for individual studies not reported. Period for the conduct of studies not specified

Table 2.6.6.2.1-12: Incidences of foetuses (litters) with hydronephrosis, misshapen kidney and increased real pelvic dilation - rat study by Anonymous 26 (Report No.: 619/94-D6154)

Symptom	Malformations in Quinoclamine study			
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day
Hydronephrosis (uni/bilateral)	--	--	--	3 (1.1 %) (2)
Variation: increased renal pelvic cavitation	21 (11) 5.9%	10 (7) 3.4%	10 (6) 2.9%	8 (8) 3.3%
Abnormality + variation combined	21(11)	10 (7)	10 (6)	11 (9)
Kidney misshapen	--	--	--	1 (1) 0.4%

Table 2.6.6.2.1-13: Comparison of incidences with hydronephrosis in the rat study of Anonymous 26 (Report No.: 619/94-D6154) and control group values from six preceding embryo-foetal studies

Symptom	Malformations in Quinoclamine study				Incidences reported in 6 studies[1] preceding the present study					
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	1	2	3	4	5	6
Hydronephrosis (uni/bilateral)	--	--	--	3 (1.1 %) 2	3 (1.0 %)	--	1 (0.3 %)	--	--	1 (0.3 %)
Variation: increased renal pelvic cavitation	21 (5.9%) 11	10 (3.4%) 7	10 (2.9%) 6	8 (3.3%) 8						
Kidney misshapen	--	--	--	1 (0.4 %)	--	--	--	--	--	--

[1]Year of study not specified

For the follow-up to the meeting of the Technical Committee on Classification and Labelling in Arona, 15-16 May 2007, comments on the defect hydronephrosis were provided by an independent expert (document in dossier: "Comments to FU I. Follow-up to the meeting of the Technical Committee on Classification and Labelling in Arona, 15-16 May 2007"). Regarding the malformations hydronephrosis and misshapen kidney the conclusion by the independent expert was that these effects were not considered to be indicative of a teratogenic effect for the following reasons:

- *If hydronephrosis had been due to a teratogenic effect of Quinoclamine, it would be expected that this finding would be observed in repeat studies performed at similar dosages in the same strain of both rats and rabbits. There was a low incidence of hydronephrosis in both the rat and rabbit studies in the studies performed by Anonymous 26/29, but there were no foetuses with hydronephrosis in either the rat or the rabbit study performed by Anonymous 25/27.*
- *There was a genetic predisposition for hydronephrosis (=severe increased renal pelvic cavitation) in the rat strain used in the rat study by Anonymous 26 -one of the control dams had severe increased renal pelvic cavitation.*
- *In the studies of Anonymous 26/29 marked maternal toxicity was observed in the two high dose rat dams with foetuses with misshapen kidney and/or hydronephrosis and in one of the high dose rabbit dams with a foetus with hydronephrosis (Tables below)*

Table 1: Rat study by Anonymous 26 (Report No.: 619/94-D6154)

Observation	*Control mean values*	*75 mg/kg bw/day*		
		Mean values	*Dam no. 87*	*Dam no. 92*
Clinical signs:				
Paddling (immediately after dosing)	*Not observed in any control animal*	*Observed in all 24 animals*	*DG 12-19*	*DG 11-19*
Nose rubbing (immediately after dosing)		*Observed in all 24 animals*	*DG 13-19*	*DG 12-19*
Red discharge – uro-genital area		*Only observed in one female*	*DG 14*	*-*
Body weight change: DG 6-9	*+ 14.9 g*	*-7.5 g*	*-16.5 g*	*-22.8 g*
Corrected bodyweight change (DG 6-20)	*+13.8%*	*+6.8%*	*-1.5%*	*-1.5%-2.7%*
Food consumption: DG 7-8 *Food consumption: DG 8-9*	*27.7 g* *28.8 g*	*15.5 g* *18.9 g*	*15.2 g* *13.0 g*	*9.4 g* *9.1 g*
Litter parameters:				
Hydronephrosis (bilateral)			*Foetus R6*	*-*

Hydronephrosis (unilateral) *Kidney misshapen*			*Foetus R8* *-* *Only litter in study with 3 late resorptions*	*Foetus R1* *Foetus R3*

Table 2: Rabbit study by Anonymous 29 (Report No.: 619/155-D6154)

Observation	*Control Mean values*	*17.5 mg/kg bw/day*		*30.0 mg/kg bw/day*		
		Mean values	*Dam no. 52*	*Mean values*	*Dam no. 81*	*Dam no. 91*
Bodyweight change DG 7-19	*+200 g*	*+130 g*	*+50 g*	*- 10 g*	*- 250 g*	*+50 g*
% bw change: DG 7-29 (corrected)	*- 4.3*	*-7.0*	*- 12.0*	*- 9.1*	*0.0*	*- 9.1*
Food consumption (g/day)						
DG 7-8	*128*	*162*	*167*	*132*	*0*	*118*
DG 8-9	*131*	*158*	*139*	*130*	*4*	*116*
DG 9-12	*137*	*150*	*144*	*110*	*1*	*91*
DG 12-15	*138*	*127*	*144*	*75*	*0*	*29*
Litter parameters:						
Hydronephrosis (unilateral)			*R4*		*R6* *High post-implantation loss - 6 late resorptions*	*R5*

Comments (RMS):

The two main rabbit studies, Anonymous 27 (1986) and (Anonymous 29 2002), were partly comparable, with the major differences being the length of dosing (DG 6-18 and DG 7-28 in the respective studies), the vehicle (0.25% gum tragacanth and 1% aqueous methylcellulose in the respectively studies), and slightly higher doses in the rabbit study by Anonymous 29 (up to 30 mg/kg bw/day) compared to the dose levels used in the rabbit study by Anonymous 27 (up to 22.5 mg/kg bw/day). Maternal effects, i.e. body weight reductions were more marked in the Anonymous 29 study, which might be explained by the mentioned differences in study design.

Findings of hydronephrosis were noted in the rabbit study by Anonymous 29 at 17.5 mg/kg bw/day (one foetus) and 30 mg/kg bw/day (two foetuses). The effect was dose related and no findings of hydronephrosis were presented in the historical control data for
6 studies preceding the present study. Furthermore, a dose related incidence in the variation of "increased pelvic dilation" were noted in this study that was statistically significant in the high dose group. Findings of hydronephrosis were also noted in the rat study by Anonymous 29 (2002) at the highest dose level of 75 mg/kg bw/day (three foetuses). The incidence of this effect (1.1%) was slightly outside the historical control range of the 6 preceding studies (1.0%). Furthermore, a single case of misshapen kidney occurred in this study at 75 mg/kg bw/day, while no findings of misshapen kidney were present in the historical control data.

No findings of hydronephrosis were observed in the rabbit study of Anonymous 27 (1986). However, it could be noted that the highest dose level used in this study was lower than in the study of Anonymous 29 (2002). Furthermore, the length of dosing in the rabbit study of Anonymous 27 (1986) was shorter compared to the rabbit study of Anonymous 29 (2002).

RMS conclusion: The incidences of hydronephrosis observed in the rat and rabbit were outside the historical control range. The defect hydronephrosis observed in both sexes, the increased incidence of the variation increased pelvic dilation observed at high dose level in the rabbit study by Anonymous 29 (2002), and the single case of misshapen kidney also observed in the rabbit study of Anonymous 29 (2002), could suggest an adverse effect of treatment, also taking into account that the kidney is a target organ for Quinoclamine.

Relevance of abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, and misaligned thoracic vertebral arch noted in rabbits

In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) increased incidence of abnormal terminal caudal vertebrae, misshapen nasal bone and absent frontal were reported. These effects were classified as variations by the study author (Table 2.6.6.2.1-14).

Increased incidence of abnormal terminal caudal vertebrae was noted in the rabbit study by Anonymous 29 (2002) at 17.5 mg/kg bw/day (mean % foetuses: 5.6% compared to 2.3% in control) and 30 mg/kg bw/day (mean % foetuses: 6.4% compared to 2.3% in control) (Table 2.6.6.2.1-15). This effect was classified as a variation by the study author but consisted findings such as fused and absent caudal vertebrae and was therefore considered severe by the RMS. This effect was not present as a variant in the historical control data in six preceding studies. The defect misaligned, connected or absent caudal vertebra was however present as malformation in these historical control data showing low incidences (mean % foetuses: 0-1%) (Table 2.6.6.2.1-11). Maternal toxicity was present at the dose levels of ≥17.5 mg/kg bw/day. At 17.5 mg/kg bw/ reduced maternal bodyweight change (67% of control at days 12-15) was noted. The malformation abnormal terminal caudal vertebrae could however not be explained by maternal toxicity.

Misshapen nasal bone (incidence mean% foetuses: 8.0% compared) and absent frontal (incidence mean % foetuses: 8.9%) were noted at the highest dose level in the rabbit study by Anonymous 29 (2002) (Table 2.6.6.2.1-14). These effects were not present in the historical control data in six preceding studies. Additional historical control data from the laboratory is available for the defect misshapen nasal bone showing low incidence (0.59%) (Table 2.6.6.2.1-12). These historical control data reflect cumulative defect data and the actual incidences in separate studies are not given. Furthermore, the period for the conduct of the studies is unknown. The opinion of RMS is that it is not accurate to compare the study performed in 2002 with control data produced several years from the time point of the study. According to OECD TG No. 43 (guidance document on mammalian reproductive toxicity testing and assessment) ±2 years are recommended for historical control data as a reasonable amount of time prior to the study being interpreted in order to avoid genetic drift in the laboratory animal population.

Misaligned thoracic vertebral arch (one foetuses) was noted at the highest dose level (30 mg/kg bw/day) in the rabbit study by Anonymous 29 (2002) (Table 2.6.6.2.1-15). This malformation was not present in historical control data in six preceding studies. Although the incidence in the study was low, an effect of Quinoclamine could not be excluded. The effect was not noted in the rabbit study by Anonymous 27 (1986), but it could be noted that the highest dose level used in this study (22.5 mg/kg bw/day) was less when compared to the study by Anonymous 29 (2002) (30 mg/kg bw/day).

Conclusion by RMS: Increased incidence of abnormal terminal caudal vertebrae and incidences of misshapen nasal bone, absent frontal, and misaligned thoracic vertebral arch were reported in the rabbit study of Anonymous 29 (2002). These effects were not reported in the study of Anonymous 27 (1986). However, it could be noted that these studies were not fully comparable, with the major differences being the length of dosing (Anonymous 27 study: DG 6-18; Anonymous 29 study: 7-28) and slightly higher doses in the study by Anonymous 29 (up to 30 mg/kg bw/day) compared to the dose levels used in the

study by Anonymous 27 (up to 22.5 mg/kg bw/day). It could be noted that maternal toxicity was more marked in the study of Anonymous 29 (2002) compared to the maternal toxicity in the Anonymous 27 study (1986).

In the study of Anonymous 29 (2002), the incidences of abnormal terminal caudal vertebrae noted at 17.5 mg/kg bw/day (mean % foetuses: 5.6% compared to 2.3% in control) and 30 mg/kg bw/day (mean % foetuses: 6.4% compared to 2.3% in control) were considered severe by RMS, and were outside range of historical control data (mean % foetuses: 0-1%), and could not be explained by maternal toxicity. Furthermore, the findings of misshapen nasal bone (incidence mean% foetuses: 8%) and absent frontal (incidence mean % foetuses: 8.9%) and misaligned thoracic vertebral arch (one foetus) noted at the highest dose level (30 mg/kg bw/day) were considered severe by RMS, and were not presented in historical control data in the six preceding studies. An effect of Quinoclamine could not be excluded, although the incidences were low.

Table 2.6.6.2.1-14: Rabbit study by Anonymous 29, 2002 (Report No. 619/155-D6154): Defects classified as foetal variations by study author

	Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day	Statistics
	Incidence (mean % foetuses) Number of litters affected				
Kidney, cavitation increased, severe (hydronephrosis)			1 (0.8) 1	2 (1.6) 2	DR*F+
Kidney, cavitation increased, slight	1 (0.3) 1	3 (1.6) 2	6 (4.4) 3	11 (8.8) 6*	F+
Liver, additional lobe	1 (0.4) 1	3 (1.8) 3	6 (3.5) 4	6 (4.4) 5*	F+
Thymus, cervical remnant	10 (5.4) 5	15 (8.9) 8	19 (11.8) 10	16 (17.9) 9*	F+
Skull, anterior fontanelle, lengthened	1 (0.8) 1		1 (1.8) 1	5 (7.9) 5*	F+
Skull, frontal, absent				5 (8.9) 4*	F+
Skull, maxilla, ossification incomplete		3 (2.4) 1	2 (2.4) 2	4 (6.7) 3	DR*F+
Skull, nasal, misshapen				5 (8.0) 3	DR**F+
Skull, aquamosal, misshapen	1 (0.6) 1		1 (1.1) 1	3 (5.6) 3	F+
Sterneabrae, fused slight		4 (2.3) 3	5 (3.2) 2	6 (4.4) 5*	F+
Vertebral- terminal, misshapen/misaligned/fused/absent	5 (2.3) 3	2 (1.59 2	9 (5.6) 6	9 (6.4) 7	DR*F+
Vertebral-cervical centrum, ossification asymmetric		1 (0.8) 1	2 (1.2) 2	3 (2.7) 3	DR*F+

Table 2.6.6.2.1-15: Comparison of abnormalities in the Quinoclamine study and number of abnormalities reported in the historical control data of the laboratory (as summarised in addendum to DAR)

Skeletal abnormalities										
Symptom as described in study **Symptom as described in background data	Malformations in Quinoclamine study Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day	Incidences reported in 6 studies preceding the present study 1	2	3	4	5	6
Litters: Foetuses:	21 200	18 176	19 160	16 124	20 181	21 188	21 193	21 183	20 186	20 193
Vertebra										
Fused thoracic vertebral arch(es)	1	1	1					1		

Skeletal abnormalities										
Symptom as described in study **Symptom as described in background data	Malformations in Quinoclamine study Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day	Incidences reported in 6 studies preceding the present study 1	2	3	4	5	6
Litters: Foetuses:	21 200	18 176	19 160	16 124	20 181	21 188	21 193	21 183	20 186	20 193
Small vertebral arch(es) **Thoracic vertebral arch reduced in size	1									
Thoracic vertebral arch absent						1				
Additional thoracic vertebral arch **Additional thoraco-lumbar vertebral arch	1	1								
Misaligned thoracic vertebral arch Thoracic vertebral arch(es) malformed				1						
Fused thoracic (vertebral) centra **Thoracic vertebral centra fused	2				1			1	2	1
Hemicentric thoracic centrum **Thoracic vertebral centrum hemicentric			2							
Thoracic hemivertebra	1									
Additional thoracic hemivertebra				1					2	
Absent cervical vertebra	1									
Fused caudal vertebra, Cleft (caudal) vertebra **Caudal vertebra(e) (distal) misaligned, connected or absent		1	2				1		2 (Mean % foetal: 1%)	

Table 2.6.6.2.1-16: Cumulative defect data- rabbit. Foetal defect data of the laboratory[1]

Symptom	Cumulative defect data in historical background data of laboratory[1]		
	Control group	Inactive group	Combined control & inactive group
Number of foetuses examined	4233	7743	11976
Foetuses:	Number and %	Number and %	Number and %
Increased renal pelvis cavitation (bilateral)	5 (0.12%)	4 (0.05%)	9 (0.08%)
Increased renal pelvis cavitation (unilateral)	3 (0.07%)	16 (0.21%)	19 (0.16%)
Additional liver lobe	1 (0.02%)	3 (0.04%)	4 (0.03%)
Cervical remnant of the thymus	-	-	-
Lengthened anterior fontanelle	-	-	-
Misshapen nasal bone	13 (0.59)	5 (0.12)	18 (0.29)
Incomplete ossification of the frontal bones	304 (13.83%)	368 (9.04%)	672 (10.72%)
Incomplete ossification of the maxilla bones	9 (0.41%)	2 (0.05%)	11 (0.18%)
Fusion of the sternebrae	137 (3.24%)	217 (2.80%)	354 (2.96%)
Caudal vertebra (distal) misaligned. Connected or absent	68 (1.61%)	117 (1.51%)	185 (1.54%)
Cervical vertebral centra incompletely ossified	11 (0.26%)	18 (0.23%)	29 (0.24%)

Asymmetric ossification of cervical vertebral centra	-	-	-

[1] Incidences for individual studies not reported. Period for the conduct of studies not specified

Relevance of subcutaneous oedema and retro-oesophageal aortic arch

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) the malformations severe subcutaneous oedema and retro-oesophageal aortic arch were noted in one animal each at the highest dose level (75 mg/kg bw/day). These malformations were not reported in historical control data in six studies preceding the present study (Table B.6.6.2.1-13). Comparison with incidences reported in additional historical background data of the laboratory (Table B.6.6.2.1-18) showed that these malformation could occur in control animals but the incidences were low. The opinion of RMS is however, that it is not accurate to compare the study performed in 2002 with control data produced several years from the time point of the study. According to OECD TG No. 43 (guidance document on mammalian reproductive toxicity testing and assessment) ±2 years are recommended for historical control data as a reasonable amount of time prior to the study being interpreted in order to avoid genetic drift in the laboratory animal population. Maternal toxicity was noted in the rat study by Anonymous 26 (2002). At the dose level of 75 mg/kg bw/day maternal reduced bodyweight gain (Days 17-19: 41%), body weight loss (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) and reduced food consumption was noted. The maternal toxicity could however not explain the malformations.

Conclusion by RMS: An effect of Quinoclamine could not be excluded, although the incidences of subcutaneous oedema and retro-oesophageal aortic arch were low.

Table 2.6.6.2.1-17: Rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154). Comparison of abnormalities in the Quinoclamine study and control group values from six preceding embryo-foetal studies (supplied after December 1995)

Symptom	Malformations in Quinoclamine study				Incidences reported in 6 studies preceding the present study					
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	1	2	3	4	5	6
External / visceral abnormalities										
Litters: Foetuses:	24 354	24 307	24 330	24 263	22 293	22 273	23 299	23 306	22 323	23 319
Subcutaneous oedema	--	--	--	1 (0.4 %)	--	--	--	--	--	--
Retro-oesophageal aortic arch	--	--	--	1 (0.4 %)	--	--	--	--	--	--

Table 2.6.6.2.1-18: Comparison of abnormalities in the Quinoclamine study and incidences reported in historical background data of the laboratory

Symptom as described in study Symptom as described in background data	Malformations in Quinoclamine study				Incidences reported in historical background data of the laboratory*		
	Control	5 mg/kg bw/day	20 mg/kg bw/day	75 mg/kg bw/day	Control group	Inactive group	Combined control and inactive groups
External / visceral abnormalities							
Litters: Foetuses:	24 354	24 307	24 330	24 263	6208	11892	18100
Subcutaneous oedema (malformation) Subcutaneous oedema, trunk (variation) Oedema general (malformation)	--	--	--	1	 1 0	 8 4	 9 4
Retro-oesophageal aortic arch / Interrupted aortic arch, right subclavian artery arising from descending aorta (may be retro-oesophageal)	--	--	--	1	1	0	1

* Incidences for individual studies not reported. Period for the conduct of studies not specified
** This symptom is classified as variation in the background data

Relevance of hyperextension of limb or paw, spina bifida, scoliosis and sternebral fusion:

Hyperextension of limb or paw:

Hyperextension of limb or paw was noted in all treated groups in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86). Incidences were 2 animals (1.7%), 1 animal (0.9%) and 1 animal (0.9%) for the low-, mid- and high dose, respectively (Table 2.6.6.2.1-19). Since the hyperextension of limb or paw occurred without clear dose response pattern and was within historical control data (7.2%) (Table 2.6.6.2.1-20), the effect noted at 2.5 and 7.5 mg/kg bw/day was not considered to be treatment-related, also taken into consideration that the numbers of foetuses with major abnormalities in each group was not increased at the dose levels of 1.5 and 7.5 mg/kg bw/day (Table 2.6.6.2.1-21).

Spina bifida:

Spina bifida was noted in the low and high dose group in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86). The incidences were 2 and 3 animals for the low- and high dose, respectively (Table 2.6.6.2.1-19). The incidences (1.7% and 2.6% for the low and high dose, respectively) were within historical control data (8.4%). The effect noted at the low dose (2.5 mg/kg bw/day) was not considered treatment-related considering that the numbers of foetuses with major abnormalities in each group was not increased at this dose level (Table 2.6.6.2.1-21).

Scoliosis:

Scoliosis was noted at 7.5 and 22.5 mg/kg bw/day in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86). The incidence was one animal in each group. Since the incidence of scoliosis (0.9%) occurred without clear dose response pattern and was within historical control data (3.4%) (Table 2.6.6.2.1-20) the effect noted at 7.5 mg/kg bw/day was not considered to be treatment-related, also taken into consideration that the number of

foetuses with major abnormalities in each group was not increased at the dose level of 7.5 mg/kg bw/day (Table 2.6.6.2.1-21).

Sternebral fusion:

Sternbral fusion was noted in three animals of the high dose group (22.5 mg/kg bw/day) in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86). The incidence of sterebral fusion (2.6%) was outside the historical control data (0.7%) (Table 2.6.6.2.1-20).

Conclusion by RMS: In the rabbit study by Anonymous 27 (1986) numbers of foetuses with major abnormalities in each group was increased at 22.5 mg/kg bw/day. At the highest dose level (22.5 mg/kg bw/day) hyperextension of limb or paw (one animal) and scoliosis (one animal) was noted. Increased incidences of spina bifida (three animals) and sternebral fusion (three animals) were also noted at 22.5 mg/kg bw/day. An effect of Quinoclamine could not be excluded, although the incidences were low and with exception of the occurrence of sterebral fusion within historical control data, and these defects were not found in the rabbit study of Anonymous 29 (2002).

Table 2.6.6.2.1-19: Incidences of hyperextension of limb or paw, scoliosis, spina-bifida and sternebral fusion in the rabbit study by Anonymous 27 (1986)

Symptom	**Quinoclamine dose level (mg/kg bw/day)**			
	0	**2.5**	**7.5**	**22.5**
Hyperextension of limb or paw (Arthrogryposis bilateral)[a]	-	2 (1.7%)	1 (0.9%)	1 (0.9%)
Spina bifida	-	2 (1.7%)	-	3 (2.6%)
Fused sternebrae (major fusion)	-	-	-	3 (2.6%)
Scoliosis	-	-	1 (0.9%)	1 (0.9%)

[a]Arthrogryposis has been categorized as “hyperextension of limb or paw” but may also fall under “malrotated limbs”

Table 2.6.6.2.1-20: Comparison of abnormalities in the Quinoclamine study and incidences reported in historical background data of the laboratory

IFIS-No.	**Symptoms acc. to IFIS (symptom as described in study)**	**Malformations in Quinoclamine study at 22.5 mg/kg bw/day**	**Background data of the laboratory in 1985 (range of group %)**
External / visceral abnormalities		Total no. foetuses examined*: 915	Total no. foetuses examined*: 708
10069	Hyperextension of limb or paw (Arthrogryposis, bilateral)	1 (0.9 %)	#
10072	Malrotated limb	#	0 – 7.2 % (0.4%)
10120	Spina bifida	3 (2.6 %)	0 – 8.4 % (0.2%)
Skeletal abnormalities			
10614	Fused sternebra (major fusion)	3 (2.6 %)	0 – 0.7 % (0.1%)
10116	Scoliosis	1 (0.9 %)	0 – 3.4 % (0.8%)

* Both controls and inactive treatments
#Arthrogryposis has been categorized as “hyperextension of limb or paw” but may also fall under “malrotated limbs”.

Table 2.6.6.2.1-21: Rabbit study by Anonymous 27 (1986) –group mean data

ACN (mg/kg bw/day)	foetal weight (g)	No of foetuses examined	Litters examined	External / visceral abnormalities (%)		Skeletal abnormalities (%)	
				minor	major	minor	major
0	37.4	127	16	1.5	2.6	24.0	1.3
2.5	37.1	118	16	4.2	4.1	33.2	2.1
7.5	39.2	110	16	3.9	1.3	27.5	0.6
22.5	35.6	116	16	2.3	6.0	21.7	9.0

Relevance of foetus growth retardation and minor foetal variations:

In the rat study by Anonymous 25, 1986 (Report No.: AKJ/4/86) reduced foetal weight (7%) were noted at the dose level of 75 mg/kg bw/day and the incidence of minor skeletal variants (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite; sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned) was increased. These findings were considered to be secondary to the maternal toxicity observed at this dose level. Maternal toxicity at this dose level consisted of reduced bodyweight gain (day 7-17: 25%) and enlarged spleen (4/24 animals). Increased incidence of skeletal variants (skull hyoid not ossified; vertebrae: thoracic centre one or more bilobed) was also noted at the dose level of 20 mg/kg bw/day. At this dose level maternal toxicity was not marked but consisted of enlarged spleen in one dam only.

In the rat study by Anonymous 34, 2002 (Report No.: 619/123-D6154) reduced foetal weight was noted at the dose level of 20 mg/kg bw/day (7%) and 75 mg/kg bw/day (12%), and the incidence of skeletal variations (incomplete ossification of skull bone and unossified fifth sternebrae) was increased at ≥20 mg/kg bw/day. These findings were considered to be secondary to the maternal toxicity observed at these dose levels. At 20 mg/kg bw/day maternal reduced bodyweight gain (Days 7-8: 62%, Days 17-19: 21%) and reduced food consumption were noted. At
75 mg/kg bw/day maternal reduced bodyweight gain (Days 17-19: 41%), body weight loss (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) and reduced food consumption were noted.

In the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) reduced foetal weight (5%, n.s.) was noted at the highest dose level of 22.5 mg/kg bw/day. Furthermore, increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control) was noted at this dose level. Minor maternal toxicity (reduced bodyweight gain, Days 0-28: 5%) was noted at 22.5 mg/kg bw/day. The reduced foetal weight was considered to be secondary to the maternal toxicity observed at this dose level. The finding of foetal variation (i.e. increased no. of caudal centra ≤15) could however not be explained by maternal toxicity.

In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) reduced litter weight (24%) was noted at 30 mg/kg bw/day and the incidence of specific foetal variations was increased at this dose level. The increased incidence of specific foetal variations included: kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior frontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, and asymmetric ossification of cervical vertebral centra (Table 2.6.6.2.1-22). The findings of abnormal

terminal caudal vertebra, misshapen nasal bone and absent frontal were reported by the study author as variations (Table 2.6.6.2.1-22) but were considered as malformations by the RMS. Maternal toxicity was noted at ≥17.5 mg/kg bw/day. Reduced maternal bodyweight change was noted at 17.5 mg/kg bw/day (Days 12-15: 67% of control) and 30 mg/kg bw/day (Days 4-29: 46% of control). In addition, reduced maternal body weight (7%) and mortality (one dam was killed on Day 18 of gestation due to severe inappetence and body weight loss and had red discharge from the urogenital region) was noted at 30 mg/kg bw/day. The finding of reduced skeletal ossification (incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, asymmetric ossification of cervical vertebral centra) noted at the highest dose level was considered to be secondary to maternal toxicity. Historical control data from the laboratory in question is available. However, these data reflect cumulative defect data and the actual incidences in separate studies are not given. Furthermore, the period for the conduct of the studies is unknown (Table 2.6.6.2.1-23).

Comments and conclusions (RMS): Reduced foetal weights were noted in both rats and rabbits at maternal toxicity dose levels. Furthermore, increased incidences of foetal variations were noted in both rats and rabbits.
In the rat study by Anonymous 25 (1986) increased incidences of skeletal variations were noted at 20 mg/kg bw/day (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed) and 75 mg/kg bw/day (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite; sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned). These effects were noted at maternal toxicity dose levels, although it could be noted that the maternal toxicity was not marked at 20 mg/kg bw/day (enlarged spleen in one dam only). Also in the rat study by Anonymous 26 (2002) increased incidences of skeletal variations (incomplete ossification of skull bone and unossified 5th sternebrae) were observed at maternal toxicity dose level of ≥20 mg/kg bw/day.
In the rabbit study by Anonymous 27 (1986) increased incidence of caudal centra ≤15 was noted at the highest dose level of 22.5 mg/kg bw/day. This defect occurred in the absence of marked maternal toxicity. In the rabbit study of Anonymous 29 (2002) the incidences of specific foetal defects: kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior frontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, and asymmetric ossification of cervical vertebral centra were increased at the highest dose level of
30 mg/kg bw/day. These defects occurred in the presence of maternal toxicity.

As a conclusion reduced foetal weights were noted in both species at maternal toxicity dose levels. In addition increased incidences of skeletal variations indicative of retarded foetal ossification were noted in the rat at maternal toxicity doses. In the rabbit, increased incidences of skeletal variations (increased incidence of caudal centra ≤15) were noted at a dose level without marked maternal toxicity. Increased incidences of specific variations (kidney caviation, additional liver lobe, cervical remnant of thymus, lengthened anterior frontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, asymmetric ossification of cervical vertebral centra) were noted at a maternal toxicity dose.

Table 2.6.6.2.1-22: Rabbit study by Anonymous 29, 2002 (Report No. 619/155-D6154): Foetal variations

	Control	5 mg/kg bw/day	17.5 mg/kg bw/day	30 mg/kg bw/day	Statistics
	Incidence (mean % foetuses) Number of litters affected				
Kidney, cavitation increased, severe (hydronephrosis)			1 (0.8) 1	2 (1.6) 2	DR*F+
Kidney, cavitation increased, slight	1 (0.3) 1	3 (1.6) 2	6 (4.4) 3	11 (8.8) 6*	F+
Liver, additional lobe	1 (0.4) 1	3 (1.8) 3	6 (3.5) 4	6 (4.4) 5*	F+
Thymus, cervical remnant	10 (5.4) 5	15 (8.9) 8	19 (11.8) 10	16 (17.9) 9*	F+
Skull, anterior fontanelle, lengthened	1 (0.8) 1		1 (1.8) 1	5 (7.9) 5*	F+
Skull, frontal, absent				5 (8.9) 4*	F+
Skull, maxilla, ossification incomplete		3 (2.4) 1	2 (2.4) 2	4 (6.7) 3	DR*F+
Skull, nasal, misshapen				5 (8.0) 3	DR**F+
Skull, aquamosal, misshapen	1 (0.6) 1		1 (1.1) 1	3 (5.6) 3	F+
Sterneabrae, fused slight		4 (2.3) 3	5 (3.2) 2	6 (4.4) 5*	F+
Vertebral- terminal, misshapen/misaligned/fused/absent	5 (2.3) 3	2 (1.59 2	9 (5.6) 6	9 (6.4) 7	DR*F+
Vertebral-cervical centrum, ossification asymmetric		1 (0.8) 1	2 (1.2) 2	3 (2.7) 3	DR*F+

Table 2.6.6.2.1-23: Cumulative defect data. Foetal defect data of the laboratory[1]

Symptom	Cumulative defect data in historical background data of laboratory[1]		
	Control group	Inactive group	Combined control & inactive group
Number of foetuses examined	4233	7743	11976
Foetuses:	Number and %	Number and %	Number and %
Increased renal pelvis cavitation (bilateral)	5 (0.12%)	4 (0.05%)	9 (0.08%)
Increased renal pelvis cavitation (unilateral)	3 (0.07%)	16 (0.21%)	19 (0.16%)
Additional liver lobe	1 (0.02%)	3 (0.04%)	4 (0.03%)
Cervical remnant of the thymus	-	-	-
Lengthened anterior fontanelle	-	-	-
Misshapen nasal bone	13 (0.59)	5 (0.12)	18 (0.29)
Incomplete ossification of the frontal bones	304 (13.83%)	368 (9.04%)	672 (10.72%)
Incomplete ossification of the maxilla bones	9 (0.41%)	2 (0.05%)	11 (0.18%)
Fusion of the sternebrae	137 (3.24%)	217 (2.80%)	354 (2.96%)
Caudal vertebra (distal) misaligned. Connected or absent	68 (1.61%)	117 (1.51%)	185 (1.54%)
Cervical vertebral centra incompletely ossified	11 (0.26%)	18 (0.23%)	29 (0.24%)
Asymmetric ossification of cervical vertebral centra	-	-	-

[1] Incidences for individual studies not reported. Period for the conduct of studies not specified

Relevance of abortion/implantation loss/intrauterine death:

Rat:

In the rat study by Anonymous 25 1986 (Report No.: AKJ/4/86) there were no abortions, and no effects of treatment at any dose level on implantation or on post-implantation losses, although it could be noted that post-implantation loss was slightly increased (not statistically significant) at the highest dose level of 75 mg/kg bw/day (7.2% compared to 6.3% in control) (Table 2.6.6.2.1-24). Maternal toxicity consisted of reduced bodyweight gain (day 7-17: 25%) and enlarged spleen (4/24 animals) noted at 75 mg/kg bw/day, and enlarged spleen (one dam only) noted at
20 mg/kg bw/day.

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) there were increases in both pre- and post-implantation losses noted at the highest dose level of 75 mg/kg bw/day, which resulted in statistically significant reduced live litter size (12 compared to 14.8 in control) (Table 2.6.6.2.1-25). The incidence of post-implantation loss (11% compared to 5% in control) was not statistically significant but was higher than expected from the current background data (background data 4.0%-6.5%). The incidence of pre-implantation loss (17.4% compared to 8.6% in control) was not statistically significant and within live expected from the current background data (3.9%-24.3%). Increased number of early intrauterine deaths was also noted at 75 mg/kg bw/day (1.1 compared to 0.7 in control) but the increase was not statistically significant. The maternal effects were more marked in this study compared to the study of Anonymous 25, which might be explained by a problem with the allocation of the animals indicating that the animals at 20 and 75 mg/kg bw/day were probably younger than the allocated to the control group. Maternal toxicity was noted at ≥20 mg/kg bw/day. Reduced food consumption and maternal bodyweight gain was noted at 20 mg/kg bw/day (Days 7-8: 62%, Days 7-19: 21%) and 75 mg/kg bw/day (Days 17-19: 41%). Maternal body weight loss (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) was noted in addition at 75 mg/kg bw/day.

Table 2.6.6.2.1-24: Rat study by Anonymous 25 (1986): Group mean pregnancy data

Dose level (mg/kg/day)	Number pregnant/ number mated	Mean no. of corpora lutea ±S.D.	Mean no. of implantations ±S.D.	Mean no. of live foetuses ±S.D.	Mean no of dead implantations ±S.D.	Mean preimplantation loss (%)	Mean post-implantation loss (%)	Sex ratio M:F
Control	21/24	18.6±3.3	13.8±3.9	13.0±4.0	0.8±1.2	24.6	6.3	1.12:1
5	20/24	17.8±2.5	14.9±1.8	14.3±1.9	0.6±0.9	15.8	4.0	0.99:1
20	21/24	17.5±2.8	13.9±3.5	13.0±3.5	0.9±1.6	20.5	5.9	0.90:1
75	21/24	17.2±1.5	13.5±2.0	12.5±2.9	1.0±1.4	21.2	7.2	0.98:1
Analysis of variance		NS	NS	NS	NS			
Kruskal-Wallis test						NS	NS	
Chi^2 test								NS

Table 2.6.6.2.1-25: Rat study by Anonymous 26 (2002): Group mean caesarean data

Mean intake (g/animal/day)	0 (mg/kg bw/day)	5	20	75
Pregnant / mated females (Day 20 gestation)	24/24	22/24	24/24	22/24
No. of corpora lutea per female	16.8	15.3	16.3	16.3
No. of implantations per female	15.5	14.8	14.6	13.1**
Pre-implantation loss (%)	8.6	3.3	9.1	17.4
Early intrauterine deaths	0.7	0.8	0.8	1.1
Late intrauterine deaths	0.0	0.0	0.1	0.1
Post-implantation loss (%)	5.0	5.6	6.0	11.0
No of live foetuses per female	14.8	14.0	13.8	12.0**

Rabbit:

In the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) dose levels up to 22.5 mg/kg bw/day were used. Pre-implantation loss and number of implantations were comparable in all groups, and no dams aborted or showed total resorption. Post-implantation loss, live litter size were not adversely affected by treatment (Table B.6.6.2.1-26). Maternal toxicity (reduced bodyweight gain, Days 0-28: 5%) was noted at 22.5 mg/kg bw/day but was not considered adverse.

In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) dose levels up to 30 mg/kg bw/day were used. One control female and two females in each of the groups which received Quinoclamine, aborted between Gestation Days 23-28. All these females had shown a slight weight loss between DG 4-7, prior to the start of dosing on DG 7, and all showed marked inappetence from DG 4 until they aborted. In addition, there was one female at 5 mg/kg bw/day, and 2 females each at 17.5 and 30 mg/kg bw/day which showed total resorption. These females also showed marked inappetence and weight loss from DG 4. For all these animals, foetal loss was considered to be related to the inappetence, which had started prior to the start of the dosing period, and not to treatment with Quinoclamine (Table 2.6.6.2.1-27). Pregnancy rate and pre-implantation loss were comparable in

all groups. There was an apparent dose-related increase in the incidence of post-implantation loss, but at 5 mg/kg bw/day, live litter size was comparable with that of the control group. In comparison, live litter size at 17.5 mg/kg bw/day (8.4 foetuses per female compared to 9.5 in control) and 30 mg/kg bw/day (7.8 foetuses per female compared to 9.5 in control) was slightly reduced (Table 2.6.6.2.1-28). Maternal toxicity was noted at ≥17.5 mg/kg bw/day. Reduced maternal bodyweight change was noted at 17.5 mg/kg bw/day (Days 12-15: 67% of control) and 30 mg/kg bw/day (Days 4-29: 46% of control). At 30 mg/kg bw/day, reduced body weight (Days 4-29: 7%) and food consumption was noted in addition, and one dam was killed on Day 18 of gestation (due to severe inappetence and body weight loss and had red discharge from the urogenital region).

Table B.6.6.2.1-26: Rabbit study by Anonymous 27 (1986): Average pregnancy success

ACN (mg/kg bw/day)	pregnant / mated females	No. of corpora lutea	No. of implantations	No of live foetuses	No. of dead implantations	Pre-impl. loss (%)	Post-impl. loss (%)	Sex ratio M:F
0	16/16	13.4	9.5	7.9	1.6	28.2	17.5	1.2:1
2.5	16/16	13.1	9.9	7.4	2.5	25.1	23.6	0.8:1
7.5	16/16	11.8	8.8	6.9	1.9	23.6	22.9	1.0:1
22.5	16/16	12.2	9.3	7.3	2.0	22.4	20.8	0.7:1
Analysis of variance		NS	NS	NS	NS			
Kruskal-Wallis test						NS	NS	
Chi^2 test								NS

Table B.6.6.2.1-27: Rabbit study by Anonymous 29 (2002): Female performance

Number of animals	0 (mg/kg bw/day)	5	17.5	30
In group	24	24	24	24
Not pregnant	2	2	1	3
Pregnant / (%)	22 (91.7)	22 (91.7)	23 (95.8)	21 (87.5)
Died/killed	0	1	0	1
Aborted and killed	1	2	2	2
With total embryo/foetal loss	0	1	2	2
With live foetuses on Day 29	21	18	19	16

Table B.6.6.2.1-28: Rabbit study by Anonymous 29 (2002): Group mean caesarean data

Mean intake (g/animal/day) Day of gestation	0 (mg/kg bw/day)	5	17.5	30	Statistics
Pregnant / mated females (Day 29)	12/24	18/24	19/24	16/24	J
No. of corpora lutea	11.9	13.1	11.9	11.8	J
No. of implantations	10.1	11.4	10.1	10.2	
Pre-implantation loss (%) / No. of affected dams	14.8/14	11.6/10	14.4/15	13.1/9	F+
Early intrauterine deaths	0.2	0.9	0.7	1.0	F+
Late intrauterine deaths	0.3	0.8	0.9	1.4	F+
Dead foetuses. % / No. of affected dams	0.0/0	0.0/0	0.0/0	0.1/1	F+

Post-implantation loss (%) / No. of affected dams	4.8/10	13.6/14	15.2/14	24.9/13*	F+
Mean number of foetuses per female	9.5	9.8	8.4	7.8	DR*J

Comments and conclusions:

In the rat study by Anonymous 26 (2002) increased incidence of post-implantation loss (11% compared to 5% in control) was noted at the highest dose level of 75 mg/kg bw/day. This effect was not statistically significant but the incidence was above expected background data (4.0%-6.5%). Furthermore, statistically significant reduced live litter size (12 compared to 14.8 in control) was noted at this dose level, and the number of early intrauterine deaths was increased (but not statistically significant). Maternal toxicity, such as reduced bodyweight gain (41%) and bodyweight loss, was observed at 75 mg/kg bw/day. In the study by Anonymous 25 (1986) implantation loss and live litter size were not adversely affected by treatment. However, it could be noted that the maternal effects were more marked in the study by Anonymous 26 (2002), which might be explained by a problem with the allocation of the animals indicating that younger animals were allocated in the mid- and high dose groups compared to the control group.

In the rabbit study by Anonymous 29 (2002), abortions were noted in animals of both control and treated groups, but these absortions were not considered substance related. In this study, dose related increased post-implantation loss was noted, but this effect was statististically significant only at the high dose level (30 mg/kg bw/day). At the dose level of 5 mg/kg bw/day increased post-implantation loss was observed but this effect was not statistically significant and live litter size was comparable with that of the control group. Maternal toxicity, such as mortality (one dam) and reduced bodyweight (7%), was noted at 30 mg/kg bw/day, and reduced maternal bodyweight changes were noted at 17.5 mg/kg bw/day (67% of control) and 30 mg/kg bw/day (46% of control).
As a conclusion increased incidence of post-implantation loss was noted in both sexes. In addition, reduced live litter size (statistically significant) and increased number of early intrauterine deaths (not statistically significant) was noted in the rat. These effects were noted at dose levels with maternal toxicity with exception of the post-implantation loss noted in the range-finding study to rabbit study by Anonymous 27, for which no maternal toxicity was presented.

Applicant's comment on the observed effects on foetal development

Text below is an extract from document M-CA Section 5

"During the prior evaluation of Quinoclamine, RMS Sweden decided to propose a classification "Repro cat.3 R63 Possible risk of harm to the unborn child", based on two major findings: The effect on heart vessels and the hydronephrosis.

The applicant presents data which show these effects may not be derived from direct chemical effects of Quinoclamine but rather could also be effects triggered by transient undernutrition of the pregnant animals due to reduced food uptakein the first days of the exposur period:

After Quinoclamine treatment, both in rats and in rabbits transient reduced food consumption, body weight loss and/or reduced body weight gain were observed after initiation of Quinoclamine treatment lasting up to 10 days (corresponds approx. to gestational day 17, depending on the study type and animal species). During the subsequent dosing period, food consumption and weight gain increased again but final maternal body weight remains somewhat reduced compared to control or lower dosing groups. It is obvious that the animals were transiently in a state of undernutrition.

It is well known that maternal undernutrition can alter uterine environment dramatically and lead to significant impairment of uterine conditions. Impairment of the intrauterine growth environment during critical periods may result in perturbations of development, characterized by intrauterine growth restriction (IUGR) and low birth weight (LBW). However furthermore, maternal homocysteine levels have been described to be increased in rats as a consequence of dietary restriction (Petrie et al. 2002; Okawa et al. 2006) (KCA 5.6.2/01 & 02). Homocysteine is a non-protein amino acid which provides the methyl group for essentially all biological methylation reactions as an intermediate within the metabolism of the essential amino acid methionine including epigenetic reprogramming. Furthermore, it has been proposed that hyperhomocysteinemia can also lead to delayed effects due to induction of epigenetic changes (Krishna et al. 2013) (KCA 5.6.2/33), and there is no reason why this should not also be expected during foetal development.

Homocystein is not directly obtained from the diet but biosynthesized from nutritional methionine in a multi-step process (Blom und Smulders 2011) (KCA 5.6.2/03). It is described to be cytotoxic (Lin et al. 2014; Nakanishi et al. 2005) (KCA 5.6.2/04 & 5) and can itself be re-methylated to methionine or irreversibly trans-sulfurated to cysteine. Re-methylation to methionine depends on Vitamin B12 and requires an intact folate-cycle whose functionality itself depends amongst other factors on sufficient availability of folic acid. Trans-sulfuration to cysteine depends on sufficient Vitamin B6 supply (Blom und Smulders 2011) (KCA 5.6.2/03). Hyperhomocysteinemia is often caused by a deficiency in vitamin availability, however, as mentioned above, is also observed in cases of maternal undernutrition or malnutrition.

The elucidation of the pathogenic mechanisms that lead to elevated homocysteine concentrations is an ongoing process. (Rees 2002) (KCA 5.6.2/06) reviewed the available information and concluded that a nutritional imbalance of S-containing amino acids in combination with low dietary protein content might be causative. Many semi-synthetic experimental diets contain an imbalance in S-containing amino acids, forcing the animal to synthesize a sizable part of its cysteine requirement from methionine. Unfortunately, when the diet is low in protein, the oxidation of amino acids is reduced, perturbing methionine metabolism and increasing levels of homocysteine. Similarly, (Li et al. 2009) (KCA 5.6.2/07) determined that hepatic methionine metabolism and therefore the homocysteine metabolism is regulated by nutritional status. The liver is a major site of methionine metabolism. In 20 h fasted mice the plasma. homocysteine levels were increased while liver methionine content was reduced. Correspondingly it was shown by gene expression analyses that several key enzymes involved in homocysteine metabolism were significantly influenced in response to starvation. Homocysteine concentrations decreased promptly when feeding was restored. Since only 20 hours of starvation were sufficient to induce an increase in plasma homocysteine levels, it can easily be assumed that the 10-day time period of undernutrition observed in rats during Quinoclamine exposure is also sufficient to do so. It is commonly accepted that maternal deficiencies in folic acid will induce severe malformations in the developing fetus, e.g. defects in the neural tube

and heart defects. Aortic arch defects in particular, were described in folic acid deficient rats by Baird et al. (1954) (KCA 5.6.2/08).

As already mentioned, an increase in maternal homocysteine concentrations was observed under protein deficiency. Homocysteine will be re-methylated under consumption of methylene tetrahydrofolate (active derivate of folic acid) and pools become depleted (James et al. 1997) (KCA 5.6.2/09). An excess of homocysteine is therefore in some ways similar to a dietary folate deficiency (Rees 2002) (KCA 5.6.2/06), which in turn may induce these heart defects.

Direct associations between hyperhomocysteinemia, folic acid deficiency and congenital heart defects in rat fetuses were described in (Lu et al. 2011) (KCA 5.6.2/10) and reviewed by (Mone et al. 2004) (KCA 5.6.2/11).

It was also shown by (Nagai et al. 2001) (KCA 5.6.2/12) that homocysteine inhibits angiogenesis in bovine aortic endothelial cells, human microvessel endothelial cells and in vivo using the chorioallantoic membrane (CAM) assay.

Furthermore it was shown by Rosenquist et al. (1996) (KCA 5.6.2/13) that homocysteine directly caused heart defects in avian embryos. The authors suggested either a cytotoxic effect of homocysteine or growth factor-like effects (Tsai et al. 1994) (KCA 5.6.2/14) or even a combination of both. Early vascular development was also impaired by homocysteine in chicken embryos (Oosterbaan et al. 2012) (KCA 5.6.2/15).

Within this context, it was reported by Gerard et al. (2014) (KCA 5.6.2/16) that folic acid depleted diet administered to rats alters protein abundance in the aorta of the respective animals compared to folic acid supplemented or control rats. Most of the identified proteins are involved in cytoskeleton-related processes important to cell function/maintenance, assembly/organization, and movement. The authors concluded that expression of proteins essential to vascular structure and, presumably, function is modulated by high intake as well as deprivation of folic acid.

Van Mil et al. (2010) (KCA 5.6.2/17) extensively reviewed the associations between hyperhomocysteinemia and congenital malformations in chicken and rodent studies.

It is therefore reasonable to assume, that the aortic arch abnormalities observed in rats and rabbits were not caused by Quinoclamine treatment but by an increased homocysteine concentration triggered by severe and prolonged food avoidance of maternal animals during a critical phase ofpregnancy.

Several publications describe the influence on undernutrition or malnutrition on kidney development. It was reported, that IUGR, which can be provoked by restriction in food supply, is associated with impaired kidney development in the fetus (reviewed by Vaccari et al. 2015; Lakshmy 2013, Schreuder et al. 2006; Franco et al. 2012) (KCA 5.6.2/18 & 19 & 20 & 21):

In rats, nephrogenesis begins about day 12 of gestation and is not complete until 8 days after birth. (Franco et al. 2012) (KCA 5.6.2/21). The resulting renal deficits of IUGR were manifold and the severity does not depend on the time point when undernutrition occurs during pregnancy. It was shown that developmental defects were of functional and morphological nature. Consequences of kidney damage were associated with several diseases later on in life, e.g. hypertension, impaired glucose tolerance, insulin resistance and adiposity. To date, few mechanisms are clearly identified by which kidney morphology and functionality can be affected during development under IUGR. Intrarenal Renin-Angiotensin system, renal sodium transport, apoptosis, IGF-I reduction, uric acid and nitric oxide are discussed as being potentially involved within this context, but epigenetic

impact and changes in the methylation state of DNA by associations with elevated homocysteine levels are also considered.

It is not fully clear whether hyperhomocysteinemia is also directly involved in impaired fetal kidney development. There were indications, that homocysteine induces vascular dysregulation in rat vascular smooth muscle cell functions. It is known that homocysteine dysregulates a number of vascular factors that regulate vascular smooth muscle tone and cell proliferation (Tsai et al. 1994; Qureshi et al. 2005; Iselin et al. 1998) (KCA 5.6.2/14 & 22 & 23). It has been found to impair the synthesis and bioactivity of nitric oxide (NO) (Qureshi et al. 2005; Chandler et al. 2009) (KCA 5.6.2/22 & 24). NO, originally identified as an endothelium-derived relaxing factor, is considered to be the main regulatory gaseous molecule involved in a wide range of biological processes, acting as second messenger and neurotransmitter. NO is synthesized from L-arginine via NO synthase (NOS) activation, in the presence of cofactors (reviewed in Fernandes und Hernandez 2016) (KCA 5.6.2/25). NO is known to reduce the tone of smooth muscle cells after appropriate stimulation and subsequent release (Loscalzo 2013; Furchgott und Zawadzki 1980) (KCA 5.6.2/26 & 27). The ureter is a syncytial smooth muscle that spreads its excitation electronically from cell to cell, and coordinated motility may not need an extensive neural network (Tahara 1990) (KCA 5.6.2/28).
The mechanism underlying changes in peripheral and renal vascular tone has not been fully explained. It was discussed in (Bank et al. 1994) (KCA 5.6.2/29) that since NO is synthesized continuously by endothelial cells and acts locally to modulate vascular smooth muscle tone, a reduction in its supply might in itself result in a higher setting of smooth muscle tone. Alternatively, a decrease in NO supply could allow other vasoactive substances produced by the endothelium or nerve endings or those circulating in the blood to elevate vascular smooth muscle tone. It has been proposed that the balance among vasoconstrictors versus vasodilators is important in setting vascular tone. Rather, the reduced availability of NO per se appears to cause a resetting of intrinsic vascular smooth muscle tone, presumably mediated by an increase in intracellular calcium concentration or sensitivity leading to impaired vaso-relaxation.

Iselin et al. (1998) (KCA 5.6.2/23) also demonstrated that NO may contribute to the regulation of tone of the ureters in humans. In animal models where the ureters were acutely obstructed by surgery or other manipulations, NO was found to reduce the pressure of the obstructive ureter in a dose dependent manner (Yan et al. 2012; Stief et al.1996) (KCA 5.6.2/30 & 31).
It was reviewed by Andersson und Persson (1994) (KCA 5.6.2/32) that NO influences also the lower urinary tract (urethra and urinary bladder) smooth muscles in different ways. It was, amongst others, suggested that NO may be involved in the decrease in intraurethral pressure.
An increase in the vascular tone, provoked by a homocysteine triggered NO deficit, may therefore lead to a constriction within the ureter as a tubular organ and consequently may severly constrict or even interrupt the urine flow from the kidney into the bladder. An obstruction of the free flow of urine from the kidney will lead to increased pressure within its structures. A dilatation of the renal pelvis might be the histopathological result, macroscopically termed hydronephrosis.

It was also reported that NO suppresses vascular smooth muscle cell proliferation (Tsai et al. 1994) (KCA 5.6.2/14). Its inhibition by homocysteine may therefore promote myointimal hyperplasia (Qureshi et al. 2005) (KCA 5.6.2/22). A hyperplasia of cells within the tubular ureter will presumably similarly lead to an obstruction of the ureter ending up in hydronephrosis by an interruption of urine flow and subsequent increase of renal pelvic pressure.
Hydronephrosis as a renal injury was observed after Quinoclamine treatment in rats and rabbits. According to the above presented mechanism, hydronephrosis could well be the consequence of sharply restricted dietary intake of the maternal animals rather than a direct embryotoxic effect of Quinoclamine.
Accordingly, the applicant propose no classification for Quinoclamine with respect to embryotoxicity."

RMS comments:

1. RMS does not agree that the findings of aortic arch malformations could be explained by maternal malnutrition. It could be noted that aortic arch malformations could be found in the rat at dose levels without marked maternal toxicity. In the rat study by Anonymous 25 (AKJ/4/86) no effects on food consumption or bw growth were noted at the dose level of 20 mg/kg bw/day. At this dose level innominate artery absent was noted in one foetus. At the dose level of 75 mg/kg bw/day statistically significant reduced food intake was noted during days 7-10 (25%), 10-13 (14%) while the effect on food consumption during days 13-17 (4% reduction) was not statistically significant and no effects on food consumption were noted post-treatment (days 17-20). At this dose level bodyweight was reduced during days 7-17 (25%). Abnormalities observed at this dose level consisted of innominate artery absent, situs inversus and interrupt aortic arch. It is douptful that the occurrence of these abnormalities could be explained by maternal malnutrition, taking into consideration that no malnutrition was noted at the dose level of 20 mg/kg bw/day. Anomalies of the aortic arch were also noted in two animals in the rabbit study by Anonymous 27 (AKJ/3/86) at the highest dose level of 22.5 mg/kg bw/day. No effects on food consumption were noted in this study. Thus, the observed anomalies of the aortic arch could not be explained by maternal malnutrition.

Furthermore, RMS does not agree that the findings of hydronephrosis could be explained by maternal malnutrition. It could be noted that hydronephrosis could be found in the rabbit at dose levels without effects on food consumption. In the rabbit study by Anonymous 29 (Report No.: 619/155-D6154) hydronephrosis was noted in one animal at the dose level of 17.5 mg/kg bw/day. No effects on food consumption were noted at this dose level.

2. Applicant has submitted several data from the open literature to support its view that the observed developmental effects could be effects triggered by transient undernutrition of the pregnant animals. The references are listed below. No study summaries are however available, and RMS has not evaluated these data.

1.	Petrie, L., Duthie, S.J., Rees, W.D., McConnell, J.M. (2002). Serum concentrations of homocysteine are elevated during early pregnancy in rodent models of fetal programming British Journal of Nutrition 88 (5), pp. 471–477 No GLP, published
2.	Okawa, H., Morita, T., Sugiyama, K. (2006). Increased plasma homocysteine concentration in rats from a low casein diet. Bioscience, Biotechnology, and Biochemistry 70 (12), pp. 3050–3053 No GLP, published
3.	Blom, H.J., Smulders, Y. (2011). Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. Journal of Inherited Metabolic Disease 34 (1), pp. 75–81 No GLP, published
4.	Lin, N., Qin, S., Luo, S., Cui, S., Huang, G., Zhang, X. (2014). Homocysteine induces cytotoxicity and proliferation inhibition in neural stem cells via DNA methylation in vitro. The Federation of European Biochemical Societies Journal 281 (8), pp. 2088–2096 No GLP, published
5.	Nakanishi, T., Akabane, E. R., Nanami, M., Kiyobayashi, Y., Moriguchi, R., Hasuike, Y. et al. (2005). Comparison of Cytotoxicity of Cysteine and Homocysteine for Renal Epithelial Cells. Nephron Experimental Nephrology 100 (1), pp. e11-e20 No GLP, published
6.	Rees, William D. (2002). Manipulating the sulfur amino acid content of the early diet and its implications for long-term health. Proceedings of the Nutrition Society 61 (01), pp. 71–77 No GLP, published
7.	Li, S., Arning, E., Liu, C., Vitvitsky, V., Hernandez, C., Banerjee, R. et al. (2009). Regulation of homocysteine homeostasis through the transcriptional coactivator PGC-1α. American Journal of Physiology - Endocrinology and Metabolism 296 (3), E543–8 No GLP, published
8.	Baird, C.D., Nelson, M.M., Monie, I.W., Evans, H.M. Congenital cardiovascular anomalies induced by pteroylglutamic acid deficiency during gestation in the rat. Circulation research 2 (6), pp. 544–554 No GLP, published
9.	James, S.J., Miller, B.J., Basnakian, A.G., Pogribny, I.P., Pogribna, M., Muskhelishvili, L. (2011). Relationship of hyperhomocysteinemia in pregnant rats and congenital heart defects in the newborn rats. Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences 36 (1), pp. 68–73 No GLP, published
10.	Lu, Y., Wang, H., Wang, X. (2011). Relationship of hyperhomocysteinemia in pregnant rats and congenital heart defects in the newborn rats. Zhong nan da xue xue bao. Yi xue ban = Journal of Central South University. Medical sciences 36 (1), pp. 68–73 No GLP, published
11.	Mone, S.M., Gillman, M.W., Miller, T.L., Herman, E.H., Lipshultz, S.E. (2004). Effects of environmental exposures on the cardiovascular system: prenatal period through adolescence. Pediatrics 113 (4 Suppl), pp. 1058–1069 No GLP, published
12.	Nagai, Y., Tasaki, H., Takatsu, H., Nihei, S., Yamashita, K., Toyokawa, T., Nakashima, Y. (2001). Homocysteine inhibits angiogenesis in vitro and in vivo. Biochemical and biophysical research communications 281 (3), pp. 726–731 No GLP, published
13.	Rosenquist, T. H., Ratashak, S. A., Selhub, J. (1996). Homocysteine induces congenital defects of the heart and neural tube: effect of folic acid. Proceedings of the National Academy of Sciences of the United States of America 93 (26), pp. 15227–15232 No GLP, published
14.	Tsai, J.C., Perrella, M.A., Yoshizumi, M., Hsieh, C.M., Haber, E., Schlegel, R., Lee, M.E. (1994). Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proceedings of the National Academy of Sciences of the United States of America 91 (14), pp. 6369–6373 No GLP, published
15.	Oosterbaan, Annelien M., Steegers, Eric A.P., Ursem, Nicolette T.C. (2012). The effects of homocysteine and folic acid on angiogenesis and VEGF expression during chicken vascular development. Microvascular Research 83 (2), pp. 98–104 No GLP, published
16.	Gerard, N., Chanson-Rolle, A., Rock, E., Brachet, P. (2014). Proteomic analysis identifies cytoskeleton-interacting proteins as major downstream targets of altered folate status in the aorta of adult rat. Molecular Nutrition & Food Research 58 (12), pp. 2307–2319

	No GLP, published
17.	van Mil, N.H., Oosterbaan, A.M., Steegers-Theunissen, R.P. (2015). Fetal kidney programming by severe food restriction: effects on structure, hormonal receptor expression and urinary sodium excretion in rats. Journal of the Renin-Angiotensin-Aldosterone System 16 (1), pp. 33–46 No GLP, published
18.	Vaccari, B., Mesquita, F.F., Gontijo, J.A., Boer, P.A. (2015). Fetal kidney programming by severe food restriction: effects on structure, hormonal receptor expression and urinary sodium excretion in rats. Journal of the Renin-Angiotensin-Aldosterone System 16 (1), pp. 33–46 No GLP, published
19.	Lakshmy, R. (2013). Metabolic syndrome: role of maternal undernutrition and fetal programming. Reviews in Endocrine & Metabolic Disorders 14 (3), pp. 229–240 No GLP, published
20.	Schreuder, M., Delemarre-van, Waal, H.A., van Wijk, A. (2006). Consequences of intrauterine growth restriction for the kidney. Kidney & Blood Pressure Research 29 (2), pp. 108–125 No GLP, published
21.	Franco, M.C.P., Oliveira, V., Ponzio, B., Rangel, M., Palomino, Z., Zaladek Gil, F. (2012). Influence of Birth Weight on the Renal Development and Kidney Diseases in Adulthood: Experimental and Clinical Evidence. International Journal of Nephrology 2012, Article ID 608025, 5 pages DOI: 10.1155/2012/608025 No GLP, published
22.	Qureshi, I., Chen, H., Brown, A.T., Fitzgerald, R., Zhang, X., Breckenridge, J. et al. (2005). Homocysteine-induced vascular dysregulation is mediated by the NMDA receptor. Vascular Medicine (London, England) 10 (3), pp. 215–223 No GLP, published
23.	Iselin, C.E., Ny, L., Larsson, B., Schaad, N.C., Alm, P., Graber, P. et al. (1998). The nitric oxide synthase/nitric oxide and heme oxygenase/carbon monoxide pathways in the human ureter. European Urology 33 (2), pp. 214–221 No GLP, published
24.	Chandler, D.L., Llinas, M.T., Reckelhoff, J.F., LaMarca, B., Speed, J., Granger, J.P. (2009). Effects of hyperhomocysteinemia on arterial pressure and nitric oxide production in pregnant rats. American Journal of Hypertension 22 (10), pp. 1115–1119 No GLP, published
25.	Fernandes, V.S., Hernandez, M. (2016). The role of nitric oxide and hydrogen sulfide in urinary tract function. Basic & Clinical Pharmacology & Toxicology. DOI: 10.1111/bcpt.12565 No GLP, published
26.	Loscalzo, J. (2013). The Identification of Nitric Oxide as Endothelium-derived Relaxing Factor. Circulation Research 113 (2), pp. 100–103 No GLP, published
27.	Furchgott, R. F., Zawadzki, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288 (5789), pp. 373–376 No GLP, published
28.	Tahara, H. (1990). The three-dimensional structure of the musculature and the nerve elements in the rabbit ureter. Journal of Anatomy 170, pp. 183–191 No GLP, published
29.	Bank, N., Aynedjian, H.S., Khan, G.A. (1994). Mechanism of vasoconstriction induced by chronic inhibition of nitric oxide in rats. Hypertension 24 (3), pp. 322–328 No GLP, published
30.	Yan, X., Tan, G., Cai, Y., Wang, H., Guo, Y., Chen, J. (2012). The effect of nitric oxide on the pressure of the acutely obstructed ureter. Urological Research 40 (2), pp. 163–169 No GLP, published
31.	Stief, C.G., Ückert, S., Truss, M.C., Becker, A.J., MacHtens, S., Jonas, U. (1996). A possible role for nitric oxide in the regulation of human ureteral smooth muscle tone in vitro. Urological Research 24 (6), pp. 333–337 No GLP, published
32.	Andersson, K.-E., Persson, K. (1994). Nitric oxide synthase and nitric oxide-mediated effects in lower urinary tract smooth muscles. World Journal of Urology 12 (5), pp. 274–280 No GLP, published

33.	Krishna, S.M., Dear, A., Craig, J.M., Norman, P.E., Golledge, J. (2013). The potential role of homocysteine mediated DNA methylation and associated epigenetic changes in abdominal aortic aneurysm formation Atherosclerosis 2013 Jun;228(2):295-305 No GLP, published

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

According to Regulation 1272/2008 (CLP), substances are classified for reproductive toxicity in Category 1A (known human reproductive toxicant) based largely on evidence from humans or in 1B (presumed human reproductive toxicant) or 2 (suspected human reproductive toxicant) largely based on animal data. The animal data required for 1B classification *"shall provide clear evidence of an adverse effect on sexual function or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects".*
Substances are classified in Category 2 when there is "some evidence from humans or experimental animals… of an adverse effect on sexual function or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1"

As there is no human data available for quinoclamine, the criteria for category 1A are not fulfilled.

The mainly manifestations of developmental toxicity noted in the studies that are considered potentially relevant for classification are: structural abnormalities (aortic arch abnormalities, skeletal abnormalities, hydronephrosis), altered growth and post-implantation loss noted in rats and rabbits. In the rat study by Anonymous 26 (2002), one case of severe oedema and one case of retro-oesophagal aortic arch were noted. These malformations were considered suitable for the setting of adversity but not enough for classification as the the incidences were low and observed in one species only at a high dose level.

Aortic arch malformations:
In the rat study by Anonymous 25, 1986 (Report No.: AKJ/4/86) interrupted aortic arch (one foetus) was noted at the highest dose level of 75 mg/kg bw/day. In addition, the aortic malformations situs inversus (two foetuses) and innominate artery absent (four foetuses) were noted at this dose level. The incidence of situs inversus (mean% foetus: 0.8%) was outside the historical control data for the study-performing laboratory in 1985 (mean % foetus: 0.4%), while the defects innominate artery absent and interrupted aoartic arch were not presented in this historical control data. The defect innominate artery absent was also found at 20 mg/kg bw/day (one foetus). Maternal toxicity noted at 20 mg/kg bw/day was less marked and consisted of enlarged spleen noted in one dam only. At 75 mg/kg bw/day, maternal toxicity consisted of reduced bw gain (25%) and enlarged spleen noted in four dams.

In the rabbit study by Anonymous 27, 1986 (report No.: AKJ/3/86) aortic arch malformations were noted in two foetuses at the highest dose level of 22.5 mg/kg bw/day. The incidence (1.7%) was within historical control data for the study-performing laboratory in 1985 (2.2%). No adverse maternal toxicity was observed in this study (at

22.5 mg/kg bw/day: reduced bodyweight gain: 5%).

Incidences of aortic arch malformations (interrupted aortic arch) were also noted in the range finding study to the rabbit study by Anonymous 28, 1986 (Report No.: AKJ/1/86) at the dose level of 20 mg/kg bw/day (one foetuses) and 50 mg/kg bw/day (one foetuses).

As a conclusion, aortic arch malformations were noted in several studies and in both species, and could not be explained by maternal toxicity. The defect was considered severe and relevant for a classification for reproductive toxicity, although the incidences of aortic arch malformations were low and the effect was not reproducible in the studies by Anonymous 26/29 (2002).

Skeletal abnormalities/variations:
In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) increased incidence of abnormal terminal caudal vertebrae was noted at 17.5 mg/kg bw/day (mean % foetuses: 5.6% compared to 2.3% in control) and 30 mg/kg bw/day (mean % foetuses: 6.4% compared to 2.3% in control). This effect was not present as variant in the historical control data in six preceding studies. The defect misaligned, connected or absent caudal vertebra was however present as malformation in these historical control data showing low incidences (mean % foetuses:
0-1%). Maternal toxicity was present at the dose levels of ≥17.5 mg/kg bw/day. At 17.5 mg/kg bw/day reduced maternal bodyweight change (67% of control at Days 12-15) was noted. At 30 mg/kg bw/day maternal bodyweight change was reduced (46% of control) as well as maternal body weight (7%), and one dam was killed on Day 18 following severe inappetence. Furthermore, findings of misshapen nasal bone (5 foetuses, incidence mean% foetuses: 8% compared to 0% in control), absent frontal (5 foetuses, incidence mean % foetuses: 8.9% compared to 0% in control) and misaligned thoracic vertebral arch (one foetus) were noted in this rabbit study at the highest dose level (30 mg/kg bw/day). These defects were not present in the historical control data in six studies preceding the present study. Increased incidence of skeletal foetal variations such as incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, and asymmetric ossification of cervical vertebral centra were also noted in the study at 30 mg/kg bw/day. The skeletal abnormalities mentioned above were not observed in the rabbit study by Anonymous 27 (1986). However, it could be noted that the rabbit studies by Anonymous 27 (1986) and Anonymous 29 (2002) were not fully comparable, with the major differences being the length of dosing (Anonymous 27 study: DG 6-18; Anonymous 29 study: 7-28) and slightly higher doses in the study by Anonymous 29 (up to 30 mg/kg bw/day) compared to the dose levels used in the rabbit study by Anonymous 27 (up to 22.5 mg/kg bw/day).

In the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) skeletal malformations were noted at the highest dose level (22.5 mg/kg bw/day). These malformations consisted of hyperextension of limb or paw noted in one animal, spina bifida noted in three animals, scoliosis noted in one animal and sternebral fusion noted in three animals. All incidences were within historical control data, except the incidence of fused sternebra (2.6%) which were outside background data of the laboratory in 1985 (0.7%). Increased incidence of skeletal variants were also

noted in this study at the highest dose level (22.5 mg/kg bw/day), and consisted of increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control). No adverse maternal toxicity was noted in this study (At 22.5 mg/kg bw/day: maternal bodyweight gain reduced 5%). The mentioned skeletal abnormalities/variants were not observed in the rabbit study by Anonymous 29 (2002).

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) increased incidences of skeletal variations were noted at 20 mg/kg bw/day (incomplete ossification of skull bone and unossified fifth sternebrae) and 75 mg/kg bw/day (incomplete ossification of skull bone and unossified fifth sternebrae). These findings were considered to be secondary to maternal toxicity observed at these dose levels. At these dose levels maternal bodyweight gain was reduced (At 20 mg/kg bw/day: Days 7-8: 62%, Day 17-19: 21%; At 75 mg/kg bw/day: Days 17-19: 41%) and bodyweight loss was noted at 75 mg/kg bw/day (Days 6-7: -4.6 g, Days 7-8: -2.6g, Days 8-9: -0.4g).

Increased incidence of skeletal variations were also noted in the rat study by Anonymous 25, 1986 (Report No.: AKJ/4/86. At 20 and 75 mg/kg bw/day the incidence of following skeletal variations was increased: skull: hyoid non ossified, vertebrae: thoracic centre one or more bilobed. At 75 mg/kg bw/day increased incidence of sternebral variations were noted in addition (non ossified 5th and 6th sternebrae, one or more bilobed, bipartite or misaligned). Maternal toxicity noted at 20 mg/kg bw/day consisted of enlarged spleen in one dam. At 75 mg/kg bw/day, enlarged spleen was noted in 4 dams and maternal bodyweight gain was reduced 25% (Day 7-17). The findings of skeletal variations noted in this study were considered to be secondary to the maternal toxicity, although it could be noted that the maternal toxicity was not marked at the dose level of 20 mg/kg bw/day.

As a conclusion, findings of skeletal variations were noted in both sexes. These were considered to be secondary to the maternal toxicity. Increased incidences of abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, misaligned thoracic vertebral arch were noted in the rabbit at high dose level and were outside the range of historical control data at time of study, or not present in this data. The defects were considered severe, and relevant for a classification for reproductive toxicity, although the incidences were low and the defects were observed in one species only.

Hydronephrosis:

In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154) findings of the malformation hydronephrosis were noted at dose levels of 17.5 (one animal) and 30 mg/kg bw/day (two animals). In addition, dose-related increased incidence of kidney cavitation was noted, statistically significant at 30 mg/kg bw/day. The incidence of hydronephrosis noted in this study was dose related. No findings of hydronephrosis were presented in the historical control data in six studies preceding the present study. Maternal toxicity noted at 17.5 mg/kg bw/day consisted of reduced body weight change (67% of control). At 30 mg/kg bw/day, maternal bodyweight change was reduced (46% of control) and one dam was killed following severe inappetence. There were no foetuses with hydronephrosis in the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) using doses up to 22.5 mg/kg

bw/day. In the range finding study of Anonymous 28, 1986 (Report No.: AKJ/1/86), one single case of kidney left agenesis was found at the dose level of 50 mg/kg bw/day.

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) findings of hydronephrosis were noted at the highest dose level of 75 mg/kg bw/day (3 animals). The incidence of hydronephrosis (1.1%) was slightly outside the control range (1.0%) of the six studies preceding the present study. Furthermore, a single case of misshapen kidney occurred at 75 mg/kg bw/day. No findings of misshapen kidney were presented in the historical control data. Maternal toxicity was present at the dose level of 20 mg/kg bw/day and above. Maternal bodyweight gain was reduced (At 20 mg/kg bw/day: Days 7-8: 62%, Day 17-19: 21%; At 75 mg/kg bw/day: Days 17-19: 41%) and bodyweight loss was noted at 75 mg/kg bw/day (Days 6-7: -4.6 g, Days 7-8: -2.6g, Days 8-9: -0.4g). There were no findings of hydronephrosis or misshapen kidney in the rat study by Anonymous 27, 1986 (Report No.: AKJ/3/86). However, it could be noted that the maternal effects were more marked in the study by Anonymous 26 (2002), which might be explained by a problem with the allocation of the animals indicating that younger animals were allocated in the mid- and high dose groups compared to the control group.

As a conclusion, findings of hydronephrosis were noted in both species. The incidences were outside the historical control range or not found in historical control data at time of study. Furthermore, one single case of misshapen kidney was noted in the rat study by Anonymous 26 (2002) at the highest dose level (75 mg/kg bw/day), and statistically significant increased incidence of kidney cavition was noted in the rabbit study by Anonymous 29 (2002) at the highest dose level (30 mg/kg bw/day). The defect hydronephrosis was considered severe and relevant for a classification for reproductive toxicity, although the incidences were low. The finding of kidney cavition noted in the rabbit was also considered relevant for classification, taking into account that the kidney is a target organ for quinoclamine.

Foetal growth retardation:
In the rat study by Anonymous 25, 1986 (Report No.: AKJ/4/86) reduced foetal weight (7%) were noted at the dose level of 75 mg/kg bw/day. This effect was considered to be secondary to the maternal toxicity observed at this dose level. Maternal toxicity at this dose level consisted of reduced bodyweight gain (day 7-17: 25%) and enlarged spleen (4/24 animals).

In the rat study by Anonymous 34, 2002 (Report No.: 619/123-D6154) reduced foetal weight was noted at the dose level of 20 mg/kg bw/day (7%) and 75 mg/kg bw/day (12%). This effect was considered to be secondary to the maternal toxicity observed at these dose levels. At 20 mg/kg bw/day maternal reduced bodyweight gain (Days 7-8: 62%, Days 17-19: 21%) and reduced food consumption were noted. At 75 mg/kg bw/day maternal reduced bodyweight gain (Days 17-19: 41%), body weight loss (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) and reduced food consumption were noted.

In the rabbit study by Anonymous 27, 1986 (Report No.: AKJ/3/86) reduced foetal weight (5%, n.s.) was noted at the highest dose level of 22.5 mg/kg bw/day. This effect was considered to be secondary to the maternal toxicity

observed at this dose level. Minor maternal toxicity (reduced bodyweight gain, Days 0-28: 5%) was noted at 22.5 mg/kg bw/day.

As a conclusion, reduced foetal weight was noted in the rat (statistically significant, reductions up to 12%) and rabbit (not statistically significant). The effect was suitable for the setting of adversity, but not enough for classification for reproductive toxicity, as significant effects were noted in one species only and seen in association with marked maternal toxicity.

Post-implantation loss:
In the rabbit study by Anonymous 29, 2002 (Report No.: 619/155-D6154), dose-related increased post-implantion loss were noted, but this effect was statistically significant only at the highest dose level (30 mg/kg bw/day). Maternal toxicity such as mortality (one dam) and reduced bodyweight (7%) were noted at 30 mg/kg bw/day, and reduced bodyweight changes were noted at 17.5 mg/kg bw/day (67% of control) and 30 mg/kg bw/day (46% of control).

In the rat study by Anonymous 26, 2002 (Report No.: 619/94-D6154) there were increases in both pre- and post-implantation losses noted at the highest dose level of 75 mg/kg bw/day. The incidence of post-implantation loss (11% compared to 5% in control) was not statistically significant but was higher than expected from the current background data (background data 4.0%-6.5%). The incidence of pre-implantation loss (17.4% compared to 8.6% in control) was not statistically significant and within live expected from the current background data (3.9%-24.3%). Maternal toxicity was adverse at ≥20 mg/kg bw/day. Maternal reduced bodyweight gain was noted at 20 mg/kg bw/day (Days 7-8: 62%, Days 7: 21%) and at 75 mg/kg bw/day (Days 17-19: 41%), and body weight loss was noted at 75 mg/kg bw/day (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g).

As a conclusion, the effect on post-implantation loss observed in the rat- and rabbit developmental studies by Anonymous 26/29 (2002) was considered suitable for the setting of adversity but not enough for classification, as the incidence was observed mainly at maternal toxicity dose levels.

Overall conclusion regarding adverse effects on developmental and RMS proposal for classification (RMS):
The findings of aortic arch malformations (noted in several studies and in both species), skeletal malformations i.e. abnormal terminal caudal vertebrae, misshapen nasal bone, absent frontal, misaligned thoracic vertebral arch (noted in the rabbit) and kidney effects i.e. hydronephrosis (noted in both species), misshapen kidney (one single case noted in rabbit) and increased incidence of kidney caviation (noted in rabbit) are considered relevant for a classification for reproductive toxicity. A classification for reproductive toxicity in Category 2 (Hazard Statement: H361d: Suspected of damaging fertility or the unborn child) is proposed for quinoclamine.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 2.6.6.3-1. Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
Two generation reproduction study In-house method Rat Sprague-Dawley M, F 25/sex/group *Study was checked for compliance with OECD TG 416 (2001) and following deviations were noted:* *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. Postlactational ovary (primordial and growing follicles) histopathology was not performed* *viii. For the offspring, age at vaginal opening or PPS for the F1 and F2 was not determined*	K-1616 (Quinoclamine) Purity: 98.5% 0, 1, 25, 500 ppm Corresponding to: F0: 0, 0.07, 1.6, 30.9 mg/kg bw/day in males; 0, 0.08, 1.9 and 37.7 mg/kg bw/day in females F1: 0, 0.07, 1.7 and 37.0 mg/kg bw/day in males; 0, 0.08, 2.0 and 43.8 mg/kg bw/day in females The parents of both generations were fed the appropriate diets for at least nine weeks and then subjected to two subsequent mating trials. Fresh diets were prepared and presented weekly to the rats of all generations from initiation (P1) or weaning (F1b—>F2, F2b)	1 ppm: Parental: -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 3%; P2 M: 7%; P2 F 4%) ↓ bw gain (P1 M: 4%, P2 M: 11%; P2 F: 4%) Offspring: -increased incidence of gray lung cysts in F2b offspring reared for 3 months (18 compared to 11 in control group) 25 ppm: Parental: -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 1%; P2 M: 7%; P2 F 5%) ↓ bw gain (P1 M: 2%, P2 M: 11%; P2 F: 6%) Offspring: -increased incidence of gray lung cysts in F2b offspring reared for 3 months (29 compared to 11 in control group) 500 ppm: Parental: **-clinical signs** (F0/F1: hunched posture) ↓ **bw** (P1 M: 4%; P2 M: **10%**; P2 F **10%**) ↓ **bw gain** (P1 M: 7%, P2 M: **11%**; P2 F: 9%) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) Offspring: -clinical signs (orange stained fur F2b offspring) ↓ **bw** during lactation (F1a: 13% and 7% in males and females, respectively;	RAR Vol. 3, B.6.6.1/01 Anonymous 19 (1975) Report No.: 854-111 New data for the Annex I renewal: No

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL (for sexual function and fertility, parents)** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
GLP: No		F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively) ↓ **litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) **-increased incidence of gray lung cysts** in F2b offspring reared for 3 months (39 compared to 11 in control group) NOAEL parental and offsprings: 25 ppm (1.6 mg/kg bw/day) NOAEL reproductive toxicity: 500 ppm (37 mg/kg bw/day)	

Table 2.6.6.3-2. Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 2.6.6.3-3. Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

Two generation reproductive toxicity study (RAR Vol. 3, B.6.6.2/01)

The study is no GLP study and considered limited due to several deviations from the OECD TG 416.

In the study groups of 25 male and 25 female Sprague-Dawley rats received K-1616 (quinoclamine) in the diet at dose level up to 500 ppm (corresponding to 30.9 and 37.7 mg/kg bw/day in F0 males and females, respectively, and 37.0 and 43.8 mg/kg bw/day in F1 males and females, respectively) through two successive generations. Treatment with the test substance did not affect mating performance or fertility of the male and female parental animals and no consistent differences from control values were noted in comparisons of parental food consumption, survival rates and parturition indices or postnatal and postweaning survival. In addition, evaluations of the data obtained from foetuses taken by caesarean section did not reveal any findings indication teratogenic effects of the test substance at any of these concentrations.

Differences from control group data noted at the high dose level (500 ppm) included lower growth period mean body weight values in the P1 (4% at week 13) and P2 (10% at week 9) generation males and P2 generation females (10% at week 9), reduced bodyweight gain in P1 (7%) and P2 (11%) generation males and P2 (9%) generation females, lower mean offspring weights at weaning in all filial generations (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively), an increase in the observations of hunched appearance during the growth periods of both parental generations, and an increased incidence of gray lung cysts and orange-stained fur noted in the F2b offspring at necropsy. Mean litter size in F2a and F2b generations were also reduced at this dose level.

Differences noted to a lesser degree at the mid dose level (25 ppm) included slightly lower mean body weight values in the P1 (1% at week 13) and P2 (7% at week 9) generation males and P2 (5% at week 9) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (2%) and P2 (11%) generation males and P2 (6%) generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of gray lung cysts in the F2b offspring at necropsy.

Differences noted to a lesser degree at the low dose level (1 ppm) included slightly lower mean body weight values in the P1 (3%) and P2 (7%) generation males and P2 (4%) generation females at the last weighing interval of the growth periods, reduced bodyweight gain in P1 (4%) and P2 (11%) generation males and P2 (4%) generation females, an occasional slight increase in the observations of hunched appearance in both parental generations, and an increased incidence of lung cysts in the F2b offspring at necropsy.

Increased incidence of gray lung cysts was noted in the F2b offspring reared for three months (at 1 ppm: 18 compared to 11 in control group; at 25 ppm: 29 compared to 11 in control group; at 500 ppm: 39 compared to control group). The findings of gray lung cysts in the F2b offspring was dose related although the relevance of this finding is not clear. The finding was not observed in the offspring of the F1b generation reared for 5 weeks or in other available toxicological studies on quinoclamine. Thus, it seems to be a finding occurring in adult F2b animals including control animals. In the high dose group the incidence of gray cysts was 3.5 times higher when compared to controls, and considered adverse. In the low and mid-dose groups the incidences were less marked (1.6 to 2.6 times higher when compared to controls) and not considered adverse in the absence of other effects in the offspring at these dose levels.

The NOAEL for parental animals was set at 25 ppm (1.6 mg/kg bw/day) based on clinical signs (hunched posture) noted in P1 and P2 generation animals at 500 ppm (37 mg/kg bw/day), reduced body weight noted in P2 males and females at 500 ppm, and reduced bodyweight gain noted in P2 males at 500 ppm.

The NOAEL for offsprings was set at 25 ppm (1.6 mg/kg bw/day) based on reduced body weights at weaning in all filial generations noted at 500 ppm (37 mg/kg bw/day) and gray lung cysts in in P2 offspring reared for 3 months.

The NOAEL for reproductive toxicity was set at 500 ppm (37 mg/kg bw/day).

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

According to the CLP Guidance Table 3.7.1(b) a substance should be classified for lactation effects when the following applies:

"(a) human evidence indicating a hazard to babies during the lactation period; and /or
(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
(c) absorption, metabolism, distribution and excretion studies that indicate the likehood that the substance is present in potentially toxic levels in breast milk."

No data is available to address criterias (a) and (c). A reduction in pup weight was seen at a dose level (500 ppm) associated with maternal toxicity. Thus, the effect on pup bodyweight is not considered to "provide clear evidence of adverse effect in the offspring due to transfer in the milk".

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Classification of quinoclamine as toxic for reproduction in Category 2, H361d ("Suspected of damaging fertility or the unborn child") is proposed.

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.

Adverse effects on sexual function and fertility

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.

<u>DS assessment of the relevance of abnormalities</u>

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").

<u>Adverse effects on or via lactation</u>

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[4]. 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.

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.

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

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.

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

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**.

2.6.7 Summary of neurotoxicity

Table 2.6.7-1. Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/organ** **-critical effect at LOAEL**	**Reference**
No data			

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

2.6.8.1.1 Impurities

See Annex C

2.6.8.1.2 Metabolites

Quinoclamine is not intended for feed or food products. Accordingly, plant metabolites are not relevant for the evaluation.

Some studies on phtalic acid, a major photolusis metabolite in soil and under aquatic conditions – were found in the open literature and were submitted by the applicant (Vol. 3, B.6.8.1). One study investigated the toxicokinetics of phthalic acid and another study investigated the genotoxic potential. Two more studies investigated the endocrine disruptive properties of this metabolite.

As quinoclamine is not intended for feed or food products, and as an assessment of the relevance of metabolites for groundwater was not considered necessary (Vol. 1 section 2.11.6), the studies on phthalic acid were not given further significance for the evaluation of the active substance in this report, with exception of the two studies investigating the endocrine disruptive properties of phthalic acid (summarised in table below). These two studies were considered as supporting data with regard to the endocrine disruption evaluation (section 2.6.8.3)

Study/method	Substance	Results	Reference	Comments
In a molecular docking study- using the docking software Glide (Schrödinger)- the molecular interactions of 31 ligands, including 12 diphthalates, their monophthalates and phthalic acid with selected human ketosteroid receptors, i.e., androgen (hAR), progesterone (hPR) and glucocorticoid (hGR) receptors were explored and their binding affinities were compared with that of corresponding natural steroids and a known endocrine disrupting	Phthalic acid	For human androgen receptor binding, the higher G scores (the highest was -8.19 kcal/mo1-1 for testosterone) were seen with the natural ligands (controls). Phthalic acid showed a lower G score (-5.49 kcal/mo1-1) indicating the ability to bind to human androgen receptors but with lower affinity than the natural ligands. For human progesterone receptor bindind the highest G score was seen with the natural ligand tetrahydrogestrinone (-9.43 kcal/mo1-1). Phthalic acid showed a lower G score (-6.46 kcal/mo1-1).	Sarath, J. M. K., Pradeep, S., Vijayalekshmy, A.K. S., Sudha Devi, R., Balachandran, S., Sreejith, M. N., Sailas, B. (2016). Human ketosteroid receptors interact with hazardous phthalate plasticizers and their metabolites: an in silico study. Journal of Applied Toxicology, 36 (6), p. 836–843 Study summary by the applicant is presented in Vol. 3, B.6.8.1/03	No GLP study The result of this study indicates that phtalic acid may cause endocrine disruption properties *in vitro/in vivo* *Study considered as supportive data*

xenobiotic, bisphenol A (BPA). The G score as an empirical scoring function that approximates the ligand binding free energy was established. The higher the negative value of the G score, the higher is the affinity of the ligand to bind to the receptor.		For human glucocorticoid receptor binding, again phthalic acid showed a lower G score (-6.86 kcal/mo1-1) than the natural ligand cortisol (-7.76 kcal/mo1-1). The molecular receptor interactions of those phthalates showing the highest G scores where graphically presented in the original source. For phthalic acid no molecular interactions were presented. As a conclusion, phthalic acid was able to interact with human androgen, progesterone, and glucocorticoid receptors in *in silico* docking experiments. The binding affinity was lower compared to the natural ligands to the respective receptors.		
Amnion-derived WISH cells were obtained from ATCC (the American Type Culture Collection, CCL-25) and maintained in the laboratory. Cells were grown at 37°C in an atmosphere of 5% CO_2:95% air, in a mixture of Ham's F12 and Dulbecco's modified Eagle medium (F12/DMEM; 1:1 v/v) supplemented with 10% fetal bovine serum (FBS), 30 mg/ml gentamicin and 0.25 mg/ml amphotericin B. The cells were seeded into 24-well plates at 2 x 10^5 cells per well in F12/DMEM + 10% FBS and grown to confluence (2–3 days). WISH cells were plated in 24-well plates (2 x 10^5 cells per well) and, at a 70% confluence, cells were preincubated for 1 h in the presence of 10 µM Ro 20-1724 (a cAMP phosphodiesterase inhibitor, suggesting that	Phthalic acid	It was demonstrated that phthalic acid (i) displaces [^{3}H]estradiol from its binding sites (IC_{50} = 89 nM, Ki = 66 nM), (ii) enhances the intracellular cyclic AMP concentration, without influencing adenylyl cyclase activity, (iii) stimulates or inhibits prostaglandin output, probably depending on the intracellular nucleotide level. 17β-estradiol exerts similar effects in WISH cells, and it was suggested by the authors that the molecular mechanisms underlying phthalic acid and steroid-hormone responses in this cell line are the same.	Pavan, B., Biondi, C., Ferretti, M. E., Lunghi, L., Paganetto, G. (2001). Phthalic acid mimics 17beta-estradiol actions in WISH cells. Toxicology letters 118 (3), p. 157–164. Study summary by the applicant is presented in Vol. 3, B.6.8.1/04	No GLP study The result of this study indicates that phtalic acid may cause endocrine disruption effects. *Study considered as supportive data*

cAMP level enhancement could induce estrogen receptor expression). Cells were washed with PBS, then incubated in the presence of ten different concentrations of [^{3}H] estradiol ranging from 5 to 250 nM for saturation experiments. For the competitive binding assays, cells were incubated in the presence of 10 nM [^{3}H] estradiol alone to determine specific binding, or in combination with nine different concentrations of phthalic acid ranging from 1 nM to 1 mM. Nonspecific binding was determined by adding 10^{-5} M diethylstilbestrol. All incubations were carried out at 37°C for 30 min, in a final volume of 0.5 ml of serum-free medium containing 20 mM $NaMoO_4$. The unbound ligand was removed by washing the cells twice with PBS supplemented with 20 mM $NaMoO_4$ and 1 mg/ml BSA, and once with normal PBS. Cells were disrupted with 1 N NaOH (0.25 ml) and collected from the cluster dishes. Bound radioactivity was measured by scintillation spectrometry.				

2.6.8.2 Supplementary studies on the active substance

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Dermal embryo-foetal development study	Quinoclamine Purity: 97.7%	The study was performed to investigate the effects of the test article on the embryonic and	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> -clinical signs (coloured urine)	RAR Vol. 3, B.6.8.2/01 Anonymous 30 (1996) Report No.: 1312-1416-001

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Rat In house method GLP: Yes	5, 100, 600 mg/kg bw/day Vehicle: 1% Tween 80 Day 6 to 15 *post-coitum*	fetal development of the rat when administered during the period of organogenesis. Three groups of twenty five sexually mature and mated female Sprague Dawley Crl:CD (SD)BR rats (8-12 weeks old) received Quinoclamine by dermal application at dose levels of 5, 100 and 600 mg/kg bw/day for 10 consecutive days from day 6 to 15 *post-coitum*, inclusive.	-macroscopical changes (reddish discolouration of treated skin) <u>100 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) -macroscopical changes (reddish discolouration of treated skin) <u>600 mg/kg bw/day:</u> -clinical signs (encrusted skin, coloured urine) ↓**bw loss** (Days 6-9: -0.41 g) ↓**bw gain** (Days 6-16: 31%) ↓FC -macroscopical changes (reddish discolouration of treated skin) No embryotoxicity or teratogenicity was noted in this study *The study is considered as supplementary data*	New data for the Annex I renewal: No

Treatment was associated with clinical signs (coloured urine noted at ≥5 mg/kg bw/day and encrusted skin noted at ≥100 mg/kg bw/day), reduced bodyweight growth noted at 600 mg/kg bw/day (bodyweight loss: -0.41 g, reduced bodyweight gain Days 6-16: 31%), reduced food consumption, and macroscopical changes (reddish discolouration of treated skin). No embryotoxicity or teratogenicity was noted in this study. The study was considered as supplementary data. The test substance was administered dermally instead of orally. The choice of administration route was not justified in study report.

2.6.8.3 Endocrine disrupting properties

The applicant has provided an assessment of potential endocrine properties of quinoclamine in a kind of WoE approach including an assessment of the available toxicity studies, a literature search and an assessment to identify structural alerts for hormonal activity using the OECD QSAR Toolbox (Vol. 3, B.6.8.3/01). The applicant has also provided an assessment of possible hyperglycaemic effects of quinoclamine including an assessment of available toxicity studies (Vol. 3, B.6.8.3/02).

Effects on endocrine organs (organ weight changes and histopathological changes) were noted in the standard toxicity studies on quinoclamine (Table 2.6.8.3-01 to 03 below). Changes in organ weights were noted for the adrenal (rat, dog), thyroidea (rat, dog), and ovary/uterus (dog). Histopathological changes were noted in the adrenal (rat, mouse, dog), testis (dog) and uterus (rat), and female mammary gland (rat). Post-implantation loss and reduced foetal weights were noted in the reproductive toxicity studies in the rat and rabbit.

In the assessment of possible hyperglycaemic effects of quinoclamine there were no evidence for secondary effects of hyperglycaemia (Vol. 3, B.6.8.3/02).

The open literature search performed by the applicant was restricted to results obtained for the active substance. However, having a look into the literature search for the metabolites, one in vitro study in the zebrafish was present for the metabolite phthalmic acid (Vol. 3, B.9.2.3). Phthalic acid was also able to interact with human androgen, progesterone, and glucocorticoid receptors in one in silico study (Vol. 3, B.6.8.1). Furthermore, phthalic acid was shown to mimics 17beta-estradiol actions in WISH cells in one in vitro study (Vol. 3, B.6.8.1). The results of these studies indicate that the metabolite phthalic acid may affect endocrine function.

No structural alerts were identified for quinoclamine indicating estrogenic activity in the QSAR analysis using OECD QSAR Toolbox.

Conclusion by RMS:
There were some effects on endocrine organs in the standard toxicity studies on quinoclamine, but no clear effect pattern was shown. The effects occurred mainly at high dose levels, and thus could be due to systemic toxicity. However, there were also some effects noted at lower dose levels that could not clearly be explained (90-day dog study: loss of estrous cyclic activity, increased thyroid weight). Although no clear pattern was shown these effects might indicate an endocrine activity of quinoclamine, also considered that increased incidence of post-implantation loss was noted in one rabbit study at a dose level without maternal toxicity. The effect of increased post-implantation loss could be considered as a parameter sensitive to but not diagnostic of EATS (estrogen, androgen, thyroid, steroidogenic). Furthermore, open literature data gives some indications of endocrine effects caused by the metabolite phthalic acid.

It could also be noted that the potential for endocrine effects have not been fully investigated in available toxicity studies due to limitations in the test guideline available at the time. For example, sperm parameters and oestrus cycles have not been investigated in the reproduction toxicity study. Nor have gestation length, vaginal opening or preputial separation been determined.

Furthermore, the assessment to identify structural alerts for hormonal activity using the OECD QSAR Toolbox was restricted to predict estrogen receptor binding affinity. No other pathways such as androgen receptor pathway was performed.

As a conclusion there are some effects that might indicate endocrine activity. It could also be noted that limited endocrine parameters have been investigated in available studies.
Quinoclamine is proposed to be classified as toxic for reproduction in Category 2 and as carcinogenic in Category 2, thus, meets the interim criteria for endocrine disruptions as specified in Plant Protection Product Regulation (EC) No. 1107/2009. Interim criteria will be applied until final criteria are implemented (EFSA. Technical report

on outcome of pesticides peer review meeting on recurring issues in mammalian toxicology. Approved: 25 July 2016).

The opinion of RMS is that the substance fulfils the interims criteria for endocrine disrupters based on available data. Further discussions will however be needed in the light of the new guidance document before a final decision is taken.

Table 2.6.8.3-01: Effects noted in reproductive endocrine organs

Study	NOAEL in study	Effect on endocrine organ	Other effects at the dose level of effect on endocrine organs	Comments by study author	Comments by RMS
Testes					
Oral (dietary) 2-year (RAR Vol 3, B.6.3.3.1/01) In house method Dog Beagle M, F 4/sex/dose GLP: Yes <u>Dose levels:</u> 0, 2, 10, 50, 250 and 1000 ppm (equivalent to 0, 0.06, 0.33, 1.42, 7.62 and 26.6 mg/kg bw/day in males, and 0, 0.06, 0.31, 1.39, 6.79 and 29.1 mg/kg bw/day in females)	NOAEL for both sexes was set at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) based on mortalities noted in both sexes at 1000 ppm, reduced bodyweight gain noted in both sexes at ≥250 ppm, changes in haematological parameters (indicating anaemia) noted in both sexes at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistically significant changes in relative organ weights (lung, spleen and gonads) noted in females at 1000 ppm, changes in gross pathology noted at 50 ppm (urinary bladder, spleen, ovary) and 250 ppm (urinary bladder, spleen, liver, ovary, kidneys) and 1000 ppm (urinary bladder, spleen, ovary, liver, gall bladder, kidneys, testes, ovary, heart, lung, mesenteric lymph nodes) and histopathological changes noted in the liver (in females at ≥50 ppm; in males at ≥250 ppm), urinary bladder (in both sexes at ≥50 ppm), adrenals (in both sexes at ≥250 ppm), lungs (in both sexes at ≥250 ppm), spleen (in males at 1000 ppm; in	<u>At 1000 ppm (26.6 mg/kg bw/day):</u> <u>Testes:</u> -small and soft -aspermatogenesis -athrophy -focal nonsuppurative orchitis	<u>At 1000 ppm (26.6 mg/kg bw/day):</u> **-mortality** (one of each sex sacrificed in extremis during week 65) **-clinical signs** (brown-tined urine, orange stained hair around urogenital area, during the second year of study: pale appearing oral mucosal membranes, yellowish discoloration of the eyes and thinness in the female sacrificed in extremis, and unhealthy appearance characterized by thinness and lethargy in the male sacrificed in extremis) ↓**bw** (week 52: M: 21%, F: 26%; week 104: M: 23%, F: 33%) ↓**bw gain or bw loss** (Weeks 0-52: M: 0.3% compared to 2.4% in controls, F:-0.6% compared to 1.7% in controls; Weeks 52-104: M: 0.1% compared to 1.2% in controls, F: -0.6% compared to 1.5% in controls) **-changes in haematological parameters** (all post treatment intervals: ↓haemoglobin M: up to 26%, F: up to 47% ↓haematocrit (M, F), ↓erythrocytes (M, F): ↑platelet counts (F: week 104) **-changes in biochemistry**: (↑serum glutamic-pyruvic	The animals appeared unhealthy with several further signs of general toxicity (anaemia, hepatotoxicity) and several histomorphological alterations (lung, liver, kidney, adrenals). Thus, the general bad health conditions of the affected animals might be the cause for the observed testicular lesions. The reduction in testis weigth in dogs would suit an estrogen agonist effect but lack of histopathological effect in the prostate (increased prostatic size and wegith by hyperplasia of the fibromuscular stroma and squamous metaplasia of the glandular epithelium) would argue rather against. The same applies to an androgen excess; a characterized disturbed spermatogenesis is usually accompanied by an unchanged or increased prostate weigth. In the affected dogs, however we see a significant reduction in prostate weight and no significant histological abnormalities.	Effects on testes were noted at a dose level with marked toxicity and might be due to systemic toxicity. No abnormalities were detected in the 90-day toxicity dog study using dose levels up to 30 mg/kg bw/day.

	females at ≥250 ppm), kidneys (in both sexes at 1000 ppm, mesenteric lymph node (in females at 1000 ppm), gall bladder (in both sexes at 1000 ppm), pancreas (in females at 1000 ppm), aorta (one female at 1000 ppm), testis (1000 ppm) and ovaries (1000 ppm)		transaminase (M, F), ↑alkaline phosphatase (M, F), ↑bilirubin (F: weeks 52, 78), ↑serum glutamineo-oxaloacetic transaminase (M, F)) **-changes in organ weights:** ↑rel lungs (92%) (F), ↑ rel spleen (77%) (F), ↑rel gonads (80%) (F), ↓rel gonads (55%) M, n.s), ↓rel prostate (45%), n.s)) **-macroscopical changes** in the liver (enlarged, lobes thickened and pale, rough surface and mottled, brown in colour, tough in consistency, firm), gall bladder (distended, walls thickened), kidneys (small, depressed areas on surface, contracted, polycystic-primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule), urinary bladder (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface), spleen (dark in colour or margins dark, enlarged), testes (small and soft), prostate (small at week 52), ovary (cyst on one), heart (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52), lung (raised yellow gray foci on all lobes, focal emphysematous appearing areas), cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in		

			colour), mesenteric lymph nodes (dark in colour), small intestine (walls slightly thickened) -**histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), necrosis, one female), lung (foci of foamy macrophages (M,F), focal pneumonitis (M, F), cholesterol clefts (M,F), fibrosis (one female), edema (M,F), consolidation (one male)), spleen (extramedullary haematopoesis and congestion (M,F)), liver (pigment in cytoplasm of hepatocytes, kuppfer cells and macrophages (M,F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (M,F), sinusoidal distension (F)), kidney (tubular nephropathy with fibrosis and renal tubular regeneration (M,F)), urinary bladder (pigment in mucosal cells (M,F), edema (one female), pigment laden macrophages (one female)), testis (aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis), ovary (lack of follicle development, follicular cysts (one female), mesenteric lymph nodes (edema, erythrophagocytosis, distension of medullary sinuses (F)), pancreas (edema (F)), gall bladder (hyperplasia (M,F), papillary infolding (M,F), cholelith (one female)), aorta (mineralisation in one female), small intestine (focal enteritis (one female), erosion (one female))		

Prostate					
Oral (dietary) 2-year (RAR Vol 3, B.6.3.3.1/01) In house method Dog Beagle M, F 4/sex/dose GLP: Yes <u>Dose levels:</u> 0, 2, 10, 50, 250 and 1000 ppm (equivalent to 0, 0.06, 0.33, 1.42, 7.62 and 26.6 mg/kg bw/day in males, and 0, 0.06, 0.31, 1.39, 6.79 and 29.1 mg/kg bw/day in females)	NOAEL for both sexes was set at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) based on mortalities noted in both sexes at 1000 ppm, reduced bodyweight gain noted in both sexes at ≥250 ppm, changes in haematological parameters (indicating anaemia) noted in both sexes at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistically significant changes in relative organ weights (lung, spleen and gonads) noted in females at 1000 ppm, changes in gross pathology noted at 50 ppm (urinary bladder, spleen, ovary) and 250 ppm (urinary bladder, spleen, liver, ovary, kidneys) and 1000 ppm (urinary bladder, spleen, ovary, liver, gall bladder, kidneys, testes, ovary, heart, lung, mesenteric lymph nodes) and histopathological changes noted in the liver (in females at ≥50 ppm; in males at ≥250 ppm), urinary bladder (in both sexes at ≥50 ppm), adrenals (in both sexes at ≥250 ppm), lungs (in both sexes at ≥250 ppm), spleen (in males at 1000 ppm; in females at ≥250 ppm), kidneys (in both sexes at 1000 ppm, mesenteric lymph node (in females at 1000 ppm), gall bladder (in both sexes at 1000	<u>At 1000 ppm (26.6 mg/kg bw/day):</u> <u>Prostate:</u> small at week 52	<u>At 1000 ppm (26.6 mg/kg bw/day):</u> **-mortality** (one of each sex sacrificed in extremis during week 65) **-clinical signs** (brown-tined urine, orange stained hair around urogenital area, during the second year of study: pale appearing oral mucosal membranes, yellowish discoloration of the eyes and thinness in the female sacrificed in extremis, and unhealthy appearance characterized by thinness and lethargy in the male sacrificed in extremis) **↓bw** (week 52: M: 21%, F: 26%; week 104: M: 23%, F: 33%) **↓bw gain or bw loss** (Weeks 0-52: M: 0.3% compared to 2.4% in controls, F: -0.6% compared to 1.7% in controls; Weeks 52-104: M: 0.1% compared to 1.2% in controls, F: -0.6% compared to 1.5% in controls) **-changes in haematological parameters** (all post treatment intervals: ↓haemoglobin M: up to 26%, F: up to 47% ↓haematocrit (M, F), ↓erythrocytes (M, F): ↑platelet counts (F: Week 104) **-changes in biochemistry:** (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F), ↑bilirubin (F: Weeks 52, 78), ↑serum glutamineo-oxaloacetic transaminase (M, F))	Since these animals lost body weight and showed a reduced body weight gain, this loss in prostate weight was assumed to be rather due to the poor general conditions than provoked by endocrine influence. There were no accompanying histopathological abnormalities, neither in the prostate nor in the seminal vesicles.	The effect on prostate was noted at a dose level with marked toxicity and might be due to systemic toxicity. No histopathological changes were noted in the prostate.

	ppm), pancreas (in females at 1000 ppm), aorta (one female at 1000 ppm), testis (1000 ppm) and ovaries (1000 ppm)		-**changes in organ weights:** ↑rel lungs (92%) (F), ↑ rel spleen (77%) (F), ↑rel gonads (80%) (F), ↓rel gonads (55%) M, n.s), ↓rel prostate (45%), n.s)) -**macroscopical changes** in the liver (enlarged, lobes thickened and pale, rough surface and mottled, brown in colour, tough in consistency, firm), gall bladder (distended, walls thickened), kidneys (small, depressed areas on surface, contracted, polycystic-primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule), urinary bladder (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface), spleen (dark in colour or margins dark, enlarged), testes (small and soft), prostate (small at week 52), ovary (cyst on one), heart (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52), lung (raised yellow gray foci on all lobes, focal emphysematous appearing areas), cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in colour), mesenteric lymph nodes (dark in colour), small intestine (walls slightly thickened) -**histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), necrosis, one female),		

			lung (foci of foamy macrophages (M,F), focal pneumonitis (M, F), cholesterol clefts (M,F), fibrosis (one female), edema (M,F), consolidation (one male)), spleen (extramedullary haematopoesis and congestion (M,F)), liver (pigment in cytoplasm of hepatocytes, kuppfer cells and macrophages (M,F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (M,F), sinusoidal distension (F)), kidney (tubular nephropathy with fibrosis and renal tubular regeneration (M,F)), urinary bladder (pigment in mucosal cells (M,F), edema (one female), pigment laden macrophages (one female)), testis (aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis), ovary (lack of follicle development, follicular cysts (one female), mesenteric lymph nodes (edema, erythrophagocytosis, distension of medullary sinuses (F)), pancreas (edema (F)), gall bladder (hyperplasia (M,F), papillary infolding (M,F), cholelith (one female)), aorta (mineralisation in one female), small intestine (focal enteritis (one female), erosion (one female))		
Ovary					
Oral (dietary) 2-year (RAR Vol 3, B.6.3.3.1/01)	NOAEL for both sexes was set at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) based on mortalities	50 ppm: Ovary: one small in size 250 ppm:	50 ppm: ↓bw (M: week 52: 3%, week 104: 8%; F: week 52: 2%, week 104: 5%)	After 104 weeks all high dose animals showed absence of developing follicles and therefore a loss of cyclic	Loss of cyclic activity was noted at a dose level with marked toxicity.

In house method Dog Beagle M, F 4/sex/dose GLP: Yes <u>Dose levels:</u> 0, 2, 10, 50, 250 and 1000 ppm (equivalent to 0, 0.06, 0.33, 1.42, 7.62 and 26.6 mg/kg bw/day in males, and 0, 0.06, 0.31, 1.39, 6.79 and 29.1 mg/kg bw/day in females)	noted in both sexes at 1000 ppm, reduced bodyweight gain noted in both sexes at ≥250 ppm, changes in haematological parameters (indicating anaemia) noted in both sexes at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistically significant changes in relative organ weights (lung, spleen and gonads) noted in females at 1000 ppm, changes in gross pathology noted at 50 ppm (urinary bladder, spleen, ovary) and 250 ppm (urinary bladder, spleen, liver, ovary, kidneys) and 1000 ppm (urinary bladder, spleen, ovary, liver, gall bladder, kidneys, testes, ovary, heart, lung, mesenteric lymph nodes) and histopathological changes noted in the liver (in females at ≥50 ppm; in males at ≥250 ppm), urinary bladder (in both sexes at ≥50 ppm), adrenals (in both sexes at ≥250 ppm), lungs (in both sexes at ≥250 ppm), spleen (in males at 1000 ppm; in females at ≥250 ppm), kidneys (in both sexes at 1000 ppm, mesenteric lymph node (in females at 1000 ppm), gall bladder (in both sexes at 1000 ppm), pancreas (in females at 1000 ppm), aorta (one female at 1000 ppm), testis (1000 ppm) and ovaries (1000 ppm)	Ovary: cyst on one <u>1000 ppm:</u> Ovary: lack of follicle development and follicular cysts in one	↓bw gain (Week 0-52: M: 2.6% compared to 2.4% in controls, F: 2.3% compared to 1.7% in controls; Week 52-104: M: 0.1% compared to 1.2% in controls, F: 0.9% compared to 1.5% in controls) **-changes in haematological parameters** ↓haematocrit (M, Week 76), ↓erythrocytes (M Week 76, F Week 104) **-macroscopical changes** in ovary (one small in size), urinary bladder (mucosa brown or tan in colour), spleen (dark in colour or margins dark)) **-histopathological changes** in lung (focal pneumonitis (one male)), liver (pigment in macrophages (one female), bile plugs in canaliculi (one female)), urinary bladder (pigment in mucosal cells (M, F)) <u>250 ppm:</u> **-clinical signs** (brown-tined urine) ↓bw (week 104: M: 8%, F: 6%) **↓bw gain or bw loss** (week 0-52: M: 1.9% compared to 2.4% in controls, F: 2% compared to 1.7% in controls, week 52-104: M: -0.1% compared to 1.2% in controls, F: 0.1% compared to 1.5% in controls) **-changes in haematological parameters** (↓haemoglobin (M: week 26: 16%, week 52: 17%, week 76: 12%; F: week 26: 16%,	activity. The body weight of high dose animals is markedly lower than in the other dosing groups. The bitches of the high dose group even lost body weight during the study in contrast to the other females involved who gained weight as expected. The animals appeared unhealthy with several further signs of general toxicity (anaemia, hepatotoxicity) and several histomorphological alterations (lung, liver, kidney, adrenals). It is reasonable to assume that those bad conditions may lead to infertility and it is highly likely that the lack of cyclicity is due to the reduction in body weight and should not be considered as triggered by endocrine mechanism	The effects on ovary noted at 50 ppm (one small in size) and 250 ppm (cyst on one) could not be explained by marked toxicity. However, the incidence was low.

			week 76: 20%), ↓haematocrit (M: Weeks 26, 52, 76; F: Weeks 26, 76, 104), ↓erythrocytes (M: Weeks 26, 52, 76, 104; F: Weeks 76, 104): -**changes in biochemistry**: (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F),↑serum glutamic-oxaloacetic transaminase (M, F)) -**macroscopical changes** in urinary bladder (mucosal surface brown or yellow-gray), liver (brown in colour, rough surfaced, tough in consistency, firm), spleen (dark in colour or margins dark), kidneys (depressed areas on surface), ovary (cyst on one), lung (white foci on surface) -**histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), focal nonsupparative adrenalitis (one male)), lung (foci of foamy macrophages (M,F)), spleen (extramedullary haematopoesis (F), congestion (F)), liver (pigment in cytoplasm of hepatocytes and kuppfer cells (M,F), pigment in macrophages (F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (one female), sinusoidal distension (F)), kidney (tubular nephrosis (one female), urinary bladder (pigment in mucosal cells (M,F), pigment laden macrophages (F))		

			At 1000 ppm (26.6 mg/kg bw/day): -**mortality** (one of each sex sacrificed in extremis during week 65) -**clinical signs** (brown-tined urine, orange stained hair around urogenital area, during the second year of study: pale appearing oral mucosal membranes, yellowish discoloration of the eyes and thinness in the female sacrificed in extremis, and unhealthy appearance characterized by thinness and lethargy in the male sacrificed in extremis) ↓**bw** (week 52: M: 21%, F: 26%; week 104: M: 23%, F: 33%) ↓**bw gain or bw loss** (Weeks 0-52: M: 0.3% compared to 2.4% in controls, F:-0.6% compared to 1.7% in controls; Weeks 52-104: M: 0.1% compared to 1.2% in controls, F: -0.6% compared to 1.5% in controls) -**changes in haematological parameters** (all post treatment intervals: ↓haemoglobin M: up to 26%, F: up to 47% ↓haematocrit (M, F), ↓erythrocytes (M, F): ↑platelet counts (F: Week 104) -**changes in biochemistry**: (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F), ↑bilirubin (F: Weeks 52, 78), ↑serum glutamineo-oxaloacetic transaminase (M, F))		

			-**changes in organ weights**: ↑rel lungs (92%) (F), ↑ rel spleen (77%) (F), ↑rel gonads (80%) (F), ↓rel gonads (55%) M, n.s), ↓rel prostate (45%), n.s)) -**macroscopical changes** in the liver (enlarged, lobes thickened and pale, rough surface and mottled, brown in colour, tough in consistency, firm), gall bladder (distended, walls thickened), kidneys (small, depressed areas on surface, contracted, polycystic-primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule), urinary bladder (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface), spleen (dark in colour or margins dark, enlarged), testes (small and soft), prostate (small at week 52), ovary (cyst on one), heart (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52), lung (raised yellow gray foci on all lobes, focal emphysematous appearing areas), cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in colour), mesenteric lymph nodes (dark in colour), small intestine (walls slightly thickened) -**histopathological changes** in adrenal (↑vacuolation of cortical		

			cells (M,F), necrosis, one female), lung (foci of foamy macrophages (M,F), focal pneumonitis (M, F), cholesterol clefts (M,F), fibrosis (one female), edema (M,F), consolidation (one male)), spleen (extramedullary haematopoesis and congestion (M,F)), liver (pigment in cytoplasm of hepatocytes, kuppfer cells and macrophages (M,F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (M,F), sinusoidal distension (F)), kidney (tubular nephropathy with fibrosis and renal tubular regeneration (M,F)), urinary bladder (pigment in mucosal cells (M,F), edema (one female), pigment laden macrophages (one female)), testis (aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis), ovary (lack of follicle development, follicular cysts (one female), mesenteric lymph nodes (edema, erythrophagocytosis, distension of medullary sinuses (F)), pancreas (edema (F)), gall bladder (hyperplasia (M,F), papillary infolding (M,F), cholelith (one female)), aorta (mineralisation in one female), small intestine (focal enteritis (one female), erosion (one female))		
Uterus (and ovary)					
Oral (capsules) 90-day study (Vol. 3,	NOAEL for both sexes was set at 3 mg/kg bw/day based on reduced bodyweight gain noted	Uterus (and ovary) Organ weights and adjusted body weight in	3 mg/kg bw/day: -clinical signs (coloured urine and faeces) (M, F)	The study report states that differences between the group mean uterus (and ovary) weight	It is stated by study author that ovary and uterus weight of control and

B.6.3.2.2/01) OECD 409 (1998) Dog Beagle M, F 4/sex/dose GLP: Yes <u>Dose levels:</u> 0, 3, 10 and 30 mg/kg bw/day	in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day, changes in haematological parameters (indicating haemolytic anaemia) noted in both sexes at ≥10 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 30 mg/kg bw/day, increased liver weight (noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day), increased thyroid/parathyroid weight noted in males at ≥10 mg/kg bw/day, increased spleen weight noted in females at 30 mg/kg bw/day), gross pathology changes noted in females at 30 mg/kg bw/day (enlarged spleen, mottled liver and red bladder) and histopathological changes noted in the bone marrow (both sexes at ≥10 mg/kg bw/day), liver (both sexes at ≥10 mg/kg bw/day), urinary bladder (noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day), kidney (noted in both sexes at 30 mg/kg bw/day) and spleen (noted in both sexes at 30 mg/kg bw/day)	control animals remarkable higher than in test item animals (statistical analysis not performed). Control animals (two of four animals) showed estrous cyclicity while treatment animals (12 animals) did not show activity.	<u>10 mg/kg bw/dag:</u> -clinical signs (coloured urine and faeces) (M, F) ↓bw gain (F: 12% n.s.) ↓FC (M) **-changes in haematological parameters** (↓red blood cell count (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin concentration (M, F), ↑platelet count (F, n.s.), ↑platelet crit (F, n.s.), ↑total white blood cell count (F, n.s.)) **-changes in organ weights** (↑adjusted liver (F: 27%), ↑adjusted thyroid/parathyroid (M: 33%)) **-histopathological changes** in bone marrow (haemopoiesis (M, F)), liver (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F)), urinary bladder (cystitis (one female)) <u>30 mg/kg bw/day:</u> -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (M: 31%, F: 35%) ↓FC (M, F) **-changes in haematological parameters** (↓red blood cell count (M, F), ↓haemoglobin (M: 18%, F: 19%), ↓packed cell volume (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin concentration (M, F), ↑mean cell volume (M, F), ↑platelet count (M, F), ↑platelet	of control and treated females at the terminal kill are considered to reflect differences in the stage of estrous cycle between individual animals and not to be related to treatment. No estrous cycle was determined in vaginal tissue. Based on the available information it is not possible to explain why only the control animals show estrous cyclicity. An exogenous influence by an endocrine acting substance cannot be excluded, however if Quinoclamine would exhibit endocrine activity additional effects should have been observed in other organs within this study, particularly impairment of spermatogenesis and ovarian lesion which would have become visible during 90 day of treatment.	treated females are considered to reflect differences in the stage of estrous cycle between individual animal and not to be related to treatment. No estrous cycle was determined in treated animals but in control animals (2 of 4 animals). The lack of cyclic activity might be indicative of endogenic activity.

			crit (M, F), ↑total white blood cell count (M, F)) -changes in biochemistry (↑mean total bilirubin (M, F)) **-changes in organ weights** (↑adjusted liver (M: 20%, F: 29%), ↑adjusted thyroid/parathyroid (M: 32%), ↑adjusted spleen (F: 56% n.s.)) **-macroscopic changes** in spleen (enlarged two females), liver (mootled, one female) and urinary bladder (red, one female) **-histopathological changes** in bone marrow (haemopoiesis characterised by greater cellularity (M, F)), spleen (haemopoiesis characterised by increased haemopoietic cells in the red pulp (M, F), congestion of the splenic red pulp (M, F)), liver (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F), bile duct hyperplasia (M, F)), kidney (pigment (M, F)), urinary bladder (transitional cell hyperplasia (M, F), arteritis (one male), cystitis (one female))		
Long-term toxicity and carcinogenicity (RAR Vol. 3, B.6.5.1/01) Oral (dietary)	NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm (38.3 mg/kg bw/day), changes in haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males	52 ppm (3.65 mg/kg bw/day) Uterus: Hydrometra (at 26 weeks: 3 animals compared to 1 in control group)	52 ppm: **-changes in urinalysis** (yellow/brown or orange discoloration) (M, F) -changes in organ weights (Week 27: ↑kidney (M: 8%)) **-histopathological changes** in urinary bladder (epithelial hyperplasia (M, F), kidneys (epithelial hyperplasis (M, F),	An increased number of females showing hydrometra were observed after 26 and 52 weeks of treatment. Principally hydrometra can be induced by estrogenic action, e.g. during permanent estrus in an age-dependent spontaneous disturbance of ovarian function. To discern whether hydrometra	Increased incidence of hydrometra was observed but did not show a clear relationship to treatment. Further estrogenic alterations such as ovarian atrophy, hyperplasia of vaginal and uterine tissue, testicular and prostate lesions were not presented.

No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group GLP: No <u>Dose levels:</u> <u>Carcinogenicity groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females <u>Chronic toxicology groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females Study was checked for compliance with OECD TG 453 and following deviations were noted: i. Haematological examination was not carried out at 3 months (the guideline	and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm. NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm	<u>676 ppm (49.4 mg/kg bw/day)</u> <u>Uterus:</u> Hydrometra (at 26 weeks: 4 animals compared to 0 in control group; at 52 weeks: 1 animal compared to 0 in control group)	↑ renal focal calcification (F), <u>ureter</u> (epithelial hyperplasia (M, F), <u>lungs</u> (arterial calcification (M)) <u>676 ppm:</u> -clinical signs (orange fur staining, ↓incidence of mass bearing animals) (M, F) ↓**bw gain** (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓FC (M, F) **-changes in haematological parameters** (↓packed blood cell volume (M week 27, 79; F week 53), ↓haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓red blood cell count (M week 27, 79; F: week 27, 53)) **-changes in biochemical parameters** (↑blood urea nitrogen (M n.s., F n.s.), ↓calcium (M: week 27, 79; F: n.s.), ↓inorganic phosphorous (M: n.s, F: week 27, 53), ↓lactate dehydrogenase (M: week 79, 103; F: week 103)) **-changes in organ weights** (Week 27: ↑rel kidney (M: 15%), ↑adrenals (F: 38%), Week 53: ↑kidney (M: 10%), Week 79: ↑heart (M: 18%, F: 28%), ↑brain (F: 28%), ↑spleen (F. 13%), ↑kidney (F: 19%), Week 104: ↑brain (F: 23%), ↑thyroid (F:43%), ↑(heart (F: 16%), ↑adrenals (F: 9%), ↑thymus (F: 50%)) **-changes in urinalysis** (yellow/brown or orange	was exogenously induced by Quinoclamine or displays a physiological phenomenon could not be decided solely based on the presence of hydrometra. However, considering the other endocrine sensitive reproductive organs, further estrogenic alterations should become visible e.g. ovarian atrophy, hyperplasia of vaginal and uterine tissue, testicular and prostate lesions. The respective organs were concerning this matter unremarkable. Thus it is to assume that observed hydrometra within this study was not due to exogenous endocrine influence.	

recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study) ii. Prothrombin time and activated partial thromboplastin time was not investigated iii. Urea was not investigated iv. Uterus and epididymides were not weighed v. Coagulating gland, ileum, lacrimal gland and seminal vesicle were not investigated for histopathology			discoloration (M, F), diuretic animals (M)) -**macroscopical changes** in urinary bladder (orange discoloration of the urinary bladder serosa) (M, F) and skin (orange staining (M, F)) -**histopathological changes** in urinary bladder (benign transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), kidneys (epithelial hyperplasia (M, F), renal papillary degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑ renal focal calcification, ureter (epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F), pancreas (↑pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity))		
Mammary gland					
Long-term toxicity and carcinogenicity (RAR Vol. 3, B.6.5.1/01)	NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm	<u>676 ppm (49.4 mg/kg bw/day)</u> <u>Mammary gland, females:</u>	<u>676 ppm:</u> -clinical signs (orange fur staining, ↓incidence of mass bearing animals) (M, F)	After 104 weeks of treatment there was a significant reduction in mammary tumors and reduced mammary acinar development and secretion in the high dose females compared	Reduced mammary acinar development and secretion were noted in females at a dose level with lower food consumption and reduced body weight, thus this

Oral (dietary) No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group GLP: No <u>Dose levels:</u> <u>Carcinogenicity groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females <u>Chronic toxicology groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females Study was checked for compliance with OECD TG 453 and following deviations were noted:	(38.3 mg/kg bw/day), changes in haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm. NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm	mammary acinar development and secretion reduced	**↓bw gain** (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓FC (M, F) **-changes in haematological parameters** (↓packed blood cell volume (M week 27, 79; F week 53), ↓haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓red blood cell count (M week 27, 79; F: week 27, 53)) **-changes in biochemical parameters** (↑blood urea nitrogen (M n.s., F n.s.), ↓calcium (M: week 27, 79; F: n.s.), ↓inorganic phosphorous (M: n.s, F: week 27, 53), ↓lactate dehydrogenase (M: week 79, 103; F: week 103)) **-changes in organ weights** (Week 27: ↑rel kidney (M: 15%), ↑adrenals (F: 38%), Week 53: ↑kidney (M: 10%), Week 79: ↑heart (M: 18%, F: 28%), ↑brain (F: 28%), ↑spleen (F. 13%), ↑kidney (F: 19%), Week 104: ↑brain (F: 23%), ↑thyroid (F:43%), ↑(heart (F: 16%), ↑adrenals (F: 9%), ↑thymus (F: 50%)) **-changes in urinalysis** (yellow/brown or orange discoloration (M, F), diuretic animals (M)) **-macroscopical changes** in <u>urinary bladder</u> (orange discoloration of the urinary bladder serosa) (M, F) and <u>skin</u> (orange staining (M, F)) **-histopathological changes** in <u>urinary bladder</u> (benign	to control. As stated by the study authors, this was probably related with the lower food consumption and reduced body weight observed in the same females	effect might be caused by bad health condition.

i.Haematological examination was not carried out at 3 months (the guideline recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study) ii. Prothrombin time and activated partial thromboplastin time was not investigated iii. Urea was not investigated iv. Uterus and epididymides were not weighed v. Coagulating gland, ileum, lacrimal gland and seminal vesicle were not investigated for histopathology			transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), kidneys (epithelial hyperplasia (M, F), renal papillary degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑ renal focal calcification, ureter (epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F), pancreas (↑pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity))		

Table 2.6.8.3-02: Effects on reproduction and development

Study	NOAEL in study	Effect on reproduction	Other effects at the dose level of effect on reproduction	Comments by study author	Comments by RMS
Reduced litter size/post-implantation loss/foetal weight					
Two generation reproduction study (RAR Vol. 3, B.6.6.1/01)	The NOAEL for parental animals was set at 25 ppm (1.6 mg/kg bw/day) based on clinical signs (hunched posture) noted in P1 and P2 generation animals at 500 ppm (37 mg/kg bw/day), reduced body weight	500 ppm: Reduced litter size in F2a and F2b generations (mean litter size born in F2a	500 ppm: Parental: **-clinical signs** (F0/F1: hunched posture) ↓**bw** (P1 M: 4%; P2 M: 10%; P2 F 10%) ↓**bw gain** (P1 M: 7%, P2 M: 11%; P2 F: 9%)	The lower implantation efficiency (increased pre-implantation loss and decreased fetal	Reduced litter size was noted at a dose level with parental toxicity

In-house method Rat Sprague-Dawley M, F 25/sex/group GLP: No <u>Dose levels:</u> 0, 1, 25, 500 ppm corresponding to: F0: 0, 0.07, 1.6, 30.9 mg/kg bw/day in males; 0, 0.08, 1.9 and 37.7 mg/kg bw/day in females F1: 0, 0.07, 1.7 and 37.0 mg/kg bw/day in males; 0, 0.08, 2.0 and 43.8 mg/kg bw/day in females *Study was checked for compliance with OECD TG 416 (2001) and following deviations were noted:* *i. No evaluation of the oestrus cycles was performed for either generation* *ii. No examination of sperm parameters was performed for either generation*	noted in P2 males and females at 500 ppm, and reduced bodyweight gain noted in P2 males at 500 ppm. The NOAEL for offsprings was set at 25 ppm (1.6 mg/kg bw/day) based on reduced body weights at weaning in all filial generations noted at 500 ppm (37 mg/kg bw/day) and gray lung cysts in P2 offspring reared for 3 months. The NOAEL for reproductive toxicity was set at 500 ppm (37 mg/kg bw/day).	generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group)	↓**litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) <u>Offspring:</u> -clinical signs (orange stained fur F2b offspring) ↓**bw** during lactation (F1a: 13% and 7% in males and females, respectively; F1b: 14% and 9% in males and females, respectively; F2a: 8% and 9% in males and females, respectively; F2b: 11% and 5% in males and females, respectively) ↓**litter size** in F2a and F2b generations (mean litter size born in F2a generation: 4 males and 5 females compared to 6 males and 6 females in the control group; mean litter size born in F2b generation: 5 males and 5 females compared to 7 males and 6 females in control group) **-increased incidence of gray lung cysts** in F2b offspring reared for 3 months (39 compared to 11 in control group)	viability) were not noted in the F1 generation and therefore not considered to be treatment related	

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. Postlactational ovary (primordial and growing follicles) histopathology was not performed* *viii. For the offspring, age at vaginal opening or PPS for the F1 and F2 was not determined*					
Teratology range finding study (Vol 3, B.6.6.2.1/01) No guideline claimed in study Rat Crl:CD (SD) BR F 5/group GLP: Yes <u>Dose levels:</u>	Study is a range finding study. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL	<u>200 mg/kg bw/day:</u> -increased incidence of post-implantation loss (24.5% compared to 2.4% in controls) -reduced foetal weight (27%)	<u>Maternal effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>50 mg/kg bw/day:</u> -clinical signs (staining around eye) <u>80 mg/kg bw/day:</u> -clinical signs (stained urine, stained fur around head) **- bw loss/↓bw gain** (day 7-10: -3.5 g, day 10-13: 14% (n.s)) ↓FC (Pregnancy Days 7-10: 27%, Pregnancy Days 10-13: 20%, Pregancy Days: 13-17: 17%) **-macroscopic changes** (enlarged spleen in one female)	Maternal toxicity: body weight loss and reduced food consumption noted at 80 mg/kg bw/day (at start of dosing)	Increased incidence of post-implantation loss and reduced foetal weight were noted at a dose level with marked maternal toxicity

0, 8, 50, 80, 200, 500 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17			200 mg/kg bw/day: **-mortality** (one animal died, two animals were killed in extremis) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss/↓bw gain** (day 7-10: -19.8 g, day 10-13:-1.5 g, day 13-17: 42% (n.s.)) ↓FC (Pregnancy Days 7-10: 52%, Pregnancy Days 10-13: 43%, Pregancy Days: 13-17: 29%) **-macroscopic changes** (enlarged spleen and adrenals, erosion of the stomach mucosa) ↑ **post-implantation loss** (24.5% compared to 2.4% in controls) 500 mg/kg bw/day: **-mortality** (one animal died on day 10 of pregnancy, the remaining four animals were killed in extremis on days 10 or 11 of pregnancy) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss** (-34 g, day 7-10) **↓FC** Developmental effects: 8 mg/kg bw/day: No treatment-related effects 50 mg/kg bw/day: No treatment-related effects 80 mg/kg bw/day: ↓mean foetal weight (8% n.s.) 200 mg/kg bw/day: ↑ **postimplantation loss** (24.5% compared to 2.4% in controls)		

			↓**mean foetal weight** (27%)		
Teratology study (Vol 3, B.6.6.2.1/02) No guideline claimed in study Rat Crl:CD (SD) BR F 24/group GLP: Yes <u>Dose levels:</u> 0, 5, 20 and 75 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17 *The study was checked for compliance with OECD TG 414 and following deviations were noted:* *i. Exposure time in study was once daily between days 7 and 17 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent)*	NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain (25%) noted in dams at 75 mg/kg bw/day and changes in gross pathology (enlarged spleen) noted in dams at ≥20 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight (7%) noted at 75 mg/kg bw/day and increased incidence of aortic abnormalities and skeletal variations noted at ≥20 mg/kg bw/day.	<u>75 mg/kg bw/day:</u> Reduced foetal weight (7%)	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> **-macroscopic changes** (enlarged spleen, one dam) <u>75 mg/kg bw/day:</u> **- bw gain** (25% day 7-17) ↓FC (Gestation Days 7-10: 25%, Gestation Days 10-13: 14%) **-macroscopic changes** (enlarged spleen, 4/24 dams) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> **-abnormalities** (innominate artery absent, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (7%) **-abnormalities** (innominate artery absent, four foetuses; situs inversus, two foetuses; interrupt aortic arch, one foetus) **-increased incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite; sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned)	The growth retardation (reduced foetal weight) is most probably due to reduced maternal bodyweight)	Reduced foetal weight was noted at a dose level with maternal toxicity

but also effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section) *ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill)* *iii. The choice of vehicle was not justified in study report*					
Teratology range finding study (RAR Vol. 3, B.6.6.2.1/03) No guideline claimed in study Rat Crl:CD (SD) IGSBR F	Study is a range finding study. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL	<u>50 mg/kg bw/day:</u> -increased post-implantation loss (6.2% compared to 2.8% in controls, mainly due to three deaths in one litter) <u>100 mg/kg bw/day:</u> -increased post-implantation loss (10.7% compared to 2.8% in controls)	<u>Maternal effects:</u> <u>10 mg/kg bw/day:</u> ↓**bw gain** (18%) (Day 6-20) <u>50 mg/kg bw/day:</u> ↓**bw gain** (27%) (Day 6-20) ↓FC (Days 4-20: 14%, Days 6-19: 14%, Days 19-20: 48%) ↑**postimplantation loss** (6.2% compared to 2.8% in controls, mainly due to three deaths in one litter) ↑number of early intrauterine deaths (mean number: 1.0 compared to 0.4 in control)	It is stated by the study author that the reduced fetal weight is due to the reduced maternal body weight gain and food consumption	Reduced fetal weight and increased post-implantation loss were noted at dose levels with maternal toxicity

7/group GLP: Yes <u>Dose levels:</u> 0, 10, 50, 100 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 6-19		-reduced litter size (12 compared to 12.6 in control)	↓mean litter weight (2%) <u>100 mg/kg bw/day:</u> ↓**bw gain** (41%) (Day 6-20) ↓FC (Days 4-20: 21%), Days 6-19: 21%, Days 19-20: 30%) ↓**gravid uterus weight** (17%) ↑**postimplantation loss** (10.7% compared to 2.8% in controls) ↑**number of early intrauterine deaths** (mean number: 1.2 compared to 0.4 in control) ↓**mean litter weight** (16%) ↓**mean litter size** (12 compared to 12.6 in control) <u>Developmental effects:</u> <u>10 mg/kg bw/day:</u> ↓**mean foetal weight** (8%) <u>50 mg/kg bw/day:</u> ↓**mean foetal weight** (11%) ↑**postimplantation loss** (6.2% compared to 2.8% in controls, mainly due to three deaths in one litter) ↑**number of early intrauterine deaths** (mean number: 1.0 compared to 0.4 in control) ↓**mean litter weight** (2%) <u>100 mg/kg bw/day:</u> ↓**mean foetal weight** (12%) ↑**postimplantation loss** (10.7% compared to 2.8% in control) ↑**number of early intrauterine deaths** (mean number: 1.2 compared to 0.4 in control) ↓**mean litter weight** (16%)		

			↓**mean litter size** (12 compared to 12.6 in control)		
Teratology study (RAR Vol. 3, B.6.6.2.1/04) No guideline claimed in study Rat Crl:CD (SD) IGSBR F 24/group GLP: Yes *The study was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted: i. Exposure time in study was once daily between days 6 and 19 of pregnancy (the guideline is not intended to examine solely the period of organogenesis (e.g. days 5-15 in the rodent) but also effects from preimplantation, when appropriate, through the entire period of gestation to the day before caesarean section)*	NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight gain noted in dams at ≥20 mg/kg bw/day, body weight loss noted in dams at 75 mg/kg bw/day, reduced mean gravid uterus weight noted in dams at ≥20 mg/kg bw/day, reduced mean litter weight noted at ≥20 mg/kg bw/day, increased number of post-implantation loss and early intrauterine deaths noted at 75 mg/kg bw/day, and reduced mean litter size noted at 75 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced foetal weight noted at ≥20 mg/kg bw/day, increased number of post-implantation loss and early intrauterine deaths noted at 75 mg/kg bw/day, reduced mean litter size noted at 75 mg/kg bw/day, increased incidence of skeletal variations noted at ≥20 mg/kg bw/day, and malformations noted at 75 mg/kg bw/day.	<u>75 mg/kg bw/day:</u> -reduced litter size (12 compared to 14.8 in control) -increased post-implantation loss (11% compared to 5% in control, n.s.) -reduced foetal weight (12%)	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 14 of gestation) ↓**bw gain** (Days 7-8: 62%, Days 17-19: 21%) ↓FC (Days 7-8: 14%, Days 9-12: 17%, Days 12-15: 10%, Days 15-17: 12%, Days 17-19: 12%) ↓**mean gravid uterus weight** (15%) ↓**mean litter weight** (13%) <u>75 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs from Day 10, nose rubbing) ↓**bw gain** (Days 17-19: 41%) **-bw loss** (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) ↓FC (Days 4-6: 9%, Days 6-7: 27%, Days 7-8: 44%, Days 8-9: 34%, Days 9-12: 30%, Days 12-15: 17%, Days 15-17: 13%, Days 17-19: 33%) ↓**mean gravid uterus weight** (30%) ↑**post-implantation loss** (11% compared to 5% in control, n.s.) ↑ **number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter size** (12 compared to 14.8 in control) ↓**mean litter weight** (29%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment-related effects	Lower foetal weight was associated by the authors with the significantly lower maternal weight gains. The increased incidence of post-implantation loss might be due to unfavourable study conditions (the rats were supplied time-mated)	Reduced litter size and increased post-implantation loss (n.s.) were noted at a dose level with maternal toxicity.

ii. Treatment was not extended (the guideline states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill) *iii. The choice of vehicle was not justified in study report* <u>Dose levels:</u> 0, 5, 20, 75 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 6-19			<u>20 mg/kg bw/day:</u> ↓**foetal weight** (7%) ↓**mean litter weight** (13%) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (12%) ↓**litter weight** (29%) ↑**post-implantation loss** (11% compared to 5% in control) ↑**pre-implantation loss** (17.4% compared to 8.6% in control but within current background data) ↑**number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter size** (12 compared to 14.8 in control) ↑**incidence of skeletal variations** (incomplete ossification of skull bone (frontal and nasal) and unossified fifth sternebrae) **-malformations** (subcutaneous oedema (one foetus), retro-oesophageal aortic arch (one foetus), kidney misshapen (one foetus), hydropnephrosis (three foetuses))		
Teratology range finding study (RAR Vol. 3, B.6.6.2.2/01) No guideline claimed in study Rabbit New Zealand White F 5/group	Study is a range finding study. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL	<u>20 mg/kg bw/day:</u> -increased post-implantation loss (31.1 compared to 8.7 in control) <u>50 mg/kg bw/day:</u> -increased post-implantation loss (61.0 compared to 8.7 in control) <u>80/8 mg/kg bw/day:</u>	<u>Maternal effects:</u> <u>8 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↑**post-implantation loss** (31.1 compared to 8.7 in control) <u>50 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 10: 4%, Day 14: 5%) ↓FC (days 6-10) ↑**post-implantation loss** (61.0 compared to 8.7 in control)	There is no explanation given by the study author with respect to the increased incidence of post-implantation loss. There was no growth retardation apparent in the surviving foetuses indicating severe maternal toxicity	Increased incidence of post-implantation loss was noted. The effect could not be explained by maternal toxicity

GLP: Yes <u>Dose levels:</u> 0, 8, 20, 50, 80/8a, 200/20a, 500/50a Vehicle: 0.25% gum tragacanth Gestation Days 6-18		-increased post-implantation loss (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> -increased post-implantation loss (30.0 compared to 8.7 in control)	<u>80/8 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 4%, Day 8: 3%, Day 10: 4%) ↓FC (n.s.) ↑**post-implantation loss** (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 6%, Day 10: 6%) ↓FC (Day 6-10: 80%) ↑**post-implantation loss** (30.0 compared to 8.7 in control) <u>500/50 mg/kg bw/day:</u> **-mortality** (both animals died, one died on day 9 and the other on day 10 of pregnancy) **-clinical signs** (lethargy, hunched posture, dark coloured urine) ↓**bw** (Day 8: 12%) ↓**FC** (Day 6-10: 80%) <u>Developmental effects:</u> <u>8 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> ↑**post-implantation loss** (31.1 compared to 8.7 in control) **-malformations** (spina bifida, two animals, interrupted aortic arch major, one animal, hindlimb left malrotated, one animal) <u>50 mg/kg bw/day:</u> ↑**post-implantation loss** (61.0 compared to 8.7 in control) **-malformations** (interrupted aortic arch major, one animal, kidney left agenesis, one animal)		

			80 mg/kg bw/day: ↑**post-implantation loss** (25.0 compared to 8.7 in control) 200/20 mg/kg bw/day: ↑**post-implantation loss** (30.0 compared to 8.7 in control)		
Teratology study (RAR Vol. 3, B.6.6.2.2/02) No guideline claimed in study Rabbit New Zealand White F 16/group GLP: Yes Dose levels: 0, 2.5, 7.5, 22.5 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 6-18 *The study was checked for compliance with updated OECD TG 414 (2001) and following deviations were noted:* *i. Treatment was not extended (the guideline*	NOAEL for maternal toxicity was 22.5 mg/kg bw/day. NOAEL for developmental toxicity was 7.5 mg/kg bw/day based on increased foetal variations (increased number of caudal centra ≤15) noted at 22.5 mg/kg bw/day and malformations noted at 22.5 mg/kg bw/day.	22.5 mg/kg bw/day: -reduced foetal weight (5% n.s.)	Maternal effects: 2.5 mg/kg bw/day: No treatment-related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day: ↓bw gain (Day 6-9: 0 kg compared to 0.08 kg in control, Days 0-28: 5%) Developmental effects: 2.5 mg/kg bw/day: No treatment related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day: ↓foetal weight (5% n.s.) ↑**increased incidence of skeletal variants** (increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control)) **-malformations** (scoliosis, one animal, spina-bifida, three animals, anomalies of the aortic arch, two animals, sternebral fusions, three animals, hyperextension of limb or paw, one animal)	Maternal toxicity: body weight loss and reduced body weight gain at 22.5 mg/kg bw (until day 9 of pregnancy; afterwards no differences to control) It is stated by the study author that the retardation of fetal growth was most probable a consequence of the maternal toxicity observed	Reduced foetal weight was not statistically significant reduced.

states: If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled kill) *ii. During the course of study relative humidity was within the range 54-76% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning)* *iii. The choice of vehicle was not justified in study report*					
Teratology range finding study No guideline claimed in study Rabbit Crl.NZW/Kbl BR F 7/group GLP: Yes Dose levels: 0, 5, 17.5, 30 mg/kg bw/day	Study is a range finding study. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL	<u>30 mg/kg bw/day:</u> -increased incidence of post-implantation loss (22.4% compared to 14.9% in control) -reduced foetal weight (3%)	<u>Maternal effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓**bw change** (Days 7-28: 12% of controls) ↓FC <u>30 mg/kg bw/day:</u> **-abortion** (one animal, on Day 29) ↓**bw change** (Days 7-28: 10% of controls) ↓FC (Days 7-28: 2.4%, Days 28-29: 4%) ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%)	It is stated by the study author that the reduced fetal weight is due to maternal body weight loss and reduced food consumption The increased incidence of post-implantation loss might be due to unfavourable study conditions (the rabbits were supplied time-mated, transported and had a very short acclimatization period	Increased incidence of post-implantation loss and reduced foetal weight were noted at a dose level with maternal toxicity

Vehicle: 1% aqueous methylcellulose Gestation Days 7-28			Developmental effects: <u>5 mg/kg bw/day:</u> No treatment-related effects <u>17.5 mg/kg bw/day:</u> No treatment related effects <u>30 mg/kg bw/day:</u> **-abortions** (one animal, on Day 29) ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%) ↓**mean foetal weight** (3%)	before the start of dosing)	
Teratology study (RAR Vol. 3, B.6.6.2.2/04) OECD 414 Rabbit Crl.NZW/Kbl BR F 24/group GLP: Yes *The study follows OECD TG 414 except for following deviations:* *i. Dosing of animals started on Day 7 of gestation (the guideline recommends administration to start on Day 6 of gestation)*	NOAEL for maternal toxicity was 5 mg/kg bw/day based on reduced bodyweight growth noted at 17.5 mg/kg bw/day (bodyweight change Days 12-15: 67% of control) and 30 mg/kg bw/day (bodyweight change Days 4-29: 46% of control), reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, and reduced litter weight noted at 30 mg/kg bw/day. NOAEL for developmental toxicity was 5 mg/kg bw/day based on reduced mean litter size noted at ≥17.5 mg/kg bw/day, increased post-implantation loss noted at 30 mg/kg bw/day, increased early and late intrauterine deaths noted at 30 mg/kg bw/day, reduced litter weight noted at 30 mg/kg bw/day, increased incidence of specific foetal variations noted at 30 mg/kg bw/day, and malformations noted at ≥17.5 mg/kg bw/day.	<u>17.5 mg/kg bw/day:</u> - reduced mean litter size (8.4 foetuses per female compared to 9.5 in control) <u>30 mg/kg bw/day:</u> -increased post-implantation loss (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) - reduced mean litter size (7.8 foetuses per female compared to 9.5 in control)	Maternal effects: <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day</u> ↓**bw change** (bw change Days 12-15: 67% of control) ↓**mean litter size** (8.4 foetuses per female compared to 9.5 in control) <u>30 mg/kg bw/day:</u> **-mortality** (one female killed on Day 18 of gestationc) ↓bw (Days 4-29: 7%) ↓**bw change** (Days 12-15: 0 kg compared to 0.12 kg in control, Days 4-29: 46% of control) ↓FC ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control)	The increased incidence of post-implantation loss might be due to unfavourable study conditions (the rabbits were supplied time-mated, transported and had a very short acclimatization period before the start of dosing) The study author concluded that the increased incidence of intrauterine deaths is a result of the mild maternal toxicity at 30 mg/kg bw/day	Increased incidence of post-implantation loss and reduced mean litter size was noted at a dose level with maternal toxicity

ii. During the course of study relative humidity was within the range 30-80% (the guideline recommends the relative humidity not to exceed 70% other than during room cleaning) *iii. The choice of vehicle was not justified in study report* <u>Dose levels:</u> 0, 5, 17.5, 30 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 7-28			↓**mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) <u>Developmental effects:</u> <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓**mean litter size** (8.4 foetuses per female compared to 9.5 in control) **-malformations** (hydronephrosis, one animal, increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 5.6% compared to 2.3% in control) <u>30 mg/kg bw/day:</u> ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control) ↓**mean litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) ↑ **specific foetal variations** (kidney cavitation, additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, asymmetric ossification of cervical vertebral centra) **- malformations** (hydronephrosis, 2 animals; increased incidence of abnormal terminal caudal vertebrae, mean % foetus: 6.4% compared to 2.3% in control; misshapen nasal bone (8.0%, not present in historial ctr data at time for study); misaligned thoracic vertebral arch, one foetus, increased		

			incidence of absent frontal, mean % foetus: 8.9% compared to 0.0% in control)		

Table 2.6.8.3-03: Effects on non-reproductive organs

Study	NOAEL in study	Effect on non-reproductive endocrine organ	Other effects at the dose level of effect on non-reproductive endocrine organs	Comments by study author	Comments by RMS
Adrenals					
Long-term toxicity and carcinogenicity (RAR Vol. 3, B.6.5.1/01) Oral (dietary) No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group GLP: No Dose levels: <u>Carcinogenicity groups:</u> 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females	NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm (38.3 mg/kg bw/day), changes in haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm.	<u>676 ppm:</u> Adrenal: -increased rel weight in females (week 27: 38%, week 104: 9%) -increased incidence of benign phaeochromocytoma -increased blood filled cyst in females (3 compared to 1 in control group)	<u>52 ppm:</u> **-changes in urinalysis** (yellow/brown or orange discoloration) (M, F) -changes in organ weights (Week 27: ↑kidney (M: 8%)) **-histopathological changes** in <u>urinary bladder</u> (epithelial hyperplasia (M, F), <u>kidneys</u> (epithelial hyperplasis (M, F), ↑ renal focal calcification (F), <u>ureter</u> (epithelial hyperplasia (M, F), <u>lungs</u> (arterial calcification (M)) <u>676 ppm:</u> -clinical signs (orange fur staining, ↓incidence of mass bearing animals) (M, F) **↓bw gain** (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓FC (M, F) **-changes in haematological parameters** (↓packed blood cell volume (M week 27, 79; F week 53), ↓haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓red blood cell count (M week 27, 79; F: week 27, 53)) **-changes in biochemical parameters** (↑blood urea nitrogen	The increase in adjusted organ weights in high dose females after 26 weeks of treatment was explained in the study report to be fortuitous or related to the slight growth retardation observed in the high dose group While cystic degeneration (blood filled cysts) is a common, spontaneously arising non-neoplastic lesion in the adrenal cortex of females Sprague-Dawley rats, it can also be found as a test article-related effect. It is a demanding challenge to distinguish between spontaneously occurred cystic degeneration and treatment-related damage. The incidence in blood filled cysts decreased after 78 and 104 weeks of study duration. Since there were no further clear histopathological indications of HPA axis disruption or disturbance (e.g. atrophy or hypertrophy) an endocrine mechanism should not be assumed.	Increased adrenal weight was noted in females at a dose level with general toxicity (reduced bw gain: 28%) and might be due to systemic toxicity

Chronic toxicology groups: 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females *Study was checked for compliance with OECD TG 453 and following deviations were noted:* *i.Haematological examination was not carried out at 3 months (the guideline recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study)* *ii. Prothrombin time and activated partial thromboplastin time was not investigated* *iii. Urea was not investigated* *iv. Uterus and epididymides were not weighed* *v. Coagulating gland, ileum, lacrimal gland and seminal vesicle were not investigated for histopathology*	NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm		(M n.s., F n.s.), ↓calcium (M: week 27, 79; F: n.s.), ↓inorganic phosphorous (M: n.s, F: week 27, 53), ↓lactate dehydrogenase (M: week 79, 103; F: week 103)) **-changes in organ weights** (Week 27: ↑rel kidney (M: 15%), ↑adrenals (F: 38%), Week 53: ↑kidney (M: 10%), Week 79: ↑heart (M: 18%, F: 28%), ↑brain (F: 28%), ↑spleen (F. 13%), ↑kidney (F: 19%), Week 104: ↑brain (F: 23%), ↑thyroid (F:43%), ↑(heart (F: 16%), ↑adrenals (F: 9%), ↑thymus (F: 50%)) **-changes in urinalysis** (yellow/brown or orange discoloration (M, F), diuretic animals (M)) **-macroscopical changes** in urinary bladder (orange discoloration of the urinary bladder serosa) (M, F) and skin (orange staining (M, F)) **-histopathological changes** in urinary bladder (benign transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), kidneys (epithelial hyperplasia (M, F), renal papillary degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑ renal focal calcification, ureter (epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F),		

			pancreas (↑pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity))		
Oral (dietary) 2-year (RAR Vol 3, B.6.3.3.1/01) In house method Dog Beagle M, F 4/sex/dose GLP: Yes Dose levels: 0, 2, 10, 50, 250 and 1000 ppm (equivalent to 0, 0.06, 0.33, 1.42, 7.62 and 26.6 mg/kg bw/day in males, and 0, 0.06, 0.31, 1.39, 6.79 and 29.1 mg/kg bw/day in females)	NOAEL for both sexes was set at 10 ppm (0.33 and 0.31 mg/kg bw/day in males and females, respectively) based on mortalities noted in both sexes at 1000 ppm, reduced bodyweight gain noted in both sexes at ≥250 ppm, changes in haematological parameters (indicating anaemia) noted in both sexes at ≥50 ppm, changes in biochemical parameters (indicating hepatotoxicity) noted in both sexes at ≥250 ppm, statistically significant changes in relative organ weights (lung, spleen and gonads) noted in females at 1000 ppm, changes in gross pathology noted at 50 ppm (urinary bladder, spleen, ovary) and 250 ppm (urinary bladder, spleen, liver, ovary, kidneys) and 1000 ppm (urinary bladder, spleen, ovary, liver, gall bladder, kidneys, testes, ovary, heart, lung, mesenteric lymph nodes) and histopathological changes noted in the liver (in females at ≥50 ppm; in males at ≥250 ppm), urinary bladder (in both sexes at ≥50 ppm), adrenals (in both sexes at ≥250 ppm), lungs (in both sexes at ≥250 ppm), spleen (in	250 ppm Adrenals -increased incidence of vacuolation of cortical cells was noted in males (3 animals compared to 0 in control group) 1000 ppm Adrenals -increased absolute and relative weights were noted in males, while decreased absolute weight was noted in females -increased vacuolation of cortical cells was noted in males (3 animals compared to 0 in control group) and in females (3 animals compared to 0 in control group)	250 ppm: **-clinical signs** (brown-tined urine) ↓bw (week 104: M: 8%, F: 6%) **↓bw gain or bw loss** (week 0-52: M: 1.9% compared to 2.4% in controls, F: 2% compared to 1.7% in controls, week 52-104: M: -0.1% compared to 1.2% in controls, F: 0.1% compared to 1.5% in controls) **-changes in haematological parameters** (↓haemoglobin (M: week 26: 16%, week 52: 17%, week 76: 12%; F: week 26: 16%, week 76: 20%), ↓haematocrit (M: Weeks 26, 52, 76; F: Weeks 26, 76, 104), ↓erythrocytes (M: Weeks 26, 52, 76, 104; F: Weeks 76, 104): **-changes in biochemistry:** (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F),↑serum glutamic-oxaloacetic transaminase (M, F)) **-macroscopical changes** in urinary bladder (mucosal surface brown or yellow-gray), liver (brown in colour, rough surfaced, tough in consistency, firm), spleen (dark in colour or margins dark),	According to the study authors increased cortical vacuolation must be seen as treatment related adrenal lesion. Presumably the bad health condition of the affected animals can be seen as the cause of cortical vacuolation (the dogs of the highest dose groups showed not only significant reduced body weight gain but even body weight loss after 2-years of treatment. The animals appeared unhealthy with several further signs of general toxicity (anaemia, hepatotoxicity) and several histomorphological alterations (lung, liver, kidney, gonads).	Increased incidence of vacuolation of cortical cells and increased adrenal weights were noted in males at dose levels with general toxicity (body weight loss and histopathological alterations in several organs)

	males at 1000 ppm; in females at ≥250 ppm), kidneys (in both sexes at 1000 ppm, mesenteric lymph node (in females at 1000 ppm), gall bladder (in both sexes at 1000 ppm), pancreas (in females at 1000 ppm), aorta (one female at 1000 ppm), testis (1000 ppm) and ovaries (1000 ppm)		kidneys (depressed areas on surface), ovary (cyst on one), lung (white foci on surface) **-histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), focal nonsupparative adrenalitis (one male)), lung (foci of foamy macrophages (M,F)), spleen (extramedullary haematopoesis (F), congestion (F)), liver (pigment in cytoplasm of hepatocytes and kuppfer cells (M,F), pigment in macrophages (F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (one female), sinusoidal distension (F)), kidney (tubular nephrosis (one female), urinary bladder (pigment in mucosal cells (M,F), pigment laden macrophages (F)) 1000 ppm: **-mortality** (one of each sex sacrificed in extremis during week 65) **-clinical signs** (brown-tined urine, orange stained hair around urogenital area, during the second year of study: pale appearing oral mucosal membranes, yellowish discoloration of the eyes and thinness in the female sacrificed in extremis, and unhealthy appearance characterized by thinness and lethargy in the male sacrificed in extremis) ↓**bw** (week 52: M: 21%, F:26%; week 104: M: 23%, F: 33%)		

			↓bw gain or bw loss (Weeks 0-52: M: 0.3% compared to 2.4% in controls, F: -0.6% compared to 1.7% in controls; Weeks 52-104: M: 0.1% compared to 1.2% in controls, F: -0.6% compared to 1.5% in controls) **-changes in haematological parameters** (all post treatment intervals: ↓haemoglobin M: up to 26%, F: up to 47% ↓haematocrit (M, F), ↓erythrocytes (M, F): ↑platelet counts (F: Week 104) **-changes in biochemistry:** (↑serum glutamic-pyruvic transaminase (M, F), ↑alkaline phosphatase (M, F), ↑bilirubin (F: Weeks 52, 78), ↑serum glutamineo-oxaloacetic transaminase (M, F)) -changes in organ weights: F: ↑rel lungs (92%) (F), ↑ rel spleen (77%) (F), ↑rel gonads (80%) (F), ↓rel gonads (55%) M, n.s), ↓rel prostate (45%), n.s)) **-macroscopical changes** in the liver (enlarged, lobes thickened and pale, rough surface and mottled, brown in colour, tough in consistency, firm), gall bladder (distended, walls thickened), kidneys (small, depressed areas on surface, contracted, polycystic-primarily in the medulla, cortex collapsed, thickened and opaque areas on capsule), urinary bladder (brown mucosa or tan in colour, wall thickened, omentum adhered to serosal surface), spleen (dark in colour or margins dark, enlarged),		

			testes (small and soft), prostate (small at week 52), ovary (cyst on one), heart (reddish-brown discoloration at coronary grove, right A/V valve thickened and vascular with dark raised area near point of attachment at week 52), lung (raised yellow gray foci on all lobes, focal emphysematous appearing areas), cartilage (yellow to brown in colour), trachea (brown or gray discoloration), ribs (brown of gray discoloration), tendons (brown or gray discoloration), bones (gray in colour), mesenteric lymph nodes (dark in colour), small intestine (walls slightly thickened) **-histopathological changes** in adrenal (↑vacuolation of cortical cells (M,F), necrosis, one female), lung (foci of foamy macrophages (M,F), focal pneumonitis (M, F), cholesterol clefts (M,F), fibrosis (one female), edema (M,F), consolidation (one male)), spleen (extramedullary haematopoesis and congestion (M,F)), liver (pigment in cytoplasm of hepatocytes, kuppfer cells and macrophages (M,F), periportal fibrosis (M,F), bile duct proliferation (M,F), bile plugs in canaliculi (M,F), sinusoidal distension (F)), kidney (tubular nephropathy with fibrosis and renal tubular regeneration (M,F)), urinary bladder (pigment in mucosal cells (M,F), edema (one female), pigment laden macrophages (one female)), testis		

			(aspermatogenesis, testicular atrophy, focal nonsuppurative orchitis), ovary (lack of follicle development, follicular cysts (one female), mesenteric lymph nodes (edema, erythrophagocytosis, distension of medullary sinuses (F)), pancreas (edema (F)), gall bladder (hyperplasia (M,F), papillary infolding (M,F), cholelith (one female)), aorta (mineralisation in one female), small intestine (focal enteritis (one female), erosion (one female))		
Carcinogenicity study (RAR Vol. 3 B.6.5.2/01) Oral (dietary) No guideline claims in study report Mouse Crl:CD-1 (ICR)BR 50/sex/group GLP: Yes Dose levels: 0, 3, 30 or 300 ppm (corresponding to averages of 0, 0.38, 3.82 and 40.2 mg/kg bw/day in males and 0, 0.44, 4.48 and 46.4	NOAEL was 3 ppm (0.38 and 0.44 mg/kg bw/day for males and females, respectively) based on increased mortality noted in both sexes at ≥30 ppm, reduced bodyweight gain (33% in males, 30% in females) noted at ≥300 ppm, increased relative liver weight noted in females at 300 ppm, increased relative kidney weights noted in males at ≥30 ppm and in females at 300 ppm and histopathological changes noted in the adrenal and stomach at ≥30 ppm and in the kidney, urether, urinary bladder, liver, sciatic nerve, spleen, heart and lymph nodes noted at 300 ppm, and increased incidence of malignant lymphoma noted in females at 300 ppm.	30 ppm: Adrenals. -spindle cell hyperplasia in females (32 animals compared to 29 in control group) -brown athrophy in females(13 animals compared to 2 in control group) 300 ppm: Adrenals: -spindle cell hyperplasia in males 13 animals compared to 9 in control group) -cortical hyperplasia (3 animals compared to 1 in control group) -brown athrophy in females (8 animals compared to 2 in control group)	30 ppm: ↑**mortality** (M, F) -clinical signs (orange fur staining) (M, F) -changes in organ weights (↑rel kidney, M: 14% n.s.) **-histopathological changes** in adrenal (adrenal spindle cell hyperplasia (F), brown athrophy (F)); stomach (hyperkeratosis and chronic inflammation (F)) 300 ppm: ↑**mortality** (M, F) -clinical signs (orange fur staining) (M, F) ↓**bw gain** (M: 33%, F: 30%) **-changes in organ weights** (↑rel liver (F: 20%), ↑rel kidney (M: 15% n.s., F: 24% n.s.), ↑rel heart (F), ↑brain (F)) **-histopathological changes** in adrenal (↑adrenal spindle cell hyperplasia (M), brown athropy (F)), kidney (cortical scarring (M, F), hydronephrosis (M, F)), liver	Brown atrophy found in females, might be induced by exogenous steroid hormone influence: however, it is also a common finding in aged rodents. Atrophic cortical tissue was only found in females, which is contrary to males where hyperplastic lesions were dominating the cortical histopathology Cortical hyperplasia was found in males, This might be induced by xenobiotic action, e.g. steroid hormone antagonists. However, the incidence is very low (3 animals out of 24) and taken together with the histology findings in females (atrophy) no consistent correlation is recognizable.	No conclusive pattern. Brown athrophy was noted in females at 30 ppm and 300 ppm. The dose response was not smooth. The incidence of spindle cell hyperplasia was increased in females at 30 ppm but not at 300 ppm, and in males at 300 ppm. However, the incidences were low. Also the increased incidence of cortical hyperplasia noted in males at 300 ppm was low.

mg/kg bw/day in females) *The study was checked for compliance with OECD TG 451 (adopted 7 September 2009). Following deviations were noted: i. the duration of study was 20 months (according to the guideline the duration of the study will normally be 24 months for rodents. Shorter or longer study durations may be used but should be justified). ii. cervix, coagulating gland, Hardian gland and lacrimal gland were not included in the histopathological evaluation.*			(chronic inflammation (F), brown pigmentation (F)), <u>sciatic nerve</u> (degeneration (F)), <u>spleen</u> (haemosiderosis (F), <u>heart</u> (generalised periarteritis (F), myocardial fibrosis (13 M, 2 F)), <u>stomach</u> (hyperkeratosis (M, F), epithelial hyperplasia (M), dilation of mucosal glands (M, F)), <u>urinary bladder</u> (epithelial hyperplasia (particularly F)), <u>urether</u> (dilation (M, F)), <u>lymph nodes</u> (histiocytosis (M, F), <u>lympho reticular tissue</u> (malignant lymphoma (F))		
Thyroidea					
Oral (capsules) 90-day study (Vol. 3, B.6.3.2.2/01) OECD 409 (1998) Dog Beagle M, F 4/sex/dose GLP: Yes	NOAEL for both sexes was set at 3 mg/kg bw/day based on reduced bodyweight gain noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day, changes in haematological parameters (indicating haemolytic anaemia) noted in both sexes at ≥10 mg/kg bw/day, changes in biochemical parameters (indicating liver toxicity) noted in both sexes at 30 mg/kg bw/day, increased liver weight (noted in females at ≥10 mg/kg bw/day and	<u>10 mg/kg bw/day:</u> <u>Thyroidea:</u> -increased adjusted weight in males (33%) <u>30 mg/kg bw/day:</u> -increased adjusted weight in males (32%)	<u>3 mg/kg bw/day:</u> -clinical signs (coloured urine and faeces) (M, F) <u>10 mg/kg bw/dag:</u> -clinical signs (coloured urine and faeces) (M, F) ↓bw gain (F: 12% n.s.) ↓FC (M) **-changes in haematological parameters** (↓red blood cell count (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin	As given in the study report the increase in the thyroid/parathyroid weight is of uncertain etiology and the microscopic findings were not treatment related	Increased adjusted thyroidea weight was noted in males. The effect could not be explained and might be indicative of endogenic activity.

Dose levels: 0, 3, 10 and 30 mg/kg bw/day	in males at 30 mg/kg bw/day), increased thyroid/parathyroid weight noted in males at ≥10 mg/kg bw/day, increased spleen weight noted in females at 30 mg/kg bw/day), gross pathology changes noted in females at 30 mg/kg bw/day (enlarged spleen, mottled liver and red bladder) and histopathological changes noted in the bone marrow (both sexes at ≥10 mg/kg bw/day), liver (both sexes at ≥10 mg/kg bw/day), urinary bladder (noted in females at ≥10 mg/kg bw/day and in males at 30 mg/kg bw/day), kidney (noted in both sexes at 30 mg/kg bw/day) and spleen (noted in both sexes at 30 mg/kg bw/day)		concentration (M, F), ↑platelet count (F, n.s.), ↑platelet crit (F, n.s.), ↑total white blood cell count (F, n.s.)) **-changes in organ weights** (↑adjusted liver (F: 27%), ↑adjusted thyroid/parathyroid (M: 33%)) **-histopathological changes** in <u>bone marrow</u> (haemopoiesis (M, F)), <u>liver</u> (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F)), <u>urinary bladder</u> (cystitis (one female)) <u>30 mg/kg bw/day:</u> -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (M: 31%, F: 35%) ↓FC (M, F) **-changes in haematological parameters** (↓red blood cell count (M, F), ↓haemoglobin (M: 18%, F: 19%), ↓packed cell volume (M, F), ↑reticulocyte count (M, F), ↓mean cell haemoglobin concentration (M, F), ↑mean cell volume (M, F), ↑platelet count (M, F), ↑platelet crit (M, F), ↑total white blood cell count (M, F)) -changes in biochemistry (↑mean total bilirubin (M, F)) **-changes in organ weights** (↑adjusted liver (M: 20%, F: 29%), ↑adjusted thyroid/parathyroid (M: 32%), ↑adjusted spleen (F: 56% n.s.)) **-macroscopic changes** in <u>spleen</u> (enlarged two females), <u>liver</u>		

			(mootled, one female) and urinary bladder (red, one female) -**histopathological changes** in bone marrow (haemopoiesis characterised by greater cellularity (M, F)), spleen (haemopoiesis characterised by increased haemopoietic cells in the red pulp (M, F), congestion of the splenic red pulp (M, F)), liver (sinusoidal cell pigment characterised by presence of intracytoplasmic iron-containing pigment (M, F), bile duct hyperplasia (M, F)), kidney (pigment (M, F)), urinary bladder (transitional cell hyperplasia (M, F), arteritis (one male), cystitis (one female))		
Long-term toxicity and carcinogenicity (RAR Vol. 3, B.6.5.1/01) Oral (dietary) No guideline claims presented in study report Rat Crl:CD(SD)BR 50/sex/group GLP: No	NOAEL for systemic toxicity was 4 ppm (corresponding to 0.21 and 0.28 mg/kg bw/day for males and females, respectively) based on reduced bodyweight gain noted in females at 676 ppm (38.3 mg/kg bw/day), changes in haematological parameters noted in both sexes at 676 ppm (37.6 and 38.3 mg/kg bw/day in males and females, respectively), changes in biochemical parameters noted in both sexes at 676 ppm, changes in organ weights (increased kidney weight noted in males at ≥52 ppm and in females at 676 ppm; increased thyroid, thymus, heart and adrenals noted in females at 676 ppm), changes in urinalysis noted in both sexes at ≥52 ppm (At 52 ppm and 676 ppm: yellow/brown	676 ppm: Thyroid: -increased adjusted weight in females at 104 weeks (43%)	52 ppm: -changes in urinalysis (yellow/brown or orange discoloration) (M, F) -changes in organ weights (Week 27: ↑kidney (M: 8%)) -histopathological changes in urinary bladder (epithelial hyperplasia (M, F), kidneys (epithelial hyperplasis (M, F), ↑ renal focal calcification (F), ureter (epithelial hyperplasia (M, F), lungs (arterial calcification (M)) 676 ppm: -clinical signs (orange fur staining, ↓incidence of mass bearing animals) (M, F) ↓bw gain (toxicology evaluation: F: 28%; carcinogenicity evaluation: F: 27%) ↓FC (M, F)	All findings were according to the author spontaneous findings in laboratory rats and show no clear relationship to treatment After 104 weeks in females, thyroid weights increased at 676 ppm indicating no consistent dose-dependent effect over time or as a function of time of treatment	Adjusted thyroid weight was increased in females of the high dose at week 104 (43%), but no histopathological changes were noted

Dose levels: Carcinogenicity groups: 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.82, 37.6 mg/kg bw/day in males and 0, 0.28, 3.65, 49.4 mg/kg bw/day in females Chronic toxicology groups: 0, 4, 52, 676 ppm corresponding to 0, 0.21, 2.89, 38.3 mg/kg bw/day in males; 0, 0.28, 3.72, 51.5 mg/kg bw/day in females *Study was checked for compliance with OECD TG 453 and following deviations were noted:* *i.Haematological examination was not carried out at 3 months (the guideline recommends measurements at 3 months if effect was seen on haematological parameters in a previous 90 day study)* *ii. Prothrombin time and activated partial thromboplastin time was not investigated*	to orange discoloration; At 676 ppm: diuretic males), macroscopic changes noted in both sexes at 676 ppm (discoloration in urinary bladder and skin) and histopathological changes noted in both sexes at ≥52 ppm. NOAEL for tumour incidence was 52 ppm (corresponding to 2.82 and 3.65 mg/kg bw/day in males and females, respectively) based on benign transitional cell papillomas in urinary bladder and increased incidence of benign phaeochromocytoma in adrenals noted in both sexes at 676 ppm		-changes in haematological parameters (↓packed blood cell volume (M week 27, 79; F week 53), ↓haemoglobin (M: 8% week 27, F 5% week 27, 9% week 53), ↓red blood cell count (M week 27, 79; F: week 27, 53)) -changes in biochemical parameters (↑blood urea nitrogen (M n.s., F n.s.), ↓calcium (M: week 27, 79; F: n.s.), ↓inorganic phosphorous (M: n.s, F: week 27, 53), ↓lactate dehydrogenase (M: week 79, 103; F: week 103)) -changes in organ weights (Week 27: ↑rel kidney (M: 15%), ↑adrenals (F: 38%), Week 53: ↑kidney (M: 10%), Week 79: ↑heart (M: 18%, F: 28%), ↑brain (F: 28%), ↑spleen (F. 13%), ↑kidney (F: 19%), Week 104: ↑brain (F: 23%), ↑thyroid (F:43%), ↑(heart (F: 16%), ↑adrenals (F: 9%), ↑thymus (F: 50%)) -changes in urinalysis (yellow/brown or orange discoloration (M, F), diuretic animals (M)) -macroscopical changes in urinary bladder (orange discoloration of the urinary bladder serosa) (M, F) and skin (orange staining (M, F)) -histopathological changes in urinary bladder (benign transitional cell papilloma (M, F), epithelial hyperplasia (M, F) polyp (one female), chronic inflammation (M, F), kidneys (epithelial hyperplasia (M, F), renal papillary		

iii. Urea was not investigated *iv. Uterus and epididymides were not weighed* *v. Coagulating gland, ileum, lacrimal gland and seminal vesicle were not investigated for histopathology*			degeneration/necrosis (M, F) ↑ renal cortical scarring (M, F) pelvis polyp (one male), ↑ renal focal calcification, ureter (epithelial hyperplasia (M, F), urethra (epithelial hyperplasia (M, F)), adrenals (benign phaeochromocytoma M, F), pancreas (↑pancreatic acinar atrophy (M, F), parathyroid (epithelial hyperplasia (M), mammary gland (↓mammary acinar development and secretion (F)), lungs (arterial calcification (M, F), ovaries (lack of cyclic activity))		

2.6.9 Summary of medical data and information

2.5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

No hazardous incident had occurred with workers in the production facilities of quinoclamine and its formulated products.

2.5.9.2 Data collected on human

No experiences from humans are available

2.5.9.3 Direct observations

No experiences from humans are available

2.5.9.4 Epidemiological studies

No experiences from humans are available

2.5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), Specific signs of poisoning, clinical tests

No experiences from humans are available

2.5.9.6 Proposes treatment; first aid measures, antidotes, medical treatment

First-aid measures:

Eyes: Rinse with sufficient water if an irritating feeling is presented. Receive medical examination and treatment if necessary.

Skin: Take off contaminated clothing and wash the skin with soap and water. Receive medical examination and treatment if an irritating feeling is presented.

Inhalation: Move to a clean zone at once. Receive medical examination and treatment if necessary.

Ingestion: Get the person to vomit as soon as possible (decontamination). Receive medical examination and treatment.

Medical treatment, antidotes: Decontamination as soon as possible. Symptomatic treatment. No antidotes available

Expected symptoms of poisoning: Brown-tinged urine, anorexia and/or lethargy are expected. Systemic intoxication in human is not known.

2.6.10 Toxicological end points for risk assessment (reference values)

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The ADI represents the maximum dose of a substance that can be ingested on a daily basis without bringing unacceptable risks to human health. Ideally, the ADI is derived from a NOAEL obtained in a long-term study.

The current EU Annex I endpoint for the ADI is 0.002 mg/kg bw/day (Review Report for quinoclamine SANCO/3622/07-rev 1, 1 January 2008) based on the NOAEL of 0.2 mg/kg bw obtained in the 2 year rat study, and a safety factor of 100.

No new value is proposed for the Annex I renewal of quinoclamine. The NOAEL of 0.2 mg/kg bw/day obtained in the 2-year rat study is considered an appropriate basis for the ADI of quinoclamine. Using an uncertainty factor of 100, the proposed ADI is 0.002 mg/kg bw/day. With respect to the dose levels where neoplastic changes were noted in the rat long-term toxicity study (37.6 mg/kg bw/day) and where teratogenic effects were observed in the rabbit developmental toxicity study (17.5 mg/kg bw/day) there is a margin of safety above 1000.

	NOAEL	**Study**	**Safety factor**
ADI: 0.002 mg/kg bw/day	0.2 mg/kg bw/day	Rat 2 year Anonymous 23 1991 Study Report: AKJ/7/90	100

2.6.10.1.1 Drinking water limit

The maximum admissible concentration of an active substance is **0.1 µg/L** (according to Directive 89/778/EEC).

A health-based limit (adult) of 0.012 mg/L (12 µg/L) can be derived assuming 20% of the ADI, water consumption of 2 L/day and bodyweight of 60 kg. The calculation of this value is:

C_{max} water= (ADI x 20% x Bodyweight)/2L = (0.002x 0.2x 60 kg)/2L = 0.012 mg/L (12 µg/L). Since this value is higher than the maximum permissible groundwater concentration of 0.1 µg/L, the Cmax water calculated should not be used.

A health-based limit (infant) of 0.0027 mg/L (2.7 µg/L) can be derived assuming 20% of the ADI, water consumption of 0.75 L/day and bodyweight of 5 kg. The calculation of this value is:

C_{max} water= (ADI x 20% x Bodyweight)/0.75L = (0.002 x 0.2x 5 kg)/0.75L = 0.0027 mg/L (2.7 µg/L). Since this value is higher than the maximum permissible groundwater concentration of 0.1 µg/L, the Cmax water calculated should not be used.

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

The ARfD represents the maximum dose of a substance that can be ingested on a single occasion without bringing an unacceptable risk to human health. It is usually derived from a NOAEL based on an acute effect occurring after a single exposure in an oral study. According to the guidance document (Guidance for the setting of an acute reference dose (ARfD) 7199/VI/99 rev 6), the NOAEL obtained in the most sensitive species should be considered.

The current EU Annex I endpoint for the ARfD is 0.05 mg/kg bw/day (Review Report for quinoclamine SANCO/3622/07-rev 1, 1 January 2008) based on the 28-day rat study supported by the developmental rat study, with the use of a safety factor of 100.

No new value is proposed for the Annex I renewal of quinoclamine. The NOAEL of 5 mg/kg bw/day obtained in the 28-day rat study and the developmental toxicity studies in the rat and rabbit (NOAELs of 5 mg/kg bw/day), is considered an appropriate basis for the ARfD of quinoclamine. Using an uncertainty factor of 100, the proposed ARfD is 0.05 mg/kg bw. With respect to the dose levels where neoplastic changes were noted in the rat long-term toxicity study (37.6 mg/kg bw/day) there is a margin of safety of 752. With the respect to the dose levels where teratogenic effects were observed in the rabbit developmental toxicity study (17.5 mg/kg bw/day) there is a margin of safety of 350.

In the 28-day rat study changes indicating haematolytic anemia were noted at a dose level of 44 mg/kg bw/day.In the developmental studies in the rat and rabbit developmental effects were noted at the dose level of 20 mg/kg bw/day.

	NOAEL	Study	Safety factor
ARfD: 0.05 mg/kg bw	5 mg/kg bw/day	Rat 28-day Anonymous 15 (2002) Study Report: 619/148 Developmental rat and rabbit (maternal and developmental NOAELs of 5 mg/kg bw/day) Anonymous 25 (1986) Study Report: AKJ/4/86 Anonymous 26 (2002) Study Report: 619/94-D6154 Anonymous 29 (2002) Study Report: 619/155-D6154	100

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The current EU Annex I endpoint for the AOEL is 0.03 mg/kg bw (Review Report for quinoclamine SANCO/3622/07 – rev 1, 1 February 2008) based on NOAEL of 3 mg/kg bw/day obtained in the 90-day dog study, with the use of a safety factor of 100.

No new value is proposed for the Annex I renewal of quinoclamine. The NOAEL of 3 mg/kg bw/day obtained in the 90-day dog study, supported by the 90-day rat studies (NOAELs at 3 mg/kg bw/day) is considered an appropriate basis for the AOEL of quinoclamine. Using an uncertainty factor of 100 the proposed AOEL is 0.03 mg/kg bw/day. With respect to the lowest dose levels where teratogenic effects were observed in the rabbit study (17.5 mg/kg bw/day), there is a margin of safety of 583. Based on the teratogenic findings in rats and rabbits the classification of quinoclamine as toxic for reproduction in Category 2 is proposed. With respect to the lowest dose levels where neoplastic changes were observed in the rat long-term study (37.6 mg/kg bw/day), there is a margin of safety of 1253.

The critical effect of haemolytic anemia was considered as the most relevant endpoint for the setting of AOEL. Results of the 90-day dog study (B.6.3.2.2/01) showed changes indicating haemolytic anemia at the LOAEL of 10 mg/kg bw/day (pigment in the liver, characterised by presence of intracytoplasmic iron-containing pigment). In one 90-day oral rat study (B.6.3.2.1/01) changes indicating haemolytic anemia were noted at the LOAEL of 13 mg/kg bw/day (increased hemosiderin deposition in the spleen). In a second 90-day oral rat study (B.6.3.2.1/02) changes indicating haemolytic anaemia were noted at the LOAEL of 13.89 mg/kg bw/day (dark straw colored urine, increased relative spleen weight, histopathological changes of increased extent of pigment in the spleen and liver).

No NOAEL for females could be established in the second 90-day oral rat study, due to reduced bodyweight gain (>10%) noted in females at ≥50 ppm (≥4.56 mg/kg bw/day). However, reduced bodyweight gain was not noted at the dose level of 3.72 mg/kg bw/day in the long-term toxicity study using the same strain of animals (CD-rat). Furthermore, no adverse effect on bodyweight gain was noted in the first 90-day oral rat study with Sprague-Dawley rats tested at doses up to 65 mg/kg bw/day. Thus, the effect on bodyweight noted in the second 90-day oral rat study was considered to be covered by the NOAEL of 3 mg/kg bw/day obtained in the 90-day dog study.

In the 2-year dog study, findings indicative of anaemia (pigment in liver) were noted at the dose level of 50 ppm (1.39/1.42 mg/kg bw/day in males and females, respectively). This study was, however not considered for the AOEL setting, since the exposure duration (1/6 of the lifespan for beagle dogs) was not considered as a typically short-term exposure.

	NOAEL	Study	Safety factor
AOEL: 0.03 mg/kg bw/day	3 mg/kg bw/day	90-day dog Anonymous 20 (2002) Study Report.: 0619/134 90-day rat (NOAEL of 3 mg/kg bw/day) Anonymous 18 (2003) Study report: 0619/132	100

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

The following value for AAOEL is proposed for the renewal of quinoclamine:

An AAOEL based on the 28-day rat study and the developmental rat studies, resulting in a value of 0.05 mg/kg bw (applying an uncertainty factor of 100).

In the 28-day rat study changes indicating haematolytic anemia were noted at a dose level of 44 mg/kg bw/day. In the developmental rat studies developmental effects were noted at the dose level of 20 mg/kg bw/day.

	NOAEL	Study	Safety factor
AAOEL: 0.05 mg/kg bw	5 mg/kg bw/day	Rat 28-day Anonymous 15 (2002) Study Report: 619/148 Developmental rat (maternal NOAELs of 5 mg/kg bw/day) Anonymous 25 (1986) Study Report AKJ/4/86 Anonymous 26 (2002) Study Report: 619/94-D6154	100

2.6.11 Summary of product exposure and risk assessment

Mogeton Top is a wettable granule (WG) containing 500 g/kg quinoclamine.

The critical GAP for the re-approval of quinoclamine is based on the use of the representative formulation Mogeton Top. Mogeton Top is intended to be used as a herbicide on golf greens and in nursery stock plants (potted).

Usage information pertinent to operator exposure is summarized in the Table 2.6.11-01.

Table 2.6.11-1: Summary of use patterns for the active substance quinoclamine in Mogeton Top.

Crop (field)	F/G	Application rate		Spray dilution	Number of applications	Application equipment
		[L product/ha]	[g a.i./ha]	[L/ha]		
Golf greens	F	7.5	3750	1000	1	Downward spraying
Golf greens	F	7.5	3750	1000	1	Hand-held spraying
Nursery stock plants (potted)	F	7.5	3750	1000	1	Hand-held spraying (pots on permeable sheets, no application on flowering plants)
Nursery stock plants (potted)	F	2.88	1440	800	1	Downward spraying (pots on permeable sheets, no application on flowering plants)
Nursery stock plants (potted)	G*	7.5	3750	1000	1	Hand-held spraying (pots on permeable sheets)
Nursery stock plants (potted)	G	1.62	810	450	1	Hand-held spraying (pots on permeable sheets)

F: field use
G: greenhouse use
G*: greenhouse use including walk-in tunnel

Dermal absorption data are available for quinoclamine from an in vitro study with human/rat skin (Rijk J.C.W., 2015, Report No. 506990) reported in Vol. 3 (Product), section B.6.2). Derived from the results of this study, dermal absorption rates of 0.9% (concentrate), 1% (spray dilution at 3.74 g/L) and 3% (spray dilution at 1.8 g/L) were proposed.

The results of the exposure calculations for operators, bystanders, residents and workers are summarized in Tables 2.6.11-2 to 7.

Table 2.6.11-02: Predicted systemic exposure of operator as a proportion of the AOEL using the EFSA calculator.

Model data	PPE	Total systemic exposure (mg/kg bw/day)	% of AOEL[1)]
Golf greens, application rate 3750 g a.i./ha, vehicle mounted downward spraying, field			
50 ha/day 60 kg bw 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0291	**97.05**
	+ work wear (arms, body and legs covered)	0.0195	**61.15**
Golf greens, application rate 3750 g a.i./ha, hand-held downward spraying, field			
1 ha/day 60 kg bw 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0437	145.55
	+ work wear (arms, body and legs covered)	0.0101	**33.52**
Nursery stock plants (potted plants)*, application rate 3750 g a.i./ha, hand-held downward spraying, field			
EFSA calculator 1 ha/day 60 kg bodyweight 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0437	145.55
	+ work wear (arms, body and legs covered)	0.0101	**33.52**

Nursery stock plants (potted plants)*, application rate 1440 g a.i./ha, hand-held downward spraying, field			
EFSA calculator 1 ha/day 60 kg bodyweight 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0476	158.69
	+ work wear (arms, body and legs covered)	0.0075	**25.03**
Nursery stock plants (potted plants)*, application rate 1440 g a.i./ha, vehicle mounted downward spraying, field			
EFSA calculator 10 ha/day 60 kg bodyweight 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0322	107.46
	+ work wear (arms, body and legs covered)	0.0153	**50.84**

1) AOEL 0.03 mg/kg bw/day

Table 2.6.11-3. Predicted systemic exposure of operator as a proportion of the AOEL using the Dutch Greenhouse model.

Model data	**PPE**	**Total systemic exposure (mg/kg bw/day)**	**% of AOEL**[1)]
Nursery stock plants (potted plants), application rate 3750 g a.i./ha, downward spraying, hand-held, greenhouse[2)]			
Dutch greenhouse Model 1 ha/day 60 kg bodyweight 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Without PPE	0.1875	625
	With PPE (coverall, gloves)	0.075	250
	With PPE (coverall, gloves and respiratory protection)	0.01875	**63**
Nursery stock plants (potted plants), application rate 810 g a.i./ha, downward spraying, hand-held, greenhouse			
Dutch greenhouse Model 1 ha/day 60 kg bodyweight 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Without PPE	0.0945	315
	With PPE (coverall, gloves)	0.0216	**72**
	With PPE (coverall, gloves and respiratory protection)	0.00945	**32**

1) AOEL 0.03 mg/kg bw/day
2) Including walk-in tunnels

Table 2.6.14-4. Predicted systemic exposure to bystanders as a proportion of the AAOEL using the EFSA calculator.

Model data	**Quinoclamine**		
	Exposure pathway	**Total absorbed dose [mg/kg bw/day]**	**% of systemic AAOEL**[1)]
Golf greens, application rate 3750 g a.i./ha, vehicle mounted downward spraying, field			
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0007	1.30
	Vapour (95th percentile)	0.0002	0.46
	Surface deposits (95th percentile)	0.0008	1.54
	Entry into treated crops (95th percentile)	0.0011	2.27
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0027	5.39
	Vapour (95th percentile)	0.0011	2.14
	Surface deposits (95th percentile)	0.0096	19.25
	Entry into treated crops (95th percentile)	0.0306	61.13

Model data	Quinoclamine		
	Exposure pathway	Total absorbed dose [mg/kg bw/day]	% of systemic AAOEL[1)]
Golf greens, application rate 3750 g a.i./ha, hand-held downward spraying, field			
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0007	1.30
	Vapour (95th percentile)	0.0002	0.46
	Surface deposits (95th percentile)	0.0008	1.54
	Entry into treated crops (95th percentile)	0.0011	2.27
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0027	5.39
	Vapour (95th percentile)	0.0011	2.14
	Surface deposits (95th percentile)	0.0096	19.25
	Entry into treated crops (95th percentile)	0.0306	61.13
Nursery stock plants, scenario ornamentals, application rate 3750 g a.i./ha, hand-held downward spraying, field			
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0007	1.30
	Vapour (95th percentile)	0.0002	0.46
	Surface deposits (95th percentile)	0.0008	1.54
	Entry into treated crops (95th percentile)	0.0035	7.03
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0027	5.39
	Vapour (95th percentile)	0.0011	2.14
	Surface deposits (95th percentile)	0.0096	19.25
	Entry into treated crops (95th percentile)	0.0063	12.66
Nursery stock plants, scenario ornamentals, application rate 1440 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (adult): 60 kg 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0009	1.82
	Vapour (95th percentile)	0.0002	0.46
	Surface deposits (95th percentile)	0.0009	1.77
	Entry into treated crops (95th percentile)	0.0041	8.10
EFSA calculator Drift rate: 8.5 % (2-3 m) Body weight (child): 10 kg 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (95th percentile)	0.0035	6.96
	Vapour (95th percentile)	0.0011	2.14
	Surface deposits (95th percentile)	0.0050	9.94
	Entry into treated crops (95th percentile)	0.0073	14.58

[1)] AAOEL 0.05 mg/kg bw/day

Table 2.6.14-5: Predicted systemic exposure to residents as a proportion of the AOEL using the EFSA calculator.

Model data	Quinoclamine		
	Exposure pathway	Total absorbed dose [mg/kg bw/day]	% of systemic AOEL[1)]
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.002	0.82
	Vapour (75th percentile)	0.002	0.77
	Surface deposits (75th percentile)	0.003	0.85
	Entry into treated crops (75th percentile)	0.006	1.90[2)]
	All pathways (mean)	0.011	3.69

Model data	Quinoclamine		
	Exposure pathway	**Total absorbed dose [mg/kg bw/day]**	**% of systemic AOEL**[1)]
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.0011	3.63
	Vapour (75th percentile)	0.0011	3.57
	Surface deposits (75th percentile)	0.0036	11.97
	Entry into treated crops (75th percentile)	0.0244	81.41[2)]
	All pathways (mean)	0.0055	18.45
Nursery stock plants, scenario ornamentals, application rate 3750 g a.i./ha, hand-held downward spraying, field			
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.0002	0.82
	Vapour (75th percentile)	0.0002	0.77
	Surface deposits (75th percentile)	0.0003	0.85
	Entry into treated crops (75th percentile)	0.0035	11.72
	All pathways (mean)	0.0033	11.13
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.0011	3.63
	Vapour (75th percentile)	0.0011	3.57
	Surface deposits (75th percentile)	0.0036	11.97
	Entry into treated crops (75th percentile)	0.0063	21.09
	All pathways (mean)	0.0094	31.21
Nursery stock plants, scenario ornamentals, application rate 1440 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (adult): 60 kg 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.0003	1.17
	Vapour (75th percentile)	0.0002	0.77
	Surface deposits (75th percentile)	0.0003	0.98
	Entry into treated crops (75th percentile)	0.0041	13.50
	All pathways (mean)	0.0038	12.81
EFSA calculator Drift rate: 5.6 % (2-3 m) Body weight (child): 10 kg 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Spray drift (75th percentile)	0.0015	4.96
	Vapour (75th percentile)	0.0011	3.57
	Surface deposits (75th percentile)	0.0018	5.99
	Entry into treated crops (75th percentile)	0.0073	24.30
	All pathways (mean)	0.0090	30.09

[1)] AOEL 0.03 mg/kg bw/day
[2)] Scenario not relevant, covered by recreational exposure scenario

Table 2.6.14-6. Predicted systemic recreational exposure as a proportion of the AOEL using the EFSA calculator.

Model data	Quinoclamine	
	Total absorbed dose [mg/kg bw/day]	**% of systemic AOEL**[1)]
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field		
EFSA calculator Exposure: 2 hours Body weight (adult): 60 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	0.0046	**15.21**
EFSA calculator Exposure: 2 hours Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	0.064	213.75

Model data	Quinoclamine	
	Total absorbed dose [mg/kg bw/day]	**% of systemic AOEL**[1)]
Refined recreational exposure considering dermal exposure only for the child		
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field		
EFSA calculator Exposure: 2 hours Body weight (child): 10 kg 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	0.00975	**32.50**

[1)] AOEL 0.03 mg/kg bw/day

Table 2.6.14-7. Predicted systemic worker exposure as a proportion of the AOEL using the EFSA calculator.

Model data	Level of personal protective equipment	Operator exposure [mg/kg bw/day]	% of AOEL[1)]
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator 8 hours/day 60 kg bodyweight TC: 5800 cm^2/hour (potential exp.) 2500 cm^2/hour (work wear) 580 cm^2/hour (work wear+gloves) 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0870	290.00
	+ work wear (arms, body and legs covered)	0.0375	125.00
	+ work wear and gloves	0.0087	**29.00**
Golf greens, application rate 1875 g a.i./ha, downward spraying, vehicle mounted, field			
EFSA calculator 8 hours/day 60 kg bodyweight TC: 5800 cm^2/hour (potential exp.) 2500 cm^2/hour (work wear) 580 cm^2/hour (work wear+gloves) 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.0435	145.00
	+ work wear (arms, body and legs covered)	0.0188	**62.50**
	+ work wear and gloves	0.0044	**14.50**
Nursery stock plants (potted plants)*, application rate 3750 g a.i./ha, downward spraying, hand-held, field			
EFSA calculator 8 hours/day 60 kg bodyweight TC: 14000 cm^2/hour (potential exp.) 5000 cm^2/hour (work wear) 1400 cm^2/hour (work wear+gloves) 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.2100	700.00
	+ work wear (arms, body and legs covered)	0.0750	250.00
	+ work wear and gloves	0.0210	**70.00**
Nursery stock plants (potted plants)*, application rate 1440 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator 8 hours/day 60 kg bodyweight TC: 14000 cm^2/hour (potential exp.) 5000 cm^2/hour (work wear) 1400 cm^2/hour (work wear+gloves) 3.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.2419	806.40
	+ work wear (arms, body and legs covered)	0.0864	288.00
	+ work wear and gloves	0.0242	**80.64**
Refined exposure considering a lower dislodgeable foliar residue (DFR; 1.65 µg/cm^2 of foliage/kg a.i. applied/ha)			
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator 8 hours/day	Potential exposure	0.04785	159.5

Model data	Level of personal protective equipment	Operator exposure [mg/kg bw/day]	% of AOEL[1)]
60 kg bodyweight TC: 5800 cm^2/hour (potential exp.) 2500 cm^2/hour (work wear) 580 cm^2/hour (work wear+gloves) 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	+ work wear (arms, body and legs covered)	0.02063	**68.75**
	+ work wear and gloves	0.004785	**15.95**
Refined exposure considering shorter duration (4.5 h/day) in addition to a refined DFR-value			
Golf greens, application rate 3750 g a.i./ha, vehicle mounted and hand-held downward spraying, field			
EFSA calculator 4.5 hours/day 60 kg bodyweight TC: 5800 cm^2/hour (potential exp.) 2500 cm^2/hour (work wear) 580 cm^2/hour (work wear+gloves) 1.0 % dermal absorption (in-use) 0.9 % dermal absorption (conc.)	Potential exposure	0.02692	**89.72**
	+ work wear (arms, body and legs covered)	0.01160	**38.68**
	+ work wear and gloves	0.002692	**8.97**

[1)] AOEL 0.03 mg/kg bw/day

As a conclusion the exposure estimations indicate that levels of exposure for operators, bystanders, residents and workers will be within acceptable levels of the proposed systemic AOEL (or AAOEL where relevant) of quinoclamine.

Note that possible exposure of workers in greenhouse and possible need for re-entry period for workers has not been assessed.

2.7 Residues

2.7.1 Summary of storage stability of residues

Not relevant for the proposed representative uses of Quinoclamine.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Not relevant for the proposed representative uses of Quinoclamine.

2.7.3 Definition of the residue

Not relevant for the proposed representative uses of Quinoclamine.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Not relevant for the proposed representative uses of Quinoclamine.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Not relevant for the proposed representative uses of Quinoclamine.

2.7.6 Summary of effects of processing

Not relevant for the proposed representative uses of Quinoclamine.

2.7.7 Summary of residues in rotational crops

Not relevant for the proposed representative uses of Quinoclamine.

2.7.8 Summary of other studies

Not relevant for the proposed representative uses of Quinoclamine.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Not relevant for the proposed representative uses of Quinoclamine.

2.7.10 Proposed MRLs and compliance with existing MRLs

Not relevant for the proposed representative uses of Quinoclamine.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not relevant for the proposed representative uses of Quinoclamine.

2.8 Fate and behaviour in the environment

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Route and rate of degradation in soil, laboratory studies

Sufficient laboratory data were presented on the fate and behaviour of quinoclamine and its degradation products in soil. Evaluation of the studies is presented in Vol 3, B.8 (CA).

Degradation of quinoclamine in soil under aerobic standard conditions was investigated in two studies (Völkel, 2015 and Muttzall & Vonk, 1992). A third study (Lewis, 2003a) investigated degradation at 10ºC but is considered to provide only supportive information. One study carried out under anaerobic conditions (Lewis, 2003b) was available and two studies on photochemical transformation at the soil surface (Adam, 2016a and Bishop, 2003) of which the older study is considered to provide only indicative information. Separate laboratory studies on rate of degradation of photochemical transformation products were submitted (Adam, 2016b, Piskorski, 2016, Fiebig, 2016 and Fiebig, 2017). For new studies, the kinetic assessments were generally done as part of each study report. For old studies, new kinetic assessments were generally provided as separate reports. Originally, the applicant suggested that the old studies on aerobic soil degradation (Muttzall & Vonk, 1992) and soil photolysis (Bishop, 2003) were unreliable since bound residues were evaluated insufficiently. The RMS nevertheless requested the old studies together with new kinetic data. Following evaluation the RMS concluded that there was no generally applicable reason not to consider the old studies.

Völkel (2015) incubated quinoclamine at 20.3ºC for 142 days in four soils at a relatively high test concentration of 9.8 mg/kg, corresponding to 7350 g/ha (0-5 cm, 1.5 g/cm^3). Samples were extracted four times with acetonitrile:water (4:1) and additionally by Soxhlet extraction with acetonitrile:water (4:1) for four hours. The non-extractable fraction increased over the course of the study. After 91 days it accounted for 11.4%, 17.3%, 23.8% and 17.1% AR in the four soils. After 142 days this had increased further to 17.8%, 22.0%, 25.9% and 23.7%, respectively.

By comparison, Muttzall & Vonk (1992) incubated quinoclamine at 20ºC in four soils at 3 mg/kg, corresponding to 2250 g/ha (0-5 cm, 1.5 g/cm^3). Mass balance was only established for two of the soils. Final samples were taken after 100 days, except for two of the soils in which extractable radioactivity and amounts of quinoclamine were determined also after 129 days. Samples were extracted in methanol and the extraction repeated until the last extract contained ≤ 5% AR. One of the soils was finally extracted with water. In the two soils for which mass balance was established, the non-extractable fraction increased until sampling day 78, at which 37.8% and 43.6% were non-extractable. After 100 days the corresponding amounts were slightly lower at 34.1% and 43.5%, respectively.

In comparison, extraction was apparently more complete in Völkel (2015). Rate of degradation was also considerably longer in Völkel (2015). However, the more rapid degradation in the old study (Muttzall & Vonk, 1992) was shown not only as loss of parent compound (which in part may have been due to poor extraction) but also in higher amounts of $^{14}CO_2$. In the four soils used by Völkel (2015) $^{14}CO_2$ accounted for 12.7%, 21.1%, 31.7% and 19.7% after 91 days. After 142 days this had increased further to 23.6%, 28.0%, 44.6% and 35.1%, respectively. In Muttzall & Vonk (1992) $^{14}CO_2$ accounted for 43.4% and 35.9% after 100 days. Comparison of the results from days 91 and 100, respectively, suggests that mineralisation was lower in at least three of the soils used by Völkel (2015). The reasons for the slower degradation observed in Völkel (2015) are not known but it cannot be excluded that the relatively high test concentration was one of them. Dose dependent degradation was suggested by the results from the study on aerobic mineralisation in surface water (Völkel, 2016a). Assuming 90% crop interception for the use on golf greens and 50% interception for the use in nurseries a single application would result in 0.54-4.5 mg/kg (0-5 cm, 1.5 g/cm^3). The test concentration used by Muttzall & Vonk (1992) falls within this range whereas the test concentration used by Völkel (2015) was about twice as high as the upper end of the range. The results from Völkel (2015) may therefore potentially be less representative but there may be additional unknown reasons for the slower degradation rate. On balance, the RMS concluded that the results from both studies should be relied upon.

Muttzall & Vonk (1992) extracted soils with methanol, not followed by Soxhlet extraction. In some of the other soil studies where methanol was used for extraction, this was followed by Soxhlet extraction (Lewis 2003a and 2003b, Adam, 2016a, and Bishop, 2003). In those studies Soxhlet released additionally 3.9-9.6% AR. These are relatively minor amounts and it is not likely that the in-complete extraction in Muttzall & Vonk (1992) had a dramatic effect on the observed degradation rate. It is also not considered likely that all radioactivity that potentially could have been released in Soxhlet would consist of only one or two metabolites which may have been missed due to the in-complete extraction.

Depending on soil, Völkel (2015) observed one-four identified metabolites in the extracts: phthalic acid, 2-carboxybenzaldehyde, AN (2-amino-1,4-naphthalenedione) and AHN (2-amino-3-hydroxy-1,4-naphthalenedione). Maximum amount of individual metabolite was 2.8% AR (phthalic acid). Additional five minor metabolites were occasionally observed at even lower amounts. Muttzall & Vonk (1992) observed at least five metabolites which in total accounted for maximum 6% AR and individually maximum 4% AR. Two of the metabolites were identified as HN (2-hydroxy-1,4-naphthalenedione, max 2% AR) and DHN (1,4-dihydronaphthalenedione, max 1% AR). One additional polar metabolite (max ca 2% AR) was observed in the water extract of one of the soils.

One of the soils used by both Völkel (2015) and Muttzall & Vonk (1992) was “Speyer 2.2” however with slight difference in composition and therefore treated as different soils herein. Lewis (2003a) also used “Speyer 2.2” for incubation at 10ºC and the soil characteristics deviated more for this sample of the soil. Lewis (2003a) extracted twice with methanol and twice with water and at three sampling points additionally with Soxhlet extraction (methanol:water, 2:1). Soxhlet released 5.8-7.3% AR. At sampling point 90 days, at which Soxhlet was not used,

24.3% AR was non-extractable. At sampling point 120 days, 18.4% was non-extractable and 7.3% AR was found in Soxhlet extracts. $^{14}CO_2$ accounted for 37.9% AR by day 90, 42.3% AR by day 120. Metabolites AN (2-amino-1,4-naphthalenedione) and HCN (2-chloro-3-hydroxy-1,4-naphthalenedione) were observed as <1% AR each. Additionally, un-identified metabolites accounted for total 5.7% AR after 120 days. Lewis (2003a) used a test concentration of 3.75 mg/kg, corresponding to 2810 g/ha (0-5 cm, 1.5 g/cm^3), thus within the representative range. Since incubations at 10ºC are not required anymore Lewis (2003a) is considered only to provide supportive information.

Rate of degradation in the studies by Völkel (2015) and Muttzall & Vonk (1992) is summarised in the below table. There was no indication of pH-dependency of the degradation rate. For Lewis (2003a) the RMS calculated a DT_{50} of 43.0 days at 10 ºC (SFO, Chi2 error 8.0%, based on methanol extracts).

Table 2.8.1.1-1. Summary of kinetic evaluation of laboratory data on aerobic degradation of quinoclamine in soil (from Völkel, 2015 and Knopp & Böing, 2016a kinetic re-assessment of results in Muttzall & Vonk, 1992).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days	χ2, error-%	Modelling endpoint (20ºC, pF 2)	
						DT_{50}, days	kinetic model
Völkel, 2015	Soil I Speyer 2.1 sand	5.0 [a]	SFO	196 / 652	4.4	202	SFO
	Soil II Speyer 2.2 loamy sand	5.5 [a]	DFOP	154 / 743	2.8	149	SFO
	Soil III Speyer 2.4 loam	7.2 [a]	SFO	77.1 / 256	1.4	79	SFO
	Soil IV Speyer 5M sandy loam	7.3 [a]	SFO	117 / 390	2.8	120	SFO
Muttzall & Vonk, 1992	Speyer 2.2 loamy sand	5.5 [b]	SFO	23.9 / 79.5	2.9	23.9	SFO
	Loam soil loam	7.3 [b]	SFO	33.7 / 112.1	8.9	30.3	SFO
	Humic sand soil sand	5.2 [b]	SFO	34.1 / 113.4	3.2	34.1	SFO
	Sandy loam soil sandy loam	7.5 [b]	SFO	19.1 / 63.5	3.4	18.3	SFO
Geometric mean, days						58.0	

a In $CaCl_2$.
b In KCl.

Under anaerobic conditions (Lewis, 2003b) quinoclamine declined rapidly but mainly into non-extractable residues (80.1% AR after 120 days) whereas mineralisation was low, only 0.7% AR $^{14}CO_2$ after 120 days. Additionally, metabolites were observed in higher quantities than under aerobic conditions. These were identified as AN (max 14.6% day 14), DHN (max 6.0% day 14) and, at lower levels, HN, HCN and AHN. In this study samples were extracted twice with methanol and twice with water and at three sampling points additionally with Soxhlet extraction (methanol:water, 2:1). Soxhlet released 4.3-9.6% AR. The RMS re-calculated degradation rates

for quinoclamine and metabolite AN (based on methanol extracts). DT_{50} for the parent was 3.9 days (SFO, Chi2 error 9.3%) and DT_{50} for the metabolite was 4.0 days (SFO-SFO, Chi2 error 21.4%). Anaerobic conditions are not considered relevant for the representative use, with applications in spring or summer.

Photolysis at the soil surface was investigated under laboratory conditions (Adam, 2016a). Samples were continuously irradiated for up to 17 days, corresponding to 31.1 days of natural summer sunlight at latitudes 30 to 50ºN. Samples were extracted twice with methanol, twice with water and thereafter by Soxhlet extraction (methanol:water, 1:1). By the end of the study, non-extractable residues had reached 14.8% AR, and $^{14}CO_2$ 21.1%. Up to 19 transformation products were observed. Major products were phthalic acid (M6, max observed 20.4%), phthalamic acid (M9, max 9.0%), 2-oxalyl-benzoic acid (M10, max 10.6%) and 2-amino-oxalyl-benzoic acid (M11, max 5.3%). Remaining products were individually observed as max 3.1% AR. Quinoclamine was stable in dark controls. Under the conditions of the test DT_{50} for quinoclamine was calculated to 6.9 days (SFO, Chi2 error 2.8%). This would correspond to DT_{50} 12.6 days of natural summer sunlight at 30 to 50ºN. Hence, photolysis may significantly contribute to the degradation of quinoclamine under field conditions.

There was also an old study on photolysis on soil (Bishop, 2003). There were three un-identified products observed in this study (SP3, SP6 and SP7), individually accounting for max 5.3% AR by study end. Degradation of quinoclamine was slow; the parent accounted for ca 62% AR after 15.8 days of continuous irradiation, corresponding to 31 days of natural summer sunlight in the UK. Knopp & Böing (2016b) re-calculated the DT_{50} for the parent to 57.8 days and the DT_{90} to 322 days (DFOP, Chi2 error 1.0%) for the conditions of the test (continuous irradiation). Since >50% of the parent was still present by study end the estimated degradation rate is considered uncertain. Since both route and rate of photochemical transformation on soil was better described in the new study (Adam, 2016a) the old study is considered as indicative only.

The rate of degradation of the four main photolysis-products was investigated in separate studies. They all degraded very fast, see tables below. For M6 (Adam, 2016b) there was a relatively high variation between replicates which presumably was the reason for relatively high Chi2 error-%. For M9 (Piskorski, 2016) the RMS noted that recovery in freshly fortified samples were low (50.8-76.5%) and the DT_{50} is therefore considered as uncertain. However, there was no trend in the recoveries of the fortified samples and therefore it is unlikely that correction for these recoveries would result in a more conservative DT_{50} (out of six sampling points the lowest recoveries were observed in the second and third).

Table 2.8.1.1-2. Summary of kinetic evaluation of laboratory data on aerobic degradation of photochemical transformation product phthalic acid (M6) in soil (from Adam, 2016b).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days	χ2, error-%	Modelling endpoint (20°C, pF 2)	
						DT_{50}, days	kinetic model
Adam, 2016b	Soil I Speyer 2.1 sand	4.9 [a]	SFO	1.0 / 3.5	14.3	1.0	SFO
	Soil II Speyer 2.4 loam	7.3 [a]	SFO	0.35 / 1.2	20.8	0.35	SFO
	Soil III Speyer 5M sandy loam	7.3 [a]	SFO	0.58 / 1.9	18.9	058	SFO
Geometric mean, days						0.6	

a In $CaCl_2$.

Table 2.8.1.1-3. Summary of kinetic evaluation of laboratory data on aerobic degradation of photochemical transformation product phthalamic acid (M9) in soil (from Piskorski, 2016).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days	χ2, error-%	Modelling endpoint (20°C, pF 2)	
						DT_{50}, days	kinetic model
Piskorski, 2016	Soil I Speyer 2.1 loamy sand	4.9 [a]	SFO	1.1 / 3.5	9.6	1.1	SFO
	Soil II Speyer 2.4 loam	7.4 [a]	SFO	0.31 / 1.0	12	0.31	SFO
	Soil III Speyer 5M sandy loam	7.3 [a]	SFO	0.39 / 1.3	7.0	0.39	SFO
Geometric mean, days						0.5	

a In $CaCl_2$.

Table 2.8.1.1-4. Summary of kinetic evaluation of laboratory data on aerobic degradation of photochemical transformation product M10 in soil (from Fiebig, 2016).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days	χ2, error-%	Modelling endpoint (20°C, pF 2)	
						DT_{50}, days	kinetic model
Fiebig, 2016	LUFA 2.2 sandy loam	5.4 [a]	SFO	0.13 / 0.43	10.4	0.13	SFO
	LUFA 2.3 sandy loam	5.9 [a]	SFO	0.30 / 0.99	14.2	0.25	SFO
	LUFA 2.4 loam	7.4 [a]	SFO	0.17 / 0.57	8.6	0.15	SFO
Geometric mean, days						0.2	

a Media not reported.

Table 2.8.1.1-5. Summary of kinetic evaluation of laboratory data on aerobic degradation of photochemical transformation product M11 in soil (from Fiebig, 2017).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days	$\chi 2$, error-%	Modelling endpoint (20°C, pF 2)	
						DT_{50}, days	kinetic model
Fiebig, 2017	LUFA 2.2 sandy loam	5.4 [a]	SFO	0.54 / 1.8	9.4	0.54	SFO
	LUFA 2.3 sandy loam	5.9 [a]	SFO	1.1 / 3.7	5.0	1.0	SFO
	LUFA 2.4 loam	7.4 [a]	SFO	0.67 / 2.2	6.1	0.58	SFO
Geometric mean, days						0.7	

a Media not reported.

2.8.1.2 Rate of degradation in soil, field studies, and modelling endpoint

A new field dissipation study (Janßen, 2017) was submitted together with a storage stability study (Dautel, 2017). Old field dissipation data included Beinhauer (1997) with residue analyses in Brielbeck (1997a and b). Kinetic evaluations of all field data were done in separate reports.

The new field study (Janßen, 2017) was carried out at two field sites in Germany; Weeze in North Rhine-Westphalia (Trial 01) and Gerichshain in Saxony (Trial 02). The soils were prepared as for sowing and then left fallow. Glyphosate was applied to keep the plots free of vegetation. The field phase of the study started in August 2015 when the test substance was applied. Mogeton Top WG formulation was sprayed onto the soil surfaces at a rate of 7500 g/ha corresponding to 3750 g quinoclamine/ha (nominal). Three replicate sub-plots (A, B, C) were used at each trial site, and these were further divided into 17 “subsub-plots” each. At each sampling interval ten soil cores were taken from each treated sub-plot A-C, to a depth of 1.0 m (10 cm on day 0). Within max 6 hours after sampling, soil cores were segmented (10 cm segments), pooled and freezed. Soil samples were extracted with acetonitrile:water (1:1), and soil segments were analysed until residues of quinoclamine were less than LOD. No metabolites were included in the analyses. LOQ was 2 µg/kg and LOD 0.72 µg/kg. LOQ represented 0.04% of the nominal dose, and 0.05-0.07% of the maximum residues found. Samples were taken from the plot until day 113 (Trial 01) and day 206 (Trial 02). In the storage stability study (Dautel, 2017) stability (>80%) was demonstrated over 3 months storage at temperature ≤ -18ºC. Thereafter stability decreased to 67.5% after 6 months, and 63.6% after 9 months. In the field study samples from the first sampling points were stored longer than 3 months; at Trial 01 samples from days 0-30 were stored for 116-154 days before analyses, and at Trial 02 samples from days 0-60 were stored for 105-182 days. Hence some degradation of quinoclamine in those samples cannot be excluded. If so, the actual residues from the early sampling dates may have been higher than the measured concentrations. The effect of this would be that the DT_{50}s calculated may be longer (i.e., more conservative) than what they would have been if the samples would have been stable during storage.

Residues of quinoclamine were predominantly found in the upper 20-cm soil horizon. Residue levels expressed as µg/kg were converted into g/ha and the total residues in 0-40 cm soil calculated. Knopp (2017a) provided the kinetic analyses. $DisT_{50}$s were calculated using non-normalised data from all sampling points to 7.6 days at Trial 01 (SFO, Chi2 error 22.9%) and to 8.6 days at Trial 02 (SFO, Chi2 error 9.8%). $DegT_{50/90}$ were calculated using time-step normalised data from all sampling points and $DegT_{50/90,\ matrix}$ were calculated using time-step normalised data using only data from sampling points after cumulative rainfall of >10 mm. At Trial 01, more than 10 mm rain had fallen before sampling day 2 (normalised to sampling day 1.5). At Trial 02, more than 10 mm rain had fallen before sampling day 15 (normalised to sampling day 14.2). In the table below the results after omitting the first sampling points are shown (i.e., $DegT_{50/90,\ matrix}$).

There was also an old field study available (Beinhauer, 1997 and Brielbeck & Marx, 1997a and b). Four German field sites were included but considering the representative use only the two trials in which application was made in the spring are considered here. These sites were located in Gnaschwitz, Saxony and Rostock, Mecklemburg. Mogeton 25WP was sprayed onto the bare soil surfaces at the end of May, 1996, at a rate of 15 kg/ha, corresponding to 3750 g quinoclamine/ha. There was one treated plot per site. Samples were taken until 150-152 days after application. Twenty soil cores per plot were taken to a depth of 20 cm, segmented, pooled and kept frozen until analyses. Soil samples were extracted twice with acetone:water (2:1). LOQ was 0.02 mg/kg, representing 0.5% of the nominal dose and 0.6-2% of the maximum residue observed. LOD was not stated. No metabolites were included in the analyses.

At Gnaschwitz, residues were only found in the 0-10 cm soil layer, whereas at Rostock site, residues were determined in the 10-20 cm layer on sampling days 0-28. The total residues in the 0-20 cm layer were used for the kinetic analyses for Rostock. The RMS calculated $DisT_{50/90}$ using non-normalised data from all sampling points. $DisT_{50}$ at Gnaschwitz was 7.9 days (SFO, Chi2 error 6.8%) and at Rostock 9.2 days with $DisT_{90}$ 62.9 days (DFOP, Chi2 error 5.0%). As calculated from k2 in DFOP the $DisT_{50}$ was 24.6 days. Knopp (2017b) calculated $DegT_{50/90}$ using time-step normalised data from all sampling points and $DegT_{50/90,\ matrix}$ using time-step normalised data using only data from sampling points after cumulative rainfall of >10 mm. At Gnaschwitz, more than 10 mm rain had fallen already 1 day after application. Due to missing weather data, day lengths for June, 1996, were not normalised for the Gnaschwitz site, and the next sampling day (day 7) was also the starting point for the kinetic analysis. Additionally, data from Gnaschwitz could not be normalised with regard to moisture, hence field capacity was assumed at the site and in that respect the result may be conservative. At Rostock, more than 10 mm rain had fallen after day 7 (the next sampling date was day 14, normalised to day 15.1 which was the starting point for the kinetic analysis). In the table below the results after omitting the first sampling points are shown.

Table 2.8.1.2-1. Summary of kinetic evaluation of data on degradation of quinoclamine under field conditions, time-step normalised and using only sampling points after cumulative rainfall of >10 mm. From Knopp (2017a and b).

Study	Soil	pH	best-fit model	DT_{50} / DT_{90}, days (20ºC, pF 2)	$\chi 2$, error-%	Modelling endpoint (20ºC, pF 2)	
						DT_{50}, days	kinetic model
Janßen, 2017	Trial 01, DE loamy fine sand	5.6 [a]	SFO	4.5 / 15.0	29.1	4.5	SFO
	Trial 02, DE loam	6.6 [a]	HS	8.6 / 23.6	8.7	5.7	from k_2 in HS
Beinhauer, 1997 and Brielbeck & Marx, 1997a and b	Gnaschwitz silt	5.4 [a]	SFO	9.6 / 31.7	10.7	9.6	SFO
	Rostock silt	5.9 [a]	SFO	20.3 / 67.3	11.4	20.3	SFO
Geometric mean, days						8.4	

a In $CaCl_2$.

DT_{50} for modelling input was determined after comparison of normalised laboratory and field DT_{50}s using the EFSA Endpoint selector. According to the current guidance the geometric mean of the field results should be used, i.e. 8.4 days.

2.8.1.3 Assessment in relation to the P-criteria for soil

The criteria for persistence in soil, as stated in Annex II to Regulation (EC) 1107/2009, are DT_{50} 120 days (PBT) and 180 days (POP and vPvB). It is assumed that these criteria represent a constant rate of degradation over the decline curve, i.e. that single first order (SFO) kinetics has been assumed implicitly when the criteria were defined.

For quinoclamine, the laboratory DT_{50}s at 20ºC were variable ranging from 19 to 196 days. Two of the eight laboratory DT_{50}s were above the criterion for P in PBT and one additional value was close to the criterion. One of the DT_{50}s were also above the criterion for vP and POP. Low extraction efficiency may have contributed to short DT_{50}s in four of the soils, and high test concentration may have contributed to long DT_{50}s in the other four soils. It is not considered likely that release of additional max. ca 10% in extracts from the laboratory study with low extraction efficiency would have resulted in DT_{50}s above the criteria. Four field $DegT_{50,\ matrix}$ values were all clearly below the criteria. As an overall conclusion considering all available data, quinoclamine is not considered as a persistent or as a very persistent substance in soil.

2.8.1.4 Adsorption in soil

Two studies were available on adsorption of quinoclamine to soil. Brielbeck & Marx (1998) used two soils, and the experimental data from the study were corrected and re-calculated in accordance with guideline by Frauen & Stähler (2001). The second study (Lewis, 2000) used four soils. There was no indication of pH-dependency of adsorption over the range tested (4.0-7.6).

Table 2.8.1.4-1. Quinoclamine: Adsorption coefficient, Freundlich isotherm, Freundlich exponent ("1/n"). (K_F and $K_{F,oc}$ at 1 mg/l)

Study	Soil	OC %	Soil pH	K_d (mL/g)	$K_{d,oc}$ (mL/g)	K_F (mL/g)	$K_{F,oc}$ (mL/g)	"1/n"
Frauen & Stähler, 2001 (Brielbeck & Marx, 1998)	Soil 1 Agroplan sandy loam	0.87	6.4 [a]	13.9	1598	11.37	1307	0.686
	Soil 2 Speyer 2.1 loamy sand	0.59	6.0 [a]	4.2	712	4.79	812	0.805
Lewis, 2000	PT 102 sandy silt loam	2.8	6.7 [b]	14.87	531	16.63	594	0.810
	SK 961089 clay loam	4.7	7.6 [b]	24.07	512	25.95	552	0.838
	Speyer 2.1 sand	0.4	5.2 [b]	2.57	642	3.72	931	0.727
	SK 566696 sandy loam	0.8	4.0 [b]	6.27	784	7.92	990	0.763
Geometric mean						9.41	827	-
Arithmetic mean						-	-	0.772

a Medium not stated.
b In $CaCl_2$.

There was also an adsorption study on the metabolite AN (Dardemann, 2010) available. There were several deviations from the OECD guideline and the study was not considered acceptable by the RMS. No other experimental studies on adsorption of metabolites/transformation products were submitted. In the absence of data, $K_{F,OC}/K_{F,OM}$ should generally be set to zero in exposure modelling. However, for metabolite AN the RMS suggests that an estimated value of Koc 605.6 L/kg could be used as a surrogate endpoint in calculations of PECsw/sed. This value was estimated in KOCWIN v2.00, Kow method (Heimann, 2018). Metabolite AN was identified as a major metabolite only in sediment, and in soil only under anaerobic conditions. Anaerobic conditions in soil are not considered relevant for the representative use, with applications in spring or summer. Therefore the RMS has not identified experimental adsorption data as a data gap for metabolite AN. However, should anaerobic conditions be considered relevant for other representative uses, then it may be necessary to request further adsorption data for metabolite AN at Member State level.

2.8.2 Summary of fate and behaviour in water and sediment

This section has been written to present degradation data necessary for comparison with the CLP criteria as well as to fulfil the requirements under Regulation (EC) No 1107/2009. The comparison with the CLP criteria is presented in section 2.9.2.4.2 (Long-term aquatic hazard (including bioaccumulation potential and degradation)).

2.8.2.1 Rapid degradability of organic substances

An overview of all studies that are considered relevant for the aquatic compartment are summarised in the table below. The studies are further presented in the sections that follow. See Vol 3, B.8 (CA) for additional information. All key studies listed in the table are considered suitable for CLP.

Table 2.8.2.1-1. Summary of relevant information on rapid degradability.

Method	Results*	Key or Supportive study *	Remarks	Reference
Hydrolysis (OECD TG No 111)	Stable at pH 4 and 7 at 50ºC. At pH 9 (50ºC) the SFO DT_{50} was 9 days, this was extrapolated to DT_{50} 360 days (20ºC). A single metabolite (HCN) was observed as 50% AR after 9 days at 50ºC. Mineralisation was not measured.	Key study	No remarks. Quinoclamine is considered to be hydrolytically stable at environmentally realistic pH values and temperatures.	Lewis, 2001
Aquatic photolysis (SETAC, 1995)	Photo-chemical DT_{50} was 2.2 days (pH 5 buffer, 20º, continuous irradiation) corresponding to 4.2 days of natural sunlight in the UK (54ºN). Seven products were formed but none of them could be identified. Unknown products 2 and 5 were observed as >10% AR. Mineralisation was not significant (<1% AR).	Key study	Due to the presence of un-identified products a second study was requested for the previous review (2007). Considering all available data it is considered likely that Unknown 5 was identical to phthalic acid.	Yeomans, 2003
Aquatic photolysis (SETAC, 1995; Japanese MAFF guideline ID #2-6-2; US EPA 161-2)	Photo-chemical DT_{50} was 14.1 days (pH 5 buffer, 25º, continuous irradiation) and 11.9 days (sterile pond water, pH 6.6, 25º, continuous irradiation). These results were re-calculated to DT_{50} 3.0 and 42.9 days, respectively, for Tokyo spring conditions. Two products were identified as >10% AR; phthalic acid and 2-carboxybenzaldehyde. Mineralisation reached 2.4% AR after 11 days of continuous irradiation of the sterile natural pond.	Key study	The minor difference in DT_{50} between the systems may reflect contributing effect of indirect photolysis in the natural water.	Shah, 2006

Method	Results*	Key or Supportive study *	Remarks	Reference
	Mineralisation was not measured in the pH 5 buffer. The quantum yield (dimensionless) was calculated to 2.7 x 10^{-6} (natural water) and 2.-5 x 10^{-6} (pH 5 buffer).			
Position paper on identity of products formed in aquatic photolysis	From the arguments presented it is considered likely that Unknown 5 (in Yeomans, 2003) was identical to phthalic acid (identified in Shah, 2006)	Supportive study	-	Heimann, 2014
Calculation of quantum yield	Using data from Yeomans (2003) the quantum yield (dimensionless) was calculated to 3.55 x 10^{-5}.	Supportive study		Greenwood & Liney, 2005
Ready biodegradability (Draft OECD TG No 301, 1990)	No significant CO_2 evolution was observed in bottles with quinoclamine over 28 days. The reference substance (acetate) was degraded by 72%. It was concluded that quinoclamine is not readily biodegradable.	Key study	Test concentration (18 and 35 mg/L) was close to or above the water solubility (19.8 mg/L, 20ºC). A slight inhibitory effect of quinoclamine was noted.	Hemmink & Blom, 1992
Aerobic mineralisation in water (OECD TG No 309)	Pelagic system was used, and two test concentrations; 10 and 100 µg/L. Mineralisation reached 29.5% AR (high dose) and 50.7% (low dose) after 61 days (study end). Nine metabolites were observed. HCN 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. SFO DT_{50}s were determined to 30.6 days (low dose) and 121 days (high dose).	Key study	The results indicate dose dependency of degradation.	Völkel, 2016a
Aerobic degradation in water/sediment (OECD TG No 308)	River and pond water/sediment systems were used. Quinoclamine was relatively rapidly distributed to the sediments, with SFO $DisT_{50}$ from the water phase of 4.2 days (river system) and 3.2 days (pond system). SFO $DegT_{50}$ in the total systems were 7.0 days (river system) and 8.9 days (pond system). Eleven metabolites were observed; M1 and M2 were	Key study	The degradation of quinoclamine in the total systems was relatively rapid with AN observed as a major metabolite. This indicates that the sediments were partially anaerobic since,	Völkel, 2016b

Method	Results*	Key or Supportive study *	Remarks	Reference
	only identified as >5% AR once and not identified: M1 as max 8.4% on day 20, and M2 as max 4.8% also day 20. M3, identified as AN, was observed as max 13.2% AR in total systems on day 7, whereof >10% in the sediment. Remaining metabolites were individually observed as max 1.9% AR. For AN, SFO-SFO $DegT_{50}$ in the total systems were determined to 22.7 days (river) and 47.8 days (pond). After 60 days (study end) mineralisation reached 25.7% AR in the river system, 11.8% AR in the pond system.		in soil, degradation was more rapid under anaerobic conditions as compared to aerobic and AN was observed as a major metabolite only under anaerobic conditions in soil. This does not invalidate the study (see § 2 of the OECD TG).	
Aerobic degradation in water/sediment (BBA guideline, Part IV, 5-1)	Ditch and river water/sediment systems were used. Again, quinoclamine was relatively rapidly distributed to the sediments, with SFO $DisT_{50}$ from the water phase of 2.6 days (ditch system) and 3.5 days (river system). SFO $DegT_{50}$ in the total systems were 6.5 days (ditch system) and 6.1 days (river system). AN was the only major metabolite observed (three additional metabolites never exceeded 1%). AN was observed as max 18% AR in total systems on day 7, and >10% only in the sediment phase. Kinetic fitting of the data for AN together with parent data did not return acceptable results. Therefore decline fits were done, with SFO DT_{50} for the total systems estimated to 14.1 days (ditch system) and 8.6 days (river system). By study end (day 105) mineralisation had reached 15.5% AR (ditch system) and 30.8% (river system).	Key study	As for the study above (Völkel, 2016b) the results indicate that the sediments were partially anaerobic.	Muttzall, 1993

* Key or supportive with reference to CLH endpoints.

2.8.2.1.1 Ready biodegradability

A study on ready biodegradability was available (Hemmink & Blom, 1992). The study was conducted in accordance with Draft OECD TG No 301 "CO2 Evolution test" (1990). The reference substance (acetate) was significantly degraded within 14 days (72% degradation). No significant CO2 evolution was observed over 28 days in bottles with quinoclamine. Two test concentrations of quinoclamine were used, 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). Nevertheless, the conclusion that quinoclamine is not readily biodegradable is still considered valid, and the test is considered as relevant for the purpose of classification and labelling.

2.8.2.1.2 BOD_5/COD

No BOD_5/COD test was available.

2.8.2.2 Other convincing scientific evidence

Relevant data on abiotic degradation were available (hydrolysis, see 2.8.2.2.5 and aquatic photolysis, see 2.8.2.2.6).

Other data of relevance for classification and labelling were one study on biodegradation in surface water (see 2.8.2.2.1), two studies on biodegradation water/sediment (see 2.8.2.2.4). Additionally, studies on biodegradation in soil were available (see 2.8.1.1 for soil laboratory data and 2.8.1.2 for soil field data).

2.8.2.2.1 Aquatic simulation tests

Völkel (2016a) investigated rate of mineralisation in surface water (pelagic test, OECD TG NO 309). Two test concentrations were used and the results indicate the degradation may be dose dependent. The results from the low dose experiment are considered as more representative (more close to estimated PECsw) than the results from the high dose. 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. After 12 days, mineralisation of the reference substance (benzoic acid) reached 73.5% AR (control samples) and 62.1% (solvent control samples).

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.

SFO DT_{50}s were determined to 30.6 days (low dose, Chi2 error 6.9%) and 121 days (high dose, Chi2 error 1.7%).

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

Field dissipation studies on soil are presented in section 2.8.1.2 but these data are considered as less relevant for classification purpose since only primary degradation and no mineralisation were measured. There was no monitoring data available (see 2.8.4).

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Inherent or enhanced biodegradability tests were not provided.

2.8.2.2.4 Soil and sediment degradation data

Soil degradation data are presented in sections 2.8.1.1 (laboratory data) and 2.8.1.2 (field data).

Two water/sediment studies were available (Völkel, 2016b and Muttzall, 1993). Völkel (2016b) was carried out in accordance with OECD TG No 308 (2002). Muttzall (1993) referred to an older guideline from German authority; BBA guideline, Part IV, 5-1 (1990) but essentially followed the principles of the current OECD guideline. The applicant originally suggested that the old study on water/sediment (Muttzall, 1993) was unreliable since bound residues were evaluated insufficiently. The RMS nevertheless requested the old study together with new kinetic data. The RMS has concluded that the old study can be relied on.

In Völkel (2016b) mineralisation reached 25.7% AR in the river system and 11.8% AR in the pond system (both on day 60, study end). In Muttzall (1993) mineralisation reached 15.5% AR in the ditch system and 30.8% AR in the river system (both on day 105, study end). By day 56 (to allow a comparison with the shorter study duration of Völkel, 2016b) mineralisation was 18.8% in the ditch system and 27.4% in the river system.

In Völkel (2016b) sediments were extracted up to three times with acetonitrile:water (4:1) followed by Soxhlet extraction with the same solvent system. Soxhlet extraction released 3.9-12% AR. Muttzall (1993) extracted sediments once with acetonitrile and once with methanol. In comparison, this was a mild extraction. Non-extractable residues accounted for max 67.9% (river system) and 82.4% (pond system) by day 60 in Völkel (2016b). By comparison, non-extractable residues accounted for max 73.1% (ditch system) and 62.1% (river system) by day 56 in Muttzall (1993). By study end at day 105, these amounts had increased to 80.6% and 67.1%, respectively. The comparison of the results from days 56/60 did not indicate that bound residues were higher in Muttzall (1993) than in the new study and the RMS decided to rely on both studies.

Degradation was relatively rapid in these systems and AN (2-amino-1,4-naphthalenedione) was observed as the (sole) major metabolite. Based on a comparison with results from aerobic and anaerobic soil studies this suggests that the sediments were partially anaerobic. This is considered acceptable (“The aerobic test simulates an aerobic

water column over an aerobic sediment layer that is underlain with an anaerobic gradient." §2 in OECD TG No 308).

Table 2.8.2.2.4-1. Summary of kinetic evaluation of laboratory data on aerobic degradation of quinoclamine in total water/sediment systems (from Völkel, 2016b and Knopp & Böing, 2016c kinetic re-assessment of results in Muttzall, 1993).

Study	Soil	OC in sediment, %	pH of water	best-fit model	DT_{50} / DT_{90}, days	χ^2, error-%	Modelling endpoint (20°C, pF 2)	
							DT_{50}, days	kinetic model
Völkel, 2016b	River system	1.2	8.0	SFO	7.0 / 23.4	12.9	7.0	SFO
	Pond system	4.7	7.6	SFO	8.9 / 29.6	15.2	8.9	SFO
Muttzall, 1993	Ditch system	4.5	8.6	SFO	6.5 /21.5	4.0	6.5	SFO
	River system	1.2	8.0	SFO	6.1 / 20.3	3.5	6.1	SFO
Geometric mean, days							7.2	

Table 2.8.2.2.4-2. Summary of kinetic evaluation of laboratory data on dissipation of quinoclamine from water phase in water/sediment systems (from Völkel, 2016b and Knopp & Böing, 2016c kinetic re-assessment of results in Muttzall, 1993).

Study	Soil	OC in sediment, %	pH of water	best-fit model	DT_{50} / DT_{90}, days	χ^2, error-%	Modelling endpoint (20°C, pF 2)	
							DT_{50}, days	kinetic model
Völkel, 2016b	River water	1.2	8.0	SFO	4.2 / 13.9	4.9	n.a.	-
	Pond water	4.7	7.6	SFO	3.2 / 10.5	6.1	n.a.	-
Muttzall, 1993	Ditch water	4.5	8.6	SFO	2.6 / 8.8	9.4	n.a.	-
	River water	1.2	8.0	SFO	3.5 / 11.7	5.4	n.a.	-

n.a. Not applicable.

Table 2.8.2.2.4-3. Summary of kinetic evaluation of laboratory data on dissipation of quinoclamine from sediment phase in water/sediment systems (from Völkel, 2016b and Knopp & Böing, 2016c kinetic re-assessment of results in Muttzall, 1993).

Study	Soil	OC in sediment, %	pH of water	best-fit model	DT_{50} / DT_{90}, days	χ^2, error-%	Modelling endpoint (20°C, pF 2)	
							DT_{50}, days	kinetic model
Völkel, 2016b	River sediment	1.2	8.0	SFO	7.4 / 24.7	18.3	n.a.	-
	Pond sediment	4.7	7.6	SFO	8.4 / 28.1	17.0	n.a.	-
Muttzall, 1993	Ditch sediment	4.5	8.6	SFO	12.5 / 41.6	8.7	n.a.	-
	River sediment	1.2	8.0	SFO	11.6 / 38.5	5.0	n.a.	-

n.a. Not applicable.

Völkel (2016b) observed eleven metabolites in the river system and seven metabolites in the pond system. M1 (un-identified) was observed as max 8.4% AR on day 20 in the river system but never again above 5%. M2 (un-identified) was observed as max 4.8% AR also on day 20 in the river system. M3 (identified as AN) was observed as max 13.2% by day 7 in the river system, mainly in the sediment phase. Remaining metabolites were individually observed as max 1.9% AR.

Muttzall (1993) also observed AN as the only major metabolite; max observation was 18% in the river system on day 7, whereof 12% was found in the sediment, 6% in the water phase. Three additional minor metabolites were observed, but their amounts never exceeded 1% AR.

2.8.2.2.5 Hydrolysis

One study on hydrolysis of quinoclamine was available (Lewis, 2001). The study was carried out in accordance with OECD TG No 111 (1981). 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 DT_{50}. Under the conditions of the test 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). It accounted for 50% AR after 9 days at pH 9, 50ºC. Quinoclamine is considered to be hydrolytically stable at environmentally realistic pH values and temperatures.

2.8.2.2.6 Photochemical degradation

Three relevant reports were provided to address photochemical transformation in water; two experimental studies (Yeomans, 2003, Shah, 2006), and one position paper (Heimann, 2014). Yeomans (2003) did not identify the products formed and therefore a second study (Shah, 2006) was requested during the previous review of quinoclamine. In Shah (2006) two major products could be identified. Heinemann (2014) was a position paper submitted for the purpose of renewal, aiming to clarify the identity of products that were un-identified in the experimental study by Yeomans (2003). Yeomans referred to a test protocol which lacks detailed descriptions (SETAC, 1995). Shah (2006) additionally referred to Japanese MAFF guideline ID #2-6-2 (2000) and US EPA guideline 161-2 (1982). However, both experimental studies were essentially carried out in accordance with standard procedures for this type of study, as described in OECD TG No 316 (2008), except that spectral properties and irradiance was measured using a spectroradiometer instead of using a chemical actinometer.

In Yeomans (2003) the photochemical half-life was 2.2 days (pH 5 and 20°C, continuous irradiation, SFO), corresponding to 4.2 days in natural sunlight 12 hours per day at UK irradiation conditions. In Shah (2006) DT_{50}s were estimated to 14.1 days (pH 5 and 25°C, continuous irradiation, SFO) and 11.9 days (sterile natural pond water, pH 6.6, 25°C, continuous irradiation, SFO). These DT_{50}s were estimated to correspond to 42.9 days and 36.5 days, respectively, under natural spring conditions in Tokyo.

In Yeomans (2003) seven distinct products were formed but none of them could be identified. Two of the products (Unknown 2 and 5) were observed as >10% AR and two additional products were observed as >5% AR at two consecutive sampling points (Unknown 1 and 4), see table below. One additional product (Unknown 6) was observed close to 10% AR at the very last sampling point. Photochemical transformation of quinoclamine would be expected to occur only at the near-surface layer of natural water bodies. In the presence of sediments the rate of disappearance from water is expected to be relatively rapid for quinoclamine ($DisT_{50}$ 3-4 days). Therefore it may

be relevant to consider the time taken for the unknown products to reach >5% AR and >10% AR in the test system, noting that number of UK summer days would be twice the number of sampling days. Considering this, the RMS propose that it can be considered as less likely that under environmentally realistic conditions at least Unknowns 1, 3, 4, 6 and 7 would be formed in amounts that would call for further consideration.

Table 2.8.2.2.6-1. Quinoclamine and major products formed in aquatic photolysis study (Yeomans, 2003). Continuous irradiation of quinoclamine in sterile pH 5 buffer (% of applied, single samples, except at zero time where duplicate samples were taken).

Days after application	0	1.1	2.8	3.9	5.0
Quinoclamine (26.1 min [a])	95.7	78.3	48.5	30.2	5.0
Unknown 1	n.d.	n.d.	5.5	6.1	2.5
Unknown 2 (13.7 min [a])	n.d.	2.9	10.6	12.0	17.1
Unknown 3	n.d.	2.3	1.1	2.9	4.2
Unknown 4 (15.1 min [a])	n.d.	n.d.	3.1	6.0	6.6
Unknown 5 (16.0 [a])	n.d.	1.7	7.6	9.8	18.9
Unknown 6 (16.6 [a])	n.d.	n.d.	4.4	4.5	9.9
Unknown 7	n.d.	1.2	4.3	4.9	4.3

n.d. Not detected.
a Approximate retention times from LC-MS analyses using atmospheric pressure chemical ionisation (APcI) in the negative ion mode.

In Shah (2006), two products were identified, phthalic acid and 2-carboxybenzaldehyde, bot present as >10% AR. There were no other products formed as >10% AR or as >5% AR at two or more consecutive sampling points, see table below. It is not considered likely that the minor products observed would be formed in significant amounts under natural conditions since the systems were continuously irradiated for 11 days and since photochemical transformation would be expected to occur only at the near-surface layer of natural water bodies.

Table 2.8.2.2.6-2. Quinoclamine and major products formed in aquatic photolysis study (Shah, 2006). Continuous irradiation of quinoclamine in sterile natural pond water (pH 6.6) and sterile pH 5 buffer (% of applied, average of duplicate samples).

	Days after application						
	0	1	3	4	7	9	11
Natural pond water							
Quinoclamine (25.3 min [a])	100.4	94.9	87.3	79.3	71.3	63.6	50.4
16.3 min [a]	n.d.	1.7	2.4	3.8	4.7	4.7	5.7
17.4 min [a]	n.d.	0.5	1.8	1.9	3.3	3.9	5.1
Phthalic acid (18.7 min [a])	n.d.	0.8	1.7	2.8	4.4	4.4	6.3
2-Carboxybenzaldehyde (19.4 min [a])	n.d.	1.8	5.0	9.3	13.2	14.8	19.5
22.1 min [a]	n.d.	n.d.	0.5	0.9	1.1	1.2	1.7
22.9 min [a]	n.d.	n.d.	0.6	1.0	1.7	1.5	2.6
pH 5 buffer							
Quinoclamine (25.3 min [a])	98.4	92.6	80.1	76.9	64.2	65.0	55.7
16.3 min [a]	n.d.	1.0	1.9	1.5	1.7	1.5	1.1
Phthalic acid (18.7 min [a])	n.d.	1.7	5.0	7.1	9.2	10.5	10.8
2-Carboxybenzaldehyde (19.4 min [a])	n.d.	2.6	8.0	9.0	16.7	13.7	18.3
21.0 min [a]	n.d.	n.d.	1.9	2.3	2.3	2.4	2.8
22.1 min [a]	n.d.	n.d.	1.0	1.0	1.7	1.7	2.3
24.5 min [a]	n.d.	n.d.	n.d.	n.d.	1.4	1.3	2.3

n.d. Not detected.
a HPLC retention times.

Heimann (2014) presented arguments to clarify the identity of unknown products formed in the experimental photolysis studies, and to show that none of the products would contain the toxophore of quinoclamine. The intact quinone ring of quinoclamine was identified as an essential part of the toxophore. Based on the differences in

retention times of (on the one hand) quinoclamine and the reference substances and (on the other hand) the photolysis products formed, as well as on the molecular weights proposed for the unknown products (data from Yeomans, 2003) the RMS agrees that it can be concluded that the quinone ring structure was no longer present in the photo-transformation products formed (possibly with the exception of Unknown 4 for which a relatively high molecular weight was proposed in Yeomans, 2003).

Furthermore, from the inhibitory action of quinoclamine on plastoquinone, involved in photosynthesis, together with the comparison of aquatic ecotoxicity data between quinoclamine and two of its transformation products that has lost the quinone ring (phthalic acid and phthalamic acid) it is concluded that the quinone ring has an essential role in the toxicity of quinoclamine. Thus, it is considered likely that the major photo-transformation products had lost the toxophore.

Heimann (2014) proposed that Unknown 5 and 6 in Yeomans (2003) were identical to phthalic acid and 2-carboxybenzaldehyde identified in Shah (2006). From Heimann's evaluation of the MS data the RMS finds it reasonable to assume that Unknown 5 was identical to phthalic acid but for Unknown 6 the RMS concludes that based on the available data it cannot be demonstrated with reasonable certainty that it was identical to 2-carboxybenzaldehyde. Furthermore, a proposed identity of Unknown 2 is considered as highly uncertain.

The RMS propose that three products formed in aquatic photolysis needs to be considered further:
phthalic acid (= Unknown 5), 2-carboxybenzaldehyde and Unknown 2.

2.8.2.2.7 Other / Weight of evidence

No other data that could be of relevance for the classification and labelling were available.

2.8.2.2.8 Assessment in relation to the P-criteria for water and sediment

The criteria for persistence in water and sediment, as stated in Annex II to Regulation (EC) 1107/2009, are:
Water: DT_{50} 40 days (fresh water in PBT), 60 days (POP, marine water in PBT, and all water in vPvB),
Sediment: DT_{50} 120 days (fresh water sediment in PBT), 180 days (POP, marine sediment in PBT, and all sediments in vPvB).

Quinoclamine is hydrolytically stable. Aquatic photolysis may contribute to the degradation of the substance but due to adsorption, quinoclamine is expected to remain near the surface of water bodies only for a limited period of time. In the study on aerobic mineralisation in surface water the DT_{50} of 121 days at the high test concentration (100 µg/L) was above the criteria for persistence in PBT as well as POP and vPvB. At the low test concentration (10 µg/L) the DT_{50} of 30.6 days was below the criteria. The RMS suggest that the result from the low test concentration are more relevant than the result from the higher dose since the low test concentration was more close to the estimated PECsw at Step 3 and above. In the water/sediment studies all DT_{50}s for degradation in the

total systems (7.0-8.9 days) were below the criteria. In this case, it may be more relevant to compare these results with the criteria for sediment (since quinoclamine is expected to partition to sediment relatively rapidly) but this makes no difference with regard to the conclusion regarding the criteria for persistence. The RMS concludes that the half-lives of quinoclamine does not exceed the criteria for persistence in the aquatic environment.

2.8.3 Summary of fate and behaviour in air

Quinoclamine has a vapour pressure of 3×10^{-6} Pa (20ºC). This is below the trigger for further assessment as provided in FOCUS Air report (2007). Henry's Law constant was calculated to 3.05×10^{-5} Pa x m^3 x mol^{-1}. This also indicates a low tendency for volatilisation from moist surfaces. Atmospheric half-life for reaction with hydroxyl radicals was estimated to 5.5 hours assuming an average daily air concentrations of hydroxyl radicals of $1.5 \times 10^6/cm^3$ (12-hr day). The RMS concludes that no further data on fate of quinoclamine in air is required.

2.8.3.1 Hazardous to the ozone layer

There were no data available on the potential hazard to the ozone layer.

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

There were no data available on the potential hazard to the ozone layer.

2.8.3.1.2 Comparison with the CLP criteria

A comparison with CLP criteria cannot be made for the hazard class in question, i.e. hazardous to the ozone layer.

2.8.3.1.3 Conclusion on classification and labelling for *hazardous to the ozone layer*

Due to lack of data no classification is proposed on classification and labelling for hazards to the ozone layer according to the CLP criteria.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

The applicant stated that no environmental monitoring data were identified in the literature search and no further data were provided. Quinoclamine is not included in the Swedish monitoring program at the national level. The RMS also searched for data in the Swedish regional pesticide database but there were no findings reported. Hence, no relevant monitoring data were available.

2.8.5 Definition of the residues in the environment requiring further assessment

Substances for which further exposure/risk assessment is considered necessary are listed in the below table.

It may be noted that under anaerobic conditions (flooded soil) AN (2-amino-1,4-naphthoquinone) and DHN (1,4-dihydronaphthalene) were observed as >10% AR or as >5% at two consecutive sampling points, respectively. Considering the representative use for quinoclamine with timing of applications in April-August further assessment of AN and DHN as soil metabolites has not been requested. However, should application of quinoclamine in autumn/winter be considered for other areas of use, the RMS recommends that exposure/risk assessment should be presented for AN and DHN.

2.8.5-1. Definition of the residues in the environment requiring further assessment.

Compartment	Residue	Justification
Soil	quinoclamine	by default
	phthalic acid (M6)	soil photolysis: max 20.4% AR
	phthalamic acid (M9)	soil photolysis: max 8.8-9.0% AR at 2 consecutive points
	2-oxalyl-benzoic acid (M10)	soil photolysis: max 10.6% AR
	2-amino-oxalyl-benzoic acid (M11)	soil photolysis: max 5.2-5.3% AR at 2 consecutive points
Groundwater	quinoclamine	by default
	phthalic acid (M6)	from soil
	phthalamic acid (M9)	from soil
	2-oxalyl-benzoic acid (M10)	from soil
	2-amino-oxalyl-benzoic acid (M11)	from soil
Surface water	quinoclamine	by default
	phthalic acid (M6) (unknown 5)	from soil, additionally from aquatic photolysis: max 18.9% AR
	phthalamic acid (M9)	from soil
	2-oxalyl-benzoic acid (M10)	from soil
	2-amino-oxalyl-benzoic acid (M11)	from soil
	unknown 2	aquatic photolysis: max 17.1% AR
	2-carboxybenzaldehyde	aquatic photolysis: max 19.5% AR
Sediment	quinoclamine	by default
	phthalic acid (M6)	from soil
	phthalamic acid (M9)	from soil
	2-oxalyl-benzoic acid (M10)	from soil
	2-amino-oxalyl-benzoic acid (M11)	from soil
	2-amino-1,4-naphthoquinone, AN	water/sediment: max 18% AR of which 12% AR in sediment
Air	quinoclamine	by default

2.8.6 Summary of exposure calculations and product assessment

2.8.6.1 Summary of calculations of PECsoil

Acceptable PECsoil were calculated for quinoclamine and four transformation products formed in soil photolysis study: M6, M9, M10 and M11. PECsoil,plateau was not required for any of the substances.

PECsoil were calculated using the standard equations and assumptions, for the following areas of use:
Golf greens, single application of 3750 or 1875 g/ha,
Nursery stock plants, single application of 3750, 1875, 1440 or 810 g/ha.
For golf greens 90% crop interception was assumed, and this was justified by the dense grass on greens, with grass blades horizontally situated. For nursery stock plants 50% crop interception was assumed. This was considered as a realistic worst case since the substrate in the pots is expected to bind quinoclamine due to its high content of organic matter and since pots are normally placed close to each other with nearly 80%, or at least 50%, of the ground occupied by pots. For hand-held applications, it was added that the operator will target the application to the substrate surface of the pots.

2.8.6.2 Summary of calculations of PECgw

Acceptable PECgw were calculated for quinoclamine and four transformation products formed in soil photolysis study: M6, M9, M10 and M11. Standard modelling with PELMO, PEARL and MACRO was done.
The transformation products were modelled separately, i.e. applied as parent substance with application rate corrected for molecular weight and formation. Due to misunderstandings in the communication between the RMS and the applicant, 25% formation of each metabolite was assumed for correction of the application rate. This is conservative since the maximum observed of these products was 20.4% (M6).

Worst case PECgw were calculated for the following areas of use:
Golf greens, single application of 3750 g/ha,
Nursery stock plants, single applications of 3750 g/ha.

“Grass/alfalfa” and “Beans (field)”/”Beans (vegetables)” were used as model crops, respectively.
Three different dates for application were considered: 1 April, 1 June and 1 August.
The application rates were corrected for crop interception and each substance applied to soil surface in the models. Crop interception was assumed to be 90% for golf greens and 50% for nursery stock plants (for justification, see above, PECsoil).

PECgw (80^{th} percentile over 20 years simulation, 1 m depth) were calculated to $\leq$ 0.000 µg/L for all compounds.

2.8.6.3 Summary of calculations of PECsw and PECsed

PECsw/sed were calculated at Steps 1-2 for quinoclamine, AN, M6, M9, M10, M11, 2-carboxybenzaldehyde and Unknown 2.

At Steps 3, 3b and 4 acceptable PECsw/sed were available for quinoclamine. The endpoints used for the parent deviated slightly from the final endpoints but the RMS accepted the modelling since the deviations were minor or resulted in more conservative estimates of exposure.
By contrast, the calculations done for metabolites and transformation products were not considered acceptable by the RMS. This was due both to approaches used and to choice of input values at Steps 3, 3b and 4.

Hence, a number of data gaps were identified:
- PECsw/sed at Steps 3, 3b and 4, as necessary, for metabolite AN using acceptable endpoints as input,
- PECsw/sed at Steps 3, 3b and 4, as necessary, for transformation products formed in soil photolysis. The RMS propose that these should be applied as parent substances with application rate corrected for molecular weight and % maximum observed in the soil photolysis study, and with spray drift excluded. Based on current results at Step 2 no further modelling would be required for M10.
- PECsw/sed at Steps 3, 3b and 4, as necessary, for transformation products formed in aquatic photolysis. The RMS propose that these PECsw could be calculated from the PECsw for the parent with correction for molecular weight and % maximum observed in the aquatic photolysis studies. It was noted that for M6, PECsw from formation on soil would need to be added to the PECsw from formation in water.
- The RMS understands that generating all these PECsw/sed for the metabolite and transformation products is work intense but depending on the outcome of the risk assessment, it may not be necessary to provide PECsw/sed for all use scenarios.

Step 3b was proposed by the applicant as a standard scenario for this crop/product combination. The RMS agrees that the standard FOCUS scenarios may be less relevant for uses on golf greens and nursery stock plants (potted plants) and that modifications of the standard assumptions may be necessary.

For use on golf greens, PECsw/sed was reduced at Step 3b by the following means:
- loading of streams from upstream catchment was excluded, since two golf courses would not be located that close to each other (less than 5 km),
- drainage was reduced due to small size of the treated area, hence loading by drainage was reduced by 90% for tractor drawn applications and by 97.5% for hand-held spot treatment,
- run-off was reduced since applications will only be made to grass surrounded by grass in fairway and rough. The fractional reduction in run-off volume and flux and in erosion mass and flux was 0.9 for tractor drawn applications and 0.975 for hand-held applications.

Additionally at Step 4 the following risk reduction was assumed for the golf green scenario at Step 4:
- spray drift was reduced by 95% nozzle reduction,
- run-off was further reduced by assumption of a 20 m vegetated buffer zone (fractional reduction 0.98/0.995 for tractor drawn applications, and 0.995/0.99875 for hand-held applications).

For applications to nursery stock plants in greenhouses, there was no drift loading assumed at Step 3b and no run-off assumed (only D-scenarios run).
At Step 4, 95% nozzle reduction was assumed, and 20 m vegetated buffer (fractional reductions for tractor drawn as well as hand-held applications were 0.8/0.95).

The RMS noted that the reductions assumed go beyond the limits recommended in the FOCUS Landscape and Mitigation report but the RMS accepted the applicant's justifications for the approach.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1. Birds

The available data on avian toxicity of technical and formulated Quinoclamine are summarised in the table below, with toxicity data used for the risk assessment marked in bold.

Since the available avian short-term dietary LDD50 (394 mg a.s./kg bw/day) is lower than the acute oral LD50 (> 2000 mg a.s./kg bw), the dietary toxicity has to be considered in the acute risk assessment in accordance with the Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2009/1438).

Table 2.9.1.1-1. Available data on avian toxicity of Quinoclamine, with toxicity data used for risk assessment marked in bold.

Species	Test substance	Time scale	Endpoint	Toxicity (mg a.s./kg b.w./day)	Remarks	Reference
Bobwhite quail	Quinoclamine	Acute oral	LD50	> 2000	LD50 > highest dose tested	Anonymous 36 1986 Report 6025-602 (In DAR 2007)
Bobwhite quail	Mogeton 25% WP	Acute oral	LD50	> 500	Old formulation; LD50 > highest dose tested	Anonymous 37 2002c Report 318948 (In DAR 2007)
Bobwhite quail	Quinoclamine	Dietary 5 d	*LDD50*	*394*	*Input for geomean calculation*	Anonymous 38 2001a, 2001b, 2002a Report 318869 (In DAR 2007)
Bobwhite quail	Mogeton 25% WP	Dietary 5 d	LDD50	> 206	Old formulation; LD50 > highest dose tested	Anonymous 39 2001c, 2001d, 2002d Report 318972 (In DAR 2007)
Mallard duck	Quinoclamine	Dietary 5 d	LDD50 *LDD50 (extrapol.)*	> 686 *1107* [a]	LD50 > highest dose tested *Input for geomean calculation*	Anonymous 40 2005 Report 05/896-113TÖ (In DAR 2007)
Japanese quail	Mogeton 50% WG	Dietary 5 d	LDD50 *LDD50 (extrapol.)*	> 1268 *2393* [b]	LD50 > highest dose tested *Input for geomean calculation*	Anonymous 41 2008 Report 07/586-113FÜ
Birds geomean LDD50 (n = 3)	Quinoclamine Mogeton 50 %WG	Dietary 5d	**LDD50**	**1014** [c]	**Acute risk assessment endpoint** *(birds geomean)*	Derived based on lowest reliable LDD50 for bobwhite quail and extrapolated LDD50 for two other species
Bobwhite quail	Quinoclamine	Reproduction 22 weeks	**NOAEL** EC10	**36.2** ♀ 37.6 ♂ 35.4	**Chronic risk assessment endpoint** *(NOAEL is preferred, EC10 is considered to be very uncertain since no confidence limits are available)*	Anonymous 42 2002b Report 318915 (In DAR 2007)

a) Calculated according to EFSA 2009/1438, chapter 2.1.2 based on 5 test animals per dose and no mortality.
b) Calculated according to EFSA 2009/1438, chapter 2.1.2 based on 10 test animals per dose and no mortality.
c) Geomean LDD50 calculated according to EFSA 2009/1438, chapter 2.4.1.

Based on three avian species a geometric mean LDD_{50} (1014 mg a.s./kg bw/d) has been calculated and was proposed for the risk assessment by the applicant. This approach was tentatively accepted by the RMS. However, it may need to be further discussed whether the resulting geomean LDD_{50} in this case is sufficiently robust, since extrapolated LDD50 values for two of the species were used as input for the calculation. It should be noted that the extrapolation method referred to (as proposed in EFSA 2009/1438) is not always protective it since assumes "*a 50% binominal probability bound that mortality could have occurred but had simply been missed by chance in the test*". Moreover, the mixing of active substance data and formulation data may be an additional source of uncertainty of the calculated geomean value. Member States are asked to provide their views on this issue during the peer review.

2.9.1.2. Mammals

An overall summary of the available data on mammalian toxicity of Quinoclamine is given in section 2.6 of this volume. More detailed information on study design and observed effects is available in Volume 3, Annex B.6.

Endpoints relevant for the acute risk assessment on wild mammals are presented in the table below. For the acute risk assessment, the RMS suggests that the previously EU-agreed LD50 of 500 mg a.s./kg b.w. from the study by Ruddock (2002) should be maintained. It is noted that in this study the female mortality was higher than the mortality of males. However, based on a statistical re-evaluation of the dataset the difference between sexes was less than 25% (Von den Berg 2017, reported in Volume 3, Annex B.9 on the active substance, section B.9.1.2.1). Hence, the combined LD50 of 500 mg a.s./kg b.w. was accepted.

Table 2.9.1.2-1. Available data on acute oral toxicity of Quinoclamine to mammals.

Species	Test substance	Time scale	Endpoint	Toxicity (mg a.s./kg b.w.)	Remarks	Reference
Rat	Quinoclamine	Acute oral, single dose	**LD50**	**500** ♀♂ 200-500 ♀ >500 ♂	Previously EU-agreed endpoint for risk assessment	Anonymous 3 2002 Report 619/141-D6144 (In DAR 2007)
Rat	Quinoclamine	Acute oral, single dose	♀ LD50	300-2000	Only females tested; 5 animals exposed to 300 and 1 animal to 2000 mg/kg	Anonymous 4 2016 Report G427 /154-768
Rat	Mogeton 50% WG	Acute oral, single dose	♀♂ LD50	> 1255		Anonymous 12 1998 Report 619/007

The selection of endpoint for the reproductive risk assessment for wild mammals was discussed during the previous peer review, and the agreed NOAEL was 17.5 mg/kg bw per day (EFSA conclusion 2007). For the purpose pf renewal, the applicant provided an overview of the previous discussions, and the rationale for the agreed value. However, since new guidance has been developed (EFSA 2009/1438) and based on considerations for other compounds following the previous evaluation, there is a need for re-consideration of the selection of endpoint for this assessment. An overview of the available data relevant for the long-term risk assessment to mammals is given in the table below.

Table 2.9.1.2-2. Summary of animal studies on adverse effects on sexual function and fertility – generational studies.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL (for sexual function and fertility, parents)** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
Two generation reproduction study In-house method Rat Sprague-Dawley	Quinoclamine Purity: 98.5% 0, 1, 25, 500 ppm Corresponding to: F0: 0, 0.07, 1.6, 30.9 mg/kg	**1 ppm:** <u>Parental:</u> -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 3%; P2 M: 7%; P2 F 4%) ↓ bw gain (P1 M: 4%, P2 M: 11%; P2 F: 4%) Offspring:	RAR Vol. 3, B.6.6.1/01 Anonymous 19 1975 Report 854-111

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
M, F 25/sex/group GLP: No	bw/day in males; 0, 0.08, 1.9 and 37.7 mg/kg bw/day in females F1: 0, 0.07, 1.7 and 37.0 mg/kg bw/day in males; 0, 0.08, 2.0 and 43.8 mg/kg bw/day in females The parents of both generations were fed the appropriate diets for at least nine weeks and then subjected to two subsequent mating trials. Fresh diets were prepared and presented weekly to the rats of all generations from initiation (P1) or weaning (F1b—>F2, F2b)	-increased incidence of grey cysts in the lung in F2b offspring reared for 3 months (18 compared to 11 in control group) **25 ppm:** <u>Parental:</u> -clinical signs (hunched posture F0/F1) ↓ bw (P1 M: 1%; P2 M: 7%; P2 F 5%) ↓ bw gain (P1 M: 2%, P2 M: 11%; P2 F: 6%) <u>Offspring:</u> -increased incidence of grey cysts in the lung in F2b offspring **500 ppm:** <u>Parental:</u> -clinical signs (F0/F1: hunched posture F0/F1) ↓ **bw** (P1 M: 4%; P2 M: **10%**; P2 F **10%**) ↓ **bw gain** (P1 M: 7%, P2 M: **11%**; P2 F: 9%) ↓ litter size in F2a and F2b generations <u>Offspring:</u> -clinical signs (orange stained fur F2b offspring) ↓ **bw** during lactation (F1a: 13% (m) and 7% (f); F1b: 14% (m) and 9% (f); F2a: 8% (m) and 9% (f); F2b: 11% (m) and 5% (f) -increased incidence of grey cysts in the lung in F2b offspring NOAEL parental and pups: 25 ppm (1.6 mg/kg bw/day) NOAEL reproductive toxicity: 500 ppm (37 mg/kg bw/day)	
Teratology range finding study No guideline claimed in study Rat Crl:CD (SD) BR F 5/group GLP: Yes	Quinoclamine Purity: 98.1% 0, 8, 50, 80, 200, 500 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17	**Maternal effects:** <u>8 mg/kg bw/day:</u> No treatment-related effects <u>50 mg/kg bw/day:</u> -clinical signs (staining around eye) <u>80 mg/kg bw/day:</u> -clinical signs (stained urine, stained fur around head) **- bw loss/↓bw gain** (day 7-10: -3.5 g), day 10-13:14% (n.s)) ↓FC <u>200 mg/kg bw/day:</u> **-mortality** (one animal died, two animals were killed in extremis) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss/↓bw gain** (day 7-10: -19.8 g, day 10-13: -1.5 g, day 13-17: 42% (n.s.)) ↓FC **-macroscopic changes** (enlarged spleen)	RAR Vol. 3, B.6.6.2.1/01 Anonymous 33 1986; Anonymous 33 1989 (addendum) Report AKJ/2/86; Report AKJ/2A/89 (addendum)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL (for sexual function and fertility, parents)** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
		↑ **post-implantation loss** (24.5% compared to 2.4% in controls) <u>500 mg/kg bw/day:</u> **-mortality** (one animal died, the remaining four animals were killed in extremis on days 10 or 11 of pregnancy) **-clinical signs** (lethargy, hunched posture, piloerection, stained urine, soft stained faeces, stained fur around anus, vagina, head) **- bw loss** (-34 g, day 7-10) **↓FC** **-macroscopic changes** (enlarged spleen and adrenals, erosion of the stomach mucosa) **Developmental effects:** <u>80 mg/kg bw/day:</u> ↓mean foetal weight (8% n.s.) <u>200 mg/kg bw/day:</u> ↑ **postimplantation loss** (24.5% compared to 2.4% in controls) **↓mean foetal weight (27%)** *Study is a range finding study only. Due to low number of animals used in the it is not considered appropriate to establish a NOAEL/LOAEL*	
Teratology study No guideline claimed in study Rat Crl:CD (SD) BR F 24/group GLP: Yes	Quinoclamine Purity: 98.1% 0, 5, 20 and 75 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 7-17	**Maternal effects:** <u>5 mg/kg bw/day:</u> No treatment related effects <u>20 mg/kg bw/day:</u> **-macroscopic changes** (enlarged spleen, one dam) <u>75 mg/kg bw/day:</u> **- bw gain** (25% day 7-17) ↓ FC **-macroscopic changes** (enlarged spleen, 4/24 dams) **Developmental effects:** <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> **↑incidence of abnormalities** (innominate artery absent) **↑incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed) <u>75 mg/kg bw/day:</u> **↓foetal weight** (7%) **↑incidence of abnormalities** (innominate artery absent, situs inversus, interrupt aortic arch) **↑incidence of skeletal variants** (skull: hyoid not ossified; vertebrae: thoracic centre one or more bilobed/bipartite;	RAR Vol. 3, B.6.6.2.1/02 Anonymous 25 1989 Report AKJ/4/86

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
		sternebrae: 5th and 6th sternebrae not ossified, one or more bilobed, bipartite or misaligned) NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	
Teratology range finding study No guideline claimed in study Rat Crl:CD (SD) IGSBR F 7/group GLP: Yes	Quinoclamine Purity: 99.0% 0, 10, 50, 100 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 6-19	**Maternal effects:** <u>10 mg/kg bw/day:</u> ↓**bw gain** (18%) (Day 6-20) <u>50 mg/kg bw/day:</u> ↓**bw gain** (27%) (Day 6-20) ↑**postimplantation loss** (6.2% compared to 2.8% in controls) ↓**mean litter weight** (2%) <u>100 mg/kg bw/day:</u> ↓**bw gain** (41%) (Day 6-20) ↓**gravid uterus weight** (17%) ↑**postimplantation loss** (10.7% compared to 2.8% in controls) ↓**mean litter weight** (16%) ↓**mean litter size** (12 compared to 12.6 in control) **Developmental effects:** <u>10 mg/kg bw/day:</u> ↓**mean foetal weight** (8%) -minor foetal variations (filamentous tissue at the top of the tail (two animals)) <u>50 mg/kg bw/day:</u> ↓**mean foetal weight** (11%) -minor foetal variations (filamentous tissue at the top of the tail (one animals)) <u>100 mg/kg bw/day:</u> ↓**mean foetal weight** (12%) -minor foetal variations (filamentous tissue at the top of the tail (seven animals)) *Study is a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL*	RAR Vol. 3, B.6.6.2.1/03 Anonymous 34 2002 Report 619/123-D6154
Teratology study No guideline claimed in study Rat Crl:CD (SD) IGSBR F 24/group GLP: Yes	Quinoclamine Purity: 99.0% 0, 5, 20, 75 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 6-19	**Maternal effects:** <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs) ↓**bw gain** (Days 7-8: 62%, Days 17-19: 21%) ↓FC ↓**mean gravid uterus weight** (15%) ↓**mean litter weight** (13%) <u>75 mg/kg bw/day:</u> -clinical signs (paddling of the forelimbs, nose rubbing) ↓**bw gain** (Days 17-19: 41%)	RAR Vol. 3, B.6.6.2.1/04 Anonymous 26 2002 Report 619/94-D6154

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL (bold text)	Reference
		-bw loss (Days 6-7: -4.6 g, Days 7-8: -2.6 g, Days 8-9: -0.4 g) ↓FC ↓**mean gravid uterus weight** (30%) ↑**post-implantation loss** (11% compared to 5% in control) ↑ **number of early intrauterine deaths** (1.1 compared to 0.7 in control) ↓**mean litter weight** (29%) ↓**number of live foetuses per female** (12 compared to 14.9 in control) **Developmental effects:** <u>5 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↓**foetal weight** (7%) ↑**skeletal variations** (incomplete ossification of skull bone and unossified fifth sternebrae) <u>75 mg/kg bw/day:</u> ↓**foetal weight** (12%) ↑**foetal variations** (incomplete ossification of skull bone and unossified fifth sternebrae) ↑**malformations** (subcutaneous oedema (one animal), retro-oesophageal aortic arch (one foetus), kidney misshapen (one animal), hydropnephrosis (three animals)) NOAEL maternal: 5 mg/kg bw/day NOAEL developmental: 5 mg/kg bw/day	
Teratology range finding study No guideline claimed in study Rabbit New Zealand White F 5/group GLP: Yes	Quinoclamine Purity: 98.1% 0, 8, 20, 50, 80/8[a], 200/20[a], 500/50[a] Vehicle: 0.25% gum tragacanth Gestation Days 6-18	**Maternal effects:** <u>8 mg/kg bw/day:</u> No treatment-related effects <u>20 mg/kg bw/day:</u> ↑**post-implantation loss** (31.1 compared to 8.7 in control) <u>50 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓FC ↑**post-implantation loss** (61.0 compared to 8.7 in control) <u>80/8 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 4%, Day 8: 3%, Day 10: 4%) ↑**post-implantation loss** (25.0 compared to 8.7 in control) <u>200/20 mg/kg bw/day:</u> -clinical signs (coloured urine) ↓bw (Day 7: 6%, Day 10: 6%) ↓FC ↑**post-implantation loss** (30.0 compared to 8.7 in control)	RAR Vol. 3, B.6.6.2.1/04 Anonymous 28 1986 Report AKJ/1/86

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - **NOAEL/LOAEL (for sexual function and fertility, parents)** - **target tissue/organ** - **critical effects at the LOAEL** (bold text)	Reference
		500/50 mg/kg bw/day: **-mortality** (both animals died, one died on day 9 and the other on day 10 of pregnancy)[b] **-clinical signs** (lethargy, hunched posture, dark coloured urine) ↓**bw** (Day 8: 12%) ↓FC **Developmental effects:** 8 mg/kg bw/day: No treatment related effects 20 mg/kg bw/day: ↑**post-implantation loss** (31.1 compared to 8.7 in control) **-malformations** (spina bifida (two animals), interrupted aortic arch major (one animal), hindlimb left malrotated (one animal)) 50 mg/kg bw/day: ↑**post-implantation loss** (61.0 compared to 8.7 in control) **-malformations** (interrupted aortic arch major (one animal), kidney left agenesis (one animal)) 80 mg/kg bw/day: ↑**post-implantation loss** (25.0 compared to 8.7 in control) 200/20 mg/kg bw/day: ↑**post-implantation loss** (30.0 compared to 8.7 in control) *Study is a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL*	
Teratology study No guideline claimed in study Rabbit New Zealand White F 16/group GLP: Yes	Quinoclamine Purity: 98.1% 0, 2.5, 7.5, 22.5 mg/kg bw/day Vehicle: 0.25% gum tragacanth Gestation Days 6-18	**Maternal effects:** 2.5 mg/kg bw/day: No treatment-related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day: ↓ bw gain (Days 0-28: 5%) **Developmental effects:** 2.5 mg/kg bw/day: No treatment related effects 7.5 mg/kg bw/day: No treatment-related effects 22.5 mg/kg bw/day:	RAR Vol. 3, B.6.6.2.1/04 Anonymous 27 1986 Report AKJ/3/86

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
		↓**foetal weight** (5% n.s.) ↑**foetal variations** (increased no. of caudal centra ≤15 (84.9% compared to 59.9% in control)) ↑**malformations** (scoliosis (one animal), spina-bifida (three animals), anomalies of the aortic arch (two animals), sternebral fusions (three animals), hyperextension of limb or paw (one animal)) NOAEL maternal toxicity: 22.5 mg/kg bw/day LOAEL maternal toxicity: not established NOAEL developmental toxicity: 7.5 mg/kg bw/day	
Teratology range finding study No guideline claimed in study Rabbit Crl.NZW/Kbl BR F 7/group GLP: Yes	Quinoclamine Purity: 99.0% 0, 5, 17.5, 30 mg/kg bw/day Vehicle: 1% aqueous methylcellulose Gestation Days 7-28	**Maternal effects:** <u>5 mg/kg bw/day:</u> No treatment-related effects <u>17.5 mg/kg bw/day:</u> -abortion (one animal Day 24) ↓**bw change** (Days 7-28: 12% of controls) ↓FC <u>30 mg/kg bw/day:</u> **-abortions** (two animals, on Day 25 or 29) ↓**bw change** (Days 7-28: 10% of controls) ↓FC ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%) ↓**mean foetal weight** (3%) **Developmental effects:** <u>5 mg/kg bw/day:</u> No treatment-related effects <u>17.5 mg/kg bw/day:</u> **-abortion** (one animal Day 24) <u>30 mg/kg bw/day:</u> **-abortions** (two animals, on Day 25 or 29) ↑**post-implantation loss** (22.4% compared to 14.9% in control) ↑**number of late intrauterine deaths** (1.6 compared to 1.0 in control) ↓**mean litter weight** (6%) ↓**mean foetal weight** (3%) *Study is a range finding study only. Due to low number of animals used in the study it is not considered appropriate to establish a NOAEL/LOAEL*	RAR Vol. 3, B.6.6.2.2/03 Anonymous 35 2002 Report 619/122-D6154
Teratology study OECD 414 Rabbit	Quinoclamine Purity: 99.0% 0, 5, 17.5, 30 mg/kg bw/day	**Maternal effects:** <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day</u> ↓**bw change** (bw change Days 12-15: 67% of control)	RAR Vol. 3, B.6.6.2.2/04 Anonymous 29 2002

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
Crl.NZW/Kbl BR F 24/group GLP: Yes	Vehicle: 1% aqueous methylcellulose Gestation Days 7-28	↓**litter size** (8.4 foetuses per female compared to 9.5 in control) <u>30 mg/kg bw/day:</u> **-mortality** (one female killed on Day 18 of gestation[c]) ↓bw (Days 4-29: 7%) ↓**bw change** (Days 4-29: 46% of control) ↓FC ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control) ↓**litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) ↑**specific foetal variations** (additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, misshapen nasal bone, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, abnormal terminal caudal vertebrae and asymmetric ossification of cervical vertebral centra) **-malformations** (hydronephrosis, 2 animals) **Developmental effects:** <u>5 mg/kg bw/day:</u> No treatment related effects <u>17.5 mg/kg bw/day:</u> ↓**litter size** (8.4 foetuses per female compared to 9.5 in control) ↑**malformations** (hydronephrosis, one animal) <u>30 mg/kg bw/day:</u> ↑**post-implantation loss** (%/No. of affected dams: 24.9/13 compared to 4.8/10 in control) ↑**early intrauterine deaths** (1.0 compared to 0.2 in control) ↑**late intrauterine deaths** (1.4 compared to 0.3 in control) ↓**litter size** (7.8 foetuses per female compared to 9.5 in control) ↓**litter weight** (24%) ↑ **specific foetal variations** (additional liver lobe, cervical remnant of thymus, lengthened anterior fontanelle, misshapen nasal bone, incomplete ossification of frontal and maxilla bones, slight fusion of sternebrae, abnormal terminal caudal vertebrae and asymmetric ossification of cervical vertebral centra) **- malformations** (hydronephrosis, 2 animals) NOAEL maternal toxicity: 5 mg/kg bw/day NOAEL developmental toxicity: 5 mg/kg bw/day	Report 619/155-D6154

The selected endpoint in the previous evaluation (NOAEL 17.5 mg/kg bw per day) was derived from a teratology study on rabbit (Anonymous 29, 2002; 619/155-D6154). At this level, there was a significant but temporary effect on body weight change and a reduction of litter size (8.4 foetuses per female compared to 9.5 in the control, i.e. 12% reduction) that was not considered as adverse at that time. In the next dose level of the rabbit study (30 mg/kg bw per day) there were more adverse effects, such as decreased body weight, more consistent reduction of body weight change, litter size and litter weight. However, the toxicological re-evaluation of this study by the RMS resulted in a proposed NOAEL of 5 mg/kg bw per day. In a corresponding range finding study (Anonymous 35, 2002; 619/122-D6154) similar effects were seen, however, based on re-evaluation by the RMS this was not considered useful for NOAEL setting due to the low number of animals used.

In one teratology study on rat (Anonymous 25, 1989; AKJ/4/86), the toxicological evaluation resulted in maternal and developmental NOAEL of 5 mg/kg bw per day. This is lower than the previously agreed value for rabbit above, but was based on macroscopic changes (enlarged spleen) in one dam at 20 mg/kg bw per day, and was not considered ecotoxicologically relevant at population level. At the next dose, 75 mg/kg bw per day, there was an adverse effect on body weight gain and therefore the ecotoxicologically relevant NOAEL from this study would be 20 mg/kg bw per day. The corresponding range finding study (Anonymous 33, 1986; Anonymous 33, 1989; AKJ/2/86 and AKJ/2A/89) was re-evaluated by the RMS but was not considered useful for NOAEL setting due to the low number of animals used.

A second teratology study on rat (Anonymous 26, 2002; 619/94-D6154) resulted in the same NOAEL of 5 mg/kg bw per day, based on significant effects on body weight gain, mean litter weight (13% reduction), foetal weight (7% reduction) and gravid uterus weight (15% reduction) at 20 mg/kg bw per day that should be considered as ecotoxicologically relevant. The corresponding range finding study (Anonymous 34, 2002; 619/123-D6154) was not considered as useful for NOAEL setting due to the low number of animals used but supported the results from the final study.

A lower NOAEL value, 1.6 mg/kg bw per day, was derived from the two-generation study in rat (Anonymous 19, 1975; 854-111). This value was based on toxicologically adverse effects on body weight and body weight gain in males and females of the second generation at the highest dose level (30.9 mg as/kg bw). It should be noted that statistically significant but smaller effects on body weight and body weight gain were observed also at lower treatment levels, however, were not considered to be adverse by the RMS toxicology expert. In the previous ecotoxicological evaluation, it was considered that the observed effects on body weight and body weight gain seen in the *second* generation of the two-generation study on rats were not ecotoxicologically relevant. Therefore, the ecotoxicologically relevant NOAEL from this study was set to the highest dose, 30.9 mg/kg bw per day, which is higher than the relevant NOAEL values from available teratology studies. The relevance of effects in the second generation has however been discussed recently in expert meetings for other compounds, and it was agreed that such effects should not be excluded.

Nevertheless, the RMS proposes that given the large dose spacing in the two-generation study by Anonymous 19 (1975), the NOAEL of 1.6 mg/kg bw per day may be overly protective. Therefore, the NOAEL of 5 mg/kg bw per day from the available teratology studies by Anonymous 25 (1989) and Anonymous 26 (2002) seems reasonable. This may need further discussion. Other MS are asked to provide their views during the peer review.

According to EFSA (2009/1438), supportive information useful for the derivation of ecotoxicologically relevant NOAEL may be derived from short term studies on rodents based on OECD TG 407 and 408. Also corresponding short term data on dog is considered as potentially relevant for the risk assessment since this is an additional species that would therefore reduce uncertainty of the selected endpoint. Available relevant data are listed in the table below. One study on dogs was only accepted for range finding purposes since too few animals were used to give a reliable NOAEL (see toxicology section). More detailed summary and evaluation of the studies is given in Volume 3, Annex B.6. Based on the toxicological evaluation, NOAEL values between 3 and 10 mg/kg bw per day were concluded from these data. This is considered to support that the previously agreed NOAEL (17.5 mg/kg bw per day) may not be protective enough for wild mammals.

Table 2.9.1.2-3. Summary of relevant short-term toxicology studies.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
Oral 28-day study OECD TG 407 Rat Crl:CD®(SD)IGSBR M, F 5/sex/dose GLP: Yes	Quinoclamine (purity: 99.0%) Oral (dietary) 0, 5, 50, 500, 1000 ppm (corresponding to 0, 0.5, 4.7, 44 and 84 mg/kg bw/day for males; 0, 0.5, 5.3, 48 and 90 mg/kg bw/day for females) 28 consecutive days	<u>5 ppm:</u> No treatment related effects <u>50 ppm:</u> No treatment related effects <u>500 ppm:</u> ↓**bw gain** (M:19%, F:21%, n.s) ↓FC (F) -changes in haematological parameters,urine analysis parameters, organ weights, histopathology in the kidneys <u>1000 ppm:</u> ↓**bw gain** (M: 42%, F: 41%) ↓FC (M, F) -changes in haematological parameters, biochemistry, urine analysis parameters, organ weights, macroscopy changes, histopathology in the kidneys NOAEL (both sexes): 50 ppm (corresponds to 4.7 (m) and 5.3 (f) mg/kg bw/day) LOAEL (both sexes): 500 ppm (corresponds to 44 (m) and 48 (f) mg/kg bw/day)	RAR Vol. 3, B.6.3.1.1/01 Anonymous 15 2002 Report 619/148
Oral 28-day study No guideline stated in study report Dog Beagle	Quinoclamine (purity: 99.0%) Oral (capsules) 0, 3, 10, 30, 100 mg/kg bw/day	<u>3 mg/kg bw/day:</u> No treatment-related effects <u>10 mg/kg bw/day:</u> -clinical signs (red- or black coloured urine (F)) -changes in urinalysis parameters (↑ turbidity (M)) <u>30 mg/kg bw/day:</u>	RAR Vol. 3, B. 6.3.1.2/01 Anonymous 16 2002 Report 619/149

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
M, F 1/sex/dose GLP: Yes	28 consecutive days	clinical signs (red- or black coloured urine (M, F)) ↓ FC -changes in urinalysis parameters -organ weight changes -histopathological changes <u>100 mg/kg bw/day[b]:</u> -clinical signs (red- or black coloured urine (M, F), vomiting (M, F), subdued on Day 3 (F)) ↓ **bw loss** (Day 4: 13% (M), 18% (F)) -poor food consumption (M, F) -changes in biochemistry, organ weight, macroscopy,, histopathology *Study accepted as a range finding study only. Due to limited histopathology and low number of animals used it is considered not appropriate to establish a NOAEL/LOAEL.*	
Oral 90-day study No guideline stated in study report Rat Sprague-Dawley (SPPF) M, F 5/sex/dose GLP: No	Quinoclamine (purity not stated in study report) Oral (dietary) 0, 50, 200 and 1000 ppm (equivalent to 0, 3, 14, 62 mg/kg bw day in males and 0, 3, 13, 65 mg/kg bw day in females) 13 weeks	<u>50 ppm:</u> ↓FC (F: 12%) ↓water consumption (15%) (F) -changes in biochemistry -organ weight changes <u>200 ppm:</u> ↓bw gain (M: 6%) ↓FC (F: 11%) ↓water consumption (12%) (F) -changes in biochemistry, organ weight, , histopathology <u>1000 ppm:</u> ↓bw gain (F: 7%) ↓FC (M: 5%, F: 14%) ↓water consumption (M: 23%, F: 17%) -changes in biochemistry, organ weight, histopathology NOAEL (both sexes): 50 ppm (corresponds to to 3 mg/kg bw/day) LOAEL (both sexes): 200 ppm (corresponds to 14 and 13 mg/kg bw/day in male and females, respectively) *Study considered limited and accepted as supportive data only*	RAR Vol. 3, B.6.3.2.1/01 Anonymous 17 1972
Oral 90-day study OECD 408 (1998) Rat Crl:CD (SD)IGSBR M, F 10/sex/dose GLP: Yes	Quinoclamine (purity: 99%) Oral (dietary) 0, 50, 200 and 800 ppm (equivalent to 0, 3.61, 13.89, 56.74 mg/kg bw/day in males, and 0, 4.56, 17.81, 74.81 mg/kg bw/day in females)	<u>50 ppm:</u> -clinical signs (↑fur staining) (M, F) ↓**bw gain** (Start to week 13: F 17%) ↓FC (F, n.s.) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -changes in biochemistry -changes in organ weights <u>200 ppm:</u> -clinical signs (↑fur staining) (M, F) ↓**bw gain** (Start to week 13: F 21%) ↓FC (F) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -changes in myelogram data, haematological parameters, biochemistry, urinalysis, organ weights,histopathology <u>800 ppm:</u>	Vol. 3, B.6.3.2.1/02 Anonymous 18 2003

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** (bold text)	**Reference**
	13 weeks	-clinical signs (↑fur staining) (M, F) ↓**bw gain** (M: 20-28%, F: 27-38%) ↓FC (M, F) ↑hypoactivity and hyperactivity (at start of treatment) (M, F) -changes in myelogram data, haematological parameters, biochemistry, organ weights, macroscopy, histopathology NOAEL (F): not established NOAEL (M): 50 ppm (corresponds to 4.56 mg/kg bw/day) LOAEL (F): 50 ppm (corresponds to 4.56 mg/kg bw/day) LOAEL (M): 200 ppm (corresponds to 13.89 mg/kg bw/day)	
Oral 90-day study OECD 409 (1998) Dog Beagle M, F 4/sex/dose GLP: Yes	Quinoclamine (purity: 99%) Oral (capsules) 0, 3, 10 and 30 mg/kg bw/day 13 weeks	<u>3 mg/kg bw/day:</u> -clinical signs (coloured urine and faeces) (M, F) <u>10 mg/kg bw/dag:</u> -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (F: 12% n.s.) ↓FC (M) -changes in haematological parameters, organ weights, histopathology <u>30 mg/kg bw/day:</u> -clinical signs (coloured urine and faeces) (M, F) ↓**bw gain** (M: 31%, F: 35%) ↓FC (M, F) -changes in haematological parameters, biochemistry, organ weights, macroscopyin spleen,histopathology NOAEL (both sexes): 3 mg/kg bw/day LOAEL (both sexes): 10 mg/kg bw/day	Vol. 3, B.6.3.2.2/01 Anonymous 20 2002

2.9.1.3 Potential for endocrine disruption in birds and mammals

No specific data on endocrine disruption in birds is available for quinoclamine.

There were some effects on endocrine organs in the standard mammalian toxicity studies. The effects occurred mainly at high dose levels and might thus be due to systemic toxicity. No clear effect pattern was noted in the assessment of available toxicity studies. However, there were some effects noted at lower dose levels which could not be explained by general toxicity but indicate an endocrine activity (loss of estrous cyclic activity and increased relative thyroid weight noted in the 90-day dog study). In addition, increased incidence of post-implantation loss was noted in one rabbit study at a dose level without maternal toxicity. This effect could be considered as a parameter sensitive to but not diagnostic of EATS (estrogen, androgen, thyroid, steroidogenic). Furthermore, open literature data gives some indications of endocrine effects in fish caused by the metabolite phthalic acid.

It could be noted that the potential for endocrine effects have not been fully investigated in available toxicity studies due to limitations in the test guideline available at the time. For example, sperm parameters and oestrus

cycles have not been investigated in the available studies. Nor have gestation length, vaginal opening or preputial separation been determined.

It could also be noted that the assessment to identify structural alerts for hormonal activity using the OECD QSAR Toolbox was restricted to predict estrogen receptor binding affinity. No other pathways such as androgen receptor pathway was not performed.

According to the draft Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (drafted by EFSA and ECHA staff, with support from JRC 7 December 2017) missing information should be clearly reported and may lead to the need to generate additional information. This will need further discussion.

2.9.2 Summary of effects on aquatic organisms

2.9.2.1. Bioaccumulation [equivalent to section 11.4 of the CLH report template]

The potential for bioaccumulation of Quinoclamine is considered to be low, and no data on bioaccumulation were considered necessary for the evaluation under Regulation 1107/2009 since estimated and experimental log Pow are below the regulatory triggers of 4 (EG 1272/2008) and 3 (EU 283/2013). This was also the case for metabolites formed in soil and water. It should be noted though, that no such exemption exists under CLP, which requires that all information available is presented and compared with the criteria.

2.9.2.1.1. Estimated bioaccumulation

No estimated bioaccumulation data are available and is not needed according to Regulation EU 283/2013 since Log Pow for Quinoclamine is <3. This is also the case for the estimated values on the metabolites.
Calculated theoretical log Pow (KOWWIN) for Quinoclamine is 1.5 (Anonymous 2004).

Compound	**Log Pow**	
	Experimental*	**KOWWIN v. 1.68**
Quinoclamine	2.12	1.50
2-amino-1,4-naphthoquinone (AN)	1.77	1.01
2-carboxybenzoic acid (CBA; also known as 2-carboxybenzaldehyde)	-	1.25
2-oxalyl-benzoic acid (M10)	-	0.74
2-amino-oxalyl-benzoic acid (M11)	-	-0.51
Phthalamic acid (M9)	-	0.28
Phthalic acid (M6)	0.73	1.07

* Experimental database match referred to in Episuite v4.11 report (ref. Hansch, C. et al, 1995)

2.9.2.1.2. Measured partition coefficient and bioaccumulation test data

Partition co-efficient n-octanol / water was measured experimentally by an HPLC method and resulted in log Pow = 1.58 at pH 11 (30°C). It was proposed that the effect of pH is not relevant as Quinoclamine has no measurable dissociation constant (Lumsden 1998). No measured data on bioaccumulation are available.

2.9.2.2. Acute aquatic hazard

Available data on the acute toxicity of technical and formulated Quinoclamine and metabolites to fish and aquatic invertebrates are summarised in the table below, with toxicity values used for the risk assessment marked in bold.

Table 2.9.2.2-1. Summary of relevant information on acute aquatic toxicity, with toxicity values used for the risk assessment marked in bold.

Species	Test substance	Time-scale (Test type)	Endpoint	Toxicity (mg a.s./L)	Remarks	Reference
Acute toxicity to fish						
Oncorhynchus mykiss Rainbow trout	Quinoclamine	Semi static 96 h OECD 203	LC50	0.044 (48 h measured)	Key study for classification of Quinoclamine	Anonymous 43 1991a Report 912043117 (In DAR 2007)
Oncorhynchus mykiss Rainbow trout	Mogeton 25% WP	Semi-static 96 h OECD 203	LC50	0.12 (mm)		Anonymous 44 1994a Report 80-91-0045-02-93 (In DAR 2007)
Oncorhynchus mykiss Rainbow trout	Mogeton 50% WG	Semi-static 96 h OECD 203	**LC50**	**0.042 (nom)**	**First tier toxicity value for risk assessment**	Anonymous 45 1998a Report 98001/01-AAOm
Geomean Rainbow trout (n=3)	Quinoclamine Mogeton 50 %WG Mogeton 25%WP	Semi-static 96 h	***LC50***	***0.061***	***Input for fish geomean calculation***	Species-specific geomean LC50 for rainbow trout
Danio rerio Zebra fish	Mogeton 50% WG	Semi-static 96 h OECD 203	***LC50***	***0.740 (gmm)***	***Input for fish geomean calculation***	Anonymous 46 2016a Report AGK-003/4-32/A
Pimephales promelas Fathead minnow	Mogeton 50% WG	Semi-static 96 h OECD 203	***LC50***	***0.531 (nom)***	***Input for fish geomean calculation***	Anonymous 47 2016b Report AGK-003/4-32/C
Oryzias latipes Medaka	Mogeton 50% WG	Semi-static 96 h OECD 203	***LC50***	***0.894 (nom)***	***Input for fish geomean calculation***	Anonymous 48 2016c Report AGK-003/4-32/F

Species	Test substance	Time-scale (Test type)	Endpoint	Toxicity (mg a.s./L)	Remarks	Reference
Fish geomean LC50 (n = 4 from 6 tests)	Quinoclamine Mogeton 50 %WG Mogeton 25%WP	Semi-static 96 h	**LC50**	**0.38**	**Higher tier toxicity value for risk assessment**	Derived by calculating species-specific geomean LC50 first for rainbow trout, and then general geomean LC50 for 4 species.
Oncorhynchus mykiss Rainbow trout	Metabolite M6 Phthalic acid	Static 96 h OECD 203	**LC50**	**> 62.3 (gmm)**	First tier toxicity value for risk assessment	Anonymous 49 1999 [a] ADAMA Report R-11304 (In Folpet DAR 2007)
Acute toxicity to aquatic invertebrates						
Daphnia magna	Mogeton 50% WG	Static 48 h OECD 202	**EC50**	**1.03 (nom)**	First tier toxicity value for risk assessment	Heintze 1998b Report 98001/01-AADm
Daphnia magna	Metabolite M6 Phthalic acid	Static 48 h OECD 202	**EC50**	**> 100 (nom)**	First tier toxicity value for risk assessment	Gries 1999 [a] ADAMA Report R-11305 (In Folpet DAR 2007)
Daphnia magna	Metabolite M9 Phthalamic acid	Static 48 h OECD 202	**EC50**	**> 100 (nom)**	First tier toxicity value for risk assessment	Scheerbaum 2016a [a] ADAMA Report R-36854
Toxicity to algae and aquatic plants						
See Table 2.9.2.3-1 below.						

nom = nominal; ini = initial measured; mm = mean measured (arithm.); gmm = geomean measured
a) Data belong to ADAMA Makteshim Ltd

2.9.2.2.1. Acute (short-term) toxicity to fish

Considering OECD TG 203 (1992), reliable data on acute toxicity of the active substance or formulations with Quinoclamine are available for four different fish species, whereof Rainbow trout was the most sensitive. 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. Hence, the overall lowest LC50 is proposed for the first tier risk assessment. As a higher tier option a geometric mean LC50 for all four species tested was proposed by the applicant, in accordance with the EFSA Aquatic Guidance Document (2013), chapter 2.1.4.1. Since the available data indicate a comparable toxicity of the active substance and the formulation, this approach was tentatively accepted by the RMS. Other MS are invited to express their views on the approach during the peer review.

One valid study testing the acute toxicity of Metabolite M6 (Phthalic acid) to fish is also available, indicating low toxicity of this metabolite compared to the active substance.

2.9.2.2.2. Acute (short-term) toxicity to aquatic invertebrates

Considering OECD TG 202 (2004), there are no reliable data on acute toxicity of the active substance to Daphnia magna. In the available study with the active ingredient, analytical measurements were conducted only on test solutions before the test initiation, and it is not possible to know if the test concentration decreased over time. Further, no analytical measurements to prove absence of contamination of the control were conducted. According to OECD TG 202 (2004), analytical measurements should also be carried out at the end of the test to ensure stable test concentrations. It is therefore likely that the reported effect values underestimated the toxicity of the test substance.
Reliable acute toxicity data on the representative formulation are available, though, and will preliminarily be used in the risk assessment for Quinoclamine.

Reliable data are also available for Metabolite M6 (Phthalic acid) and Metabolite M9 (Phthalamic acid), showing low acute toxicity for these metabolites to *Daphnia magna*, compared to Quinoclamine.

2.9.2.2.3. Acute (short-term) toxicity to algae or aquatic plants

All available data are listed in Table 2.9.2.3-1 below.

2.9.2.2.4. Acute (short-term) toxicity to other aquatic organisms

No further data are available.

2.9.2.3. Long-term aquatic hazard

Available data on the chronic toxicity of technical and formulated Quinoclamine and metabolites to fish, aquatic invertebrates, algae and macrophytes are summarised in the table below, with toxicity values selected for the risk assessment marked in bold.

Table 2.9.2.3-1. Summary of relevant information on chronic aquatic toxicity, with toxicity values selected for the risk assessment marked in bold.

Species	Test substance	Time-scale (Test type)	Endpoint	Toxicity (mg a.s./L)	Remarks	Reference
Long-term and chronic toxicity to fish						
Oncorhynchus mykiss Rainbow trout	Quinoclamine	ELS 90 d Flow-through OECD 210	**NOEC** EC10	**0.00213 (nom)** 0.0024 (nom)	First tier toxicity value for risk assessment Key study for the classification of Quinoclamine	Anonymous 50 2015 Report AGK-001/4-43/E
Oncorhynchus mykiss Rainbow trout	Mogeton 50 % WG	ELS 90 d Pulse exposure OECD 210	**NOEC** EC10	**0.020 (nom) [a]** 0.023 (nom)	Higher tier toxicity value for risk assessment.	Anonymous 51 2016d Report AGK-003/4-43/E

Species	Test substance	Time-scale (Test type)	Endpoint	Toxicity (mg a.s./L)	Remarks	Reference
					N.B. *This kind of refinement is generally not accepted within the Northern Zone.*	
Long-term and chronic toxicity to aquatic invertebrates						
Daphnia magna	Mogeton 50% WG	21 d Pulse exposure OECD 211	**NOEC / EC10**	**0.0102 (nom)**	Higher tier toxicity value for risk assessment ***N.B.*** *This kind of refinement is generally not accepted within the Northern Zone.*	Renner 2016 Report 15 10 48 084W
Chironomus riparius	Quinoclamine	24 d Water-sediment (spiked water) OECD draft GD 1998	Emergence [b] NOEC(aq) **EC10(aq)**	 0.063 (nom) **0.052 (nom)**	First tier toxicity value for risk assessment Key study for the classification of Quinoclamine	Kleiner 2000a Report 991048113 (In DAR 2007)
Chironomus riparius	Metabolite AN 2-Amino-1,4-naphtoquinone	21 d Water-sediment (spiked water) OECD 219	Development NOEC(aq) **EC10(aq)** Emergence NOEC(aq) EC10(aq)	 < 0.145 (ini) **0.0813 (ini)** 0.674 (ini) 0.643 (ini)	First tier toxicity value for risk assessment	Juckeland 2009 Report 09 10 48 004W
Toxicity to algae						
Scenedesmus subspicatus	Mogeton 50% WG	72 h Static OECD 201	NOEC LOEC EC10 **ErC50** EbC50	- 0.014 (gmm) - **0.029 (gmm)** 0.014 (gmm)	First tier toxicity value for risk assessment No NOEC or EC10 based on geomean conc. can be derived from the study	Dengler 1998 Report 98001/01-AASs
Navicula pelliculosa	Quinoclamine	72 h Static OECD 201	NOEC ErC10 ErC50 EbC10 EbC50	0.07 (72-h meas.) 0.115 (72-h meas.) 0.468 (72-h meas.) 0.06 (72-h meas.) 0.185 (72-h meas.)	Poorly reliable but supportive	Barth 2000 Report 991048121 (In DAR 2007)
Pseudo-kirchneriella subcapitata	Metabolite M6 Phthalic acid	72 h Static OECD 201	NOEC ErC10 **ErC50** EyC10 EyC50	26.8 (ini) 48.4 (ini) **56.8 (ini)** 43.3 (ini) 49.3 (ini)	First tier toxicity value for risk assessment	Scheerbaum 2016b [c] ADAMA Report R-36849
Pseudo-kirchneriella subcapitata	Metabolite M9 Phthalamic acid	72 h Static OECD 201	pH adjusted: NOEC ErC10 **ErC50** EyC10 EyC50	 100 (nom) >100 (nom) **>100 (nom)** >100 (nom) >100 (nom)	First tier toxicity value for risk assessment	Scheerbaum 2016c [c] ADAMA Report R-36850
Toxicity to macrophytes						

Species	Test substance	Time-scale (Test type)	Endpoint	Toxicity (mg a.s./L)	Remarks	Reference
Lemna minor	Quinoclamine	7 d Semi-static OECD draft 1997	NOEC ErC10 ErC50 EyC10 EyC50	0.4 (gmm) 0.5 (gmm) 0.11 (gmm) 0.03 (gmm) 0.09 (gmm)		Kleiner 2000b Report 991048122 (In DAR 2007)
Myriophyllum spicatum	Quinoclamine	14 d Semi-static OECD draft 2013	NOEC ErC10 ErC50 EyC10 EyC50 Root number EC10 **EC50**	0.0086 (gmm) 0.0108 (gmm) 0.1347 (gmm) 0.0018 (gmm) 0.0613 (gmm) 0.0044 (gmm) **0.0515 (gmm)**	First tier toxicity value for risk assessment Key study for the classification of Quinoclamine	Juckeland 2015 Report 14 10 48 008 W
Lemna minor	Mogeton 50% WG	7 d Semi-static OECD TG 221	NOEC ErC10 ErC50 EyC10 EyC50	Not determined (<0.05) 0.0453 (nom) 0.116 (nom) 0.0309 (nom) 0.0711 (nom)		Juckeland 2008 Report 08 10 48 013 W

nom = nominal; ini = initial measured; mm = mean measured (arithm.); gmm = geomean measured
a) NOEC is proposed by RMS based on 15% (non-significant) effects on post-hatch survival 90 dpf. The available EC10 value is considered to be uncertain due to the absence of confidence limits. It should be noted that there is no clear evidence as to whether the most sensitive life-stage was exposed during the test.
b) No effects on development rate were observed in this study.
c) Data belong to ADAMA Makteshim Ltd

2.9.2.3.1. Chronic toxicity to fish

Considering OECD TG 210 (2013), one reliable chronic study on rainbow trout with the active substance is available, from which a valid endpoint for the risk assessment to fish is derived.

In addition, a higher tier chronic study on rainbow trout was submitted by the applicant, where pulse exposure of the representative formulation was tested in order to simulate more realistic conditions. The study as such was considered to be well performed. However, it should be noted that the proposed refinement based on high-resolution analysis of FOCUS surface water peaks is not accepted within the Northern Zone. Further, there is no clear evidence that the exposure phase covered the most sensitive life stage of the fish. This is further discussed in Vol. 3 CP, section B.9.4.

No chronic toxicity data for fish are available for the metabolites of Quinoclamine.

2.9.2.3.2. Chronic toxicity to aquatic invertebrates

Crustaceans

Considering OECD TG 211 (2012), there are no reliable data on chronic toxicity of the active substance or any of its metabolites to Daphnia magna. The available study was accepted in the previous evaluation, but is no longer considered to be valid. The validity criterion of at least 60 living offspring per surviving parent in the control (OECD TG 211, revised 2012) was not fulfilled (actual mean value 46.78 offspring/adult). This might

have been an effect of group exposure, which was accepted by the guideline from 1984, available when the study was conducted. According to the current guideline (and since 1998), it is recommended that parent daphnids are held individually during the reproduction test.

Analytical measurements were conducted for the three highest concentrations, although it is recommended in OECD TG 211 (2012) that analytical measurements should be conducted for at least the lowest and highest concentrations. The measured concentration in the second highest treatment was lower than 80% of the nominal concentration, hence it could have been more suitable to express effect values as measured concentrations rather than nominal values. This is however not possible for the NOEC or EC_{10}, since no analytical measurements are available at these test concentrations. It was further noted that the proposed LC_{50} value for survival was extrapolated outside the range of tested concentrations in the study. This is normally not recommended (OECD No. 54; guidance on statistical analysis).

For the representative formulation, a higher tier study with *Daphnia magna* is available, where pulse exposure of the representative formulation was tested in order to simulate more realistic conditions. The study as such was considered to be well performed. However, it should be noted that the proposed refinement based on high-resolution analysis of FOCUS surface water peaks is not accepted within the Northern Zone. This is further discussed in Vol. 3 CP, section B.9.4.

Sediment dwelling organisms

The applicant submitted two chronic water-sediment studies, one with Quinoclamine and one with Metabolite AN (2-Amino-1,4-naphtoquinone), on the sediment-dwelling midge *Chironomus riparius*. Both studies were assessed as valid according to OECD TG 219 (2004). Based on the study results, the metabolite AN appeared to be slightly less toxic than the active substance to *Chironomus.* In addition to the available aquatic (mg/L) endpoints from the studies, the applicant also proposed calculated sediment (mg/kg sed.) endpoints which were extrapolated from the aquatic toxicity endpoints. However, considering the test design (water spiked system), and the limited available data on actual concentrations in the sediment phase, the RMS found it less appropriate to estimate any toxicity endpoints for the sediment phase at all from these studies. The RMS therefore proposes that only the aquatic toxicity endpoints from the overlaying water should be used.

2.9.2.3.3. Chronic toxicity to algae or aquatic plants

Algae

Considering OECD TG 201 (2006), there are no reliable data available on toxicity of the active substance to green algae. Hence, for the time being, the risk assessment will rely on the available data on green algae for the representative formulation.

Since Quinoclamine has an herbicidal mode of action, an additional algal species (the diatom *Navicula pelliculosa*) has been tested with the active substance. The available data are not considered valid for risk

assessment, but may be useful as supportive information to conclude on the relatively low toxicity of Quinoclamine to *Navicula pelliculosa* compared to green algae.

Further, two reliable metabolite studies are available, which demonstrate low toxicity of both Metabolite M6 (Phthalic acid) and Metabolite M9 (Phthalamic acid) to green algae, compared to the toxicity of Quinoclamine.

Macrophytes

Considering OECD TG 221 (2006), reliable data on toxicity to *Lemna gibba* are available both for the active susbtance and for the representative formulation. Considering OECD TG 238 (2014), also a valid sediment-free *Myriophyllum spicatum* toxicity test with the active substance is available.

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 macrophytes.

2.9.2.3.4. Chronic toxicity to other aquatic organisms

No further data are available.

2.9.2.4. Comparison with the CLP criteria

2.9.2.4.1. Acute aquatic hazard

All available acute toxicity data are presented above in Table 2.9.2.2-1 (fish and aquatic invertebrates) and Table 2.9.2.3-1 (algae and macrophytes). A summary of the key data is given below.

Acute toxicity to fish: 96 h LC50 = 0.044 mg a.s./L
Chronic toxicity to fish: 90 d ELS NOEC = 0.00212 mg a.s./L
Acute toxicity to aquatic invertebrates (*Daphnia magna*): 48 h EC50 = 1.03 mg a.s./L
Chronic toxicity to aquatic invertebrates (Chironomus sp.): 24 d EC10 = 0.052 mg a.s./L
Toxicity to algae: 72 h ErC50 = 0.029 mg a.s./L
Toxicity to aquatic macrophytes: 7 d ErC50 = 0.11 mg a.s./L

The toxicity of Quinoclamine to algae (ErC50 = 0.029, the most sensitive group) fulfils the classification criterion of ≤ 1 mg/L for Category Acute 1 according to Regulation (EG) 1272/2008.

The proposed M-factor is 10 (appropriate for acute toxicity values within in the range 0.01 – 0.1 mg/L).

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

All available chronic toxicity data are presented above in Table 2.9.2.3-1.

Quinoclamine is not rapidly degradable (see section 2.8.2). The substance was not readily biodegradable in 28-day test for ready biodegradability. The primary degradation products cannot be demonstrated to not require classification and therefore primary degradation cannot be used to conclude the substance is rapidly degradable. In a surface water simulation test half-lives were 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. Quinoclamine is considered as hydrolytically stable at environmentally realistic temperatures and pH values. Two studies on aquatic photolysis indicated that under favourable conditions half-lives for primary degradation may be shorter than 16 days but ultimate degradation did not reach >70% within 28 days.

Considering that Quinoclamine is not rapidly degradable in the aquatic environment (see section 2.8.2), the long-term toxicity of Quinoclamine at all taxonomic levels fulfils the classification criterion of ≤ 0.1 mg/L for Category Chronic 1 according to Regulation (EG) 1272/2008. The lowest available chronic toxicity value for Quionoclamine is derived from a fish study (NOEC = 0.00213 mg/L). The proposed M-factor is 10 (appropriate for chronic toxicity values within in the range 0.001 – 0.01 mg/L).

When based on the Log Kow, the substance has a 'low potential for bioaccumulation'. Quinoclamine is not expected to bioaccumulate.

2.9.2.5. Conclusion on classification and labelling for environmental hazards

Quinoclamine shall be classified as

a) Acute (short-term) aquatic hazard
   Category Acute 1, M-factor = 10
b) Long-term aquatic hazard
   Category Chronic 1, M-factor = 10

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	Quinoclamine >95%	72-h	NOEC	0.07	Barth 2000

pelliculosa Poorly reliable but supportive		Static OECD TG 201, GLP	E_rC_{10} E_rC_{50} E_bC_{10} E_bC_{50}	0.115 0.468 0.06 0.185 (72-h meas.)	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.

Supplemental information - In depth analyses by RAC

The ECOSAR v. 1.11. QSAR program calculated Class-specific estimations for quinoclamine aquatic toxicity in classes Aliphatic amines, Vinyl/Allyl halines and Quinones. The Quinones class fits the existing data by far the best. The other classes predict e.g. acute toxicity values from 8 to 115.

Table. ECOSAR v.1.11 QSARs from quinoclamine aquatic toxicity in class Quinones

ECOSAR Class	**Organism**	**Duration and endpoint**	**Predicted mg/L**
Quinones	Fish	96-h LC_{50}	0.098
Quinones	Daphnid	48-h LC_{50}	0.495
Quinones	Green algae	96-h EC_{50} (growth)	0.068
Quinones	Fish	ChV	0.008 **!**
Quinones	Daphnid	ChV	1.770 **!**
Quinones	Green algae	ChV	0.016

! - The toxicity value was estimated through application of acute-to chronic ratios per methods outlined in the ECOSAR Methodology Document provided in the ECOSAR Help Menu

ChV = geometric mean of the LOEC and NOEC

Using log Kow 1.50

Quinoclamine was within the applicability domain of the model. Training sets for the SAR equations were small, two substances and 3 studies for the acute fish equation at best.

2.9.2.6. Assessment in relation to the B and T criteria (Regulation 1107/2009)

B-criterion:

The potential for bioaccumulation of Quinoclamine and its metabolites formed in soil and water is considered to be low. No data on bioaccumulation are considered necessary since estimated and experimental log Pow are below the regulatory trigger of 3 (EU 283/2013).

T-criterion:

An active substance fulfils the criteria for toxicity in aquatic organisms, as stated in Annex II to Regulation (EC) 1107/2009, if the long-term no-observed effect concentration for marine and freshwater organisms is less than 0.01 mg/L.

Quinoclamine fulfils the T-criterion, with a of NOEC 0.00213 mg a.s./L from the ELS study on rainbow trout (Anonymous 50, 2015).

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Based on the available data, none of the metabolites fulfils the T-criterion.

2.9.2.7 Potential for endocrine disruption in aquatic organisms

No indications of endocrine disrupting properties of Quinoclamine have been found in the available data set for aquatic organisms. However, no specific studies are available to conclude on potential for endocrine disruption. The open scientific literature presented by the applicant does not provide any indications of endocrine activity of the active substance in fish. Further considerations may be needed in order to conclude the assessment on endocrine disruption in fish.

Regarding metabolites, one published *in vitro* study gives some indications of endocrine effects in fish caused by phthalic acid (Maradonna et al. 2013). Due to uncertainties on the identity of the test substance, the study is considered less reliable but may be supportive if other evidence become available. See Vol. 3 CA, section B.9.2.3 for further information.

2.9.3 Summary of effects on arthropods

2.9.3.1 Effects on bees

The relevant available data on toxicity to bees are summarised in the table below, with endpoints selected for the risk assessment marked in bold.

Table 2.9.3.1-1. Summary of relevant information on toxicity to bees, with endpoints selected for the risk assessment marked in bold.

Species	Test substance	Time scale	Endpoint	Toxicity	Remarks	Reference
Apis mellifera L. (adult honey bee)	Mogeton 50% WG	48 h Acute	**Oral LD_{50}** **Contact LD_{50}**	**> 401.0 µg a.s./bee** **> 411.2 µg a.s./bee**	a, b a, b	Franke 2014 Report 14 10 48 151 B
Apis mellifera L. (adult honey bee)	Mogeton 50% WG	48 h Acute	Oral LD_{50} Contact LD_{50}	> 188.1 µg a.s./bee > 200 µg a.s./bee		Barth 2008 Report 08 10 48 022 B
Bombus terrestris (adult bumblebee)	Mogeton 50% WG	96 h Acute	**Oral LD_{50}** **Contact LD_{50}**	**> 287.6 µg a.s./bee** **> 498.1 µg a.s./bee**	a a	Amsel 2015 Report 14 10 48 057 A
Osmia bicornis L. (adult solitary bee)	Mogeton 50% WG	96 h Acute	**Oral LD_{50}** **Contact LD_{50}**	**101.1 µg a.s./bee** **283.5 µg a.s./bee**	a a	Schnurr 2015 Report 14 10 48 154 B
Apis mellifera L. (adult honey bee)	Mogeton 50% WG	10 d Chronic	**LD50** LD20 LD10 NOED **NOEDhpg**	**78.3 µg a.s./bee/day** 53.5 µg a.s./bee/day 43.9 µg a.s./bee/day 49.6 µg a.s./bee/day **119.2 µg a.s./bee/day**	a, b b	Ruhland 2015 Report 14 10 48 153 B
Apis mellifera L. (honey bee larvae)	Mogeton 50% WG	120 h Repeated exposure	LD50 **NOED**	50.9 µg a.s./larva **19.8 µg a.s./larva**	 a, b	Kleebaum 2015 Report 14 10 48 152 B

a) Endpoint used for the risk assessment performed according to EPPO(2010
b) Endpoint used for the risk assessment performed according to the EFSA(2013)

2.9.3.2 Effects on arthropods other than bees

The relevant available data on toxicity to arthropods other than bees are summarised in the tables below, with endpoints selected for the risk assessment marked in bold.

Standard laboratory studies with the old formulation Mogeton 25% WP tested on a range of arthropod species are available from the previous evaluation of Quinoclamine. These studies indicate a low toxicity (< 30% effect) of Quinoclamine to *Aphidius rhopalosiphi, Typhlodromus pyri, Pardosa spp.* and *Poecilus cupreus* when tested at doses comparable to the currently proposed GAP. For *Aleochara bilineata*, one older study with Mogeton 25% WP showed up to 70% effect on reproduction at a relevant dose (Ullrich 1992b), however a newer study performed with Mogeton 50% WG (identical to the representative formulation Mogeton TOP), revealed no effects at a relevant dose (Kühner 1998). Moreover, the older study by Ullrich (1992b) was considered less reliable since no toxic reference was included in the test. Therefore, this study was not used for the risk assessment.

Table 2.9.3.2-1. Standard laboratory tests to arthropods other than bees

Species	Test substance	End point	Toxicity (kg a.s./ha)	Remarks	Reference
Indicator species					
Aphidius rhopalosiphi (parasitic wasp)	Mogeton 25% WP	48-h LR50 and ER50 (fecundity)	> **3.81**	< 30 % effect up to 3.81 kg a.s./ha	Kleiner 1999a Report 99 10 48 069 (In DAR 2007)
Typhlodromus pyri (predatory mite)	Mogeton 25% WP	7-d LR50, 14-d LR50 and ER50 (fecundity)	> **3.81**	< 30 % effect up to 3.81 kg a.s./ha	Kleiner 1999b Report 99 10 48 070 (In DAR 2007)
Additional species					
Pardosa spp. (lycosid spider)	Mogeton 25% WP	14-d LR50 and ER50 (feeding capacity)	> **3.81**	< 30 % effect up to 3.81 kg a.s./ha	Kleiner 1999c Report 99 10 48 068 (In DAR 2007)
Poecilus cupreus (carabid beetle)	Mogeton 25% WP	14-d LR50 and ER50 (feeding capacity)	> **3.81**	< 30 % effect up to 3.81 kg a.s./ha	Ullrich 1992a Report 1396/30-91 (In DAR 2007)
Aleochara bilineata (rove beetle)	Mogeton 25% WP	2-month ER50 (reproduction)	< 3.81	70 % effect Study considered as less reliable	Ullrich 1992b Report 1396/20-91 (In DAR 2007)
Aleochara bilineata (rove beetle)	Mogeton 50% WG	10-week ER50 (reproduction)	> **3.75**	< 30 % effect up to 15 kg a.s./ha	Kühner 1998 Report 98001/01-NLAb (In DAR 2007)

Extended laboratory data are available for the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri*, as well as one additional species, *Orius laevigatus.* For *Typhlodromus pyri* significant effects on reproduction were observed at rates ≥ 0.527 kg a.s./ha, whereas no significant effects were observed on the other two species tested up to and including at least the double highest recommended application rate for Quinoclamine.

An aged residue study with *Typhlodromus pyri* was also submitted, showing no significant effects on mortality or reproduction after 28 days exposure to aging residues of the test substance when applied at a rate corresponding to the proposed GAP.

Table 2.9.3.2-2. Extended laboratory tests and aged residue tests to arthropods other than bees

Species	Life stage	Test substance / substrate	Time scale	End point	Dose (kg a.s./ha)	% effect	ER_{50} (kg a.s./ha)	Reference
Extended laboratory studies								
Aphidius rhopalosiphi (parasitic wasp)	Females 0-48 h old	Mogeton 50% WG barley seedlings	14 days	Mortality, repellency, repro-duction	0.527 1.054 2.108 4.214 **8.432**	< 30% (n.s.) at all doses	**ER50 > 8.432**	Sipos 2008a Report 07/586-351FD
Typhlodromus pyri (predatory mite)	Proto-nymphs	Mogeton 50% WG bean leaf disks	7 days	Mortality	0.0659 - 8.432	< 30% (n.s.) at all doses	LR50 > 8.432	Sipos 2008b Report 07/586-351RA
			14 days	Repro-duction	<u>Test 1</u> **0.527** 1.054 2.108 4.214 8.432 <u>Test 2</u> 0.0659 0.132 0.264 0.527 1.054	**-32.8% *** -53.3% * -72.6% * -46.5% * -87.3% * -13.7% (n.s.) -18.6% (n.s.) -18.7% (n.s.) -28.9% (n.s.) -23.0% (n.s.)	**ER50 > 0.527** [a)]	
Orius laevigatus (predatory bug)	Second instar nymphs	Mogeton 50% WG bean leaves	21 days	Mortality, repro-duction	1.85 2.73 4.11 6.17 **9.25**	< 30% (n.s.) at all doses	**ER50 > 9.25**	Rathke 2016a Report 160405DH / IOE16877
Aged residue studies								
Typhlodromus pyri (predatory mite)	Proto-nymphs	Mogeton 50% WG whole bean plants	28 days	Mortality	3.855 (ini)	< 30% (n.s.) at all doses	LR50 > 3.855	Rathke 2016b Report 160405DH / IRD16877
				Repro-duction	**3.855** (ini) Day 0-14 Day 14-28 (aged residues)	 -45.3% * 7.57% (n.s.)	**ER50 > 3.855**	

** significant effect*
n.s. = Not significant effect
a) The applicant proposed ER50 = 3.3 kg a.s./ha based on both tests in the study, and excluding results at 0.527 - 1.054 kg a.s./ha). This approach was considered less reliable by the RMS.

2.9.4 Summary of effects on non-target soil meso- and macrofauna

2.9.4.1 Effects on earthworms

The relevant available data on toxicity to earthworms are summarised in the table below, with endpoints selected for the risk assessment marked in bold.

Table 2.9.4.1-1. Summary of relevant information on toxicity to earthworms

Species	Test substance	Application method /OM	Time scale	End point	Toxicity (mg a.s./kg soil dw)	Remarks	Reference
Laboratory studies							
Eisenia fetida	Quinoclamine	Test item incorporated into the soil / 5% peat	Chronic, 56 days	NOEC **EC10** EC25 EC50	6.5 **5.1** 7.5 11.4	a	Friedrich 2009 Report 09 10 48 067S
Eisenia fetida	Mogeton 50 % WG	Test item incorporated into the soil / 5% peat	Chronic, 56 days	**NOEC** EC10 EC20 EC50	**6.9** 12.3 14.8 20.4		Vértesi 2008 Report 07/586-211G
Eisenia fetida	Metabolite M6 Phthalic acid	Test item incorporated into the soil / 5% peat	Chronic, 56 days	**NOEC** EC10 EC20 EC50	**68.0** > 122.4 > 122.4 > 122.4	b	Friedrich 2016a Report 16 10 48 133 S
Eisenia fetida	Metabolite M10 2-(Carboxy-carbonyl) benzoic acid	Test item incorporated into the soil / 5% peat	Chronic, 56 days	**NOEC** EC10 EC20 EC50	**61.2** > 61.2 > 61.2 > 61.2	c	Friedrich 2016b Report 16 10 48 134 S
Field studies							
Earthworms	Mogeton 50 % WG	Field study in Germany / Grassland / 5.33% OM / Application in October / Plot sprayer	1 year	**NOEC**	**3.75 kg a.s./ha** (not analytically verified)	grassland	Schulz 2011 Report 10 10 48 002 F

a) Given the observed dose response pattern with 17.7% non-significant effect on reproduction at 6.5 mg a.s./kg dw soil, the proposed NOEC at this level seems to be very uncertain. In this case, the RMS considered the EC10 of 5.1 mg a.s./kg dry soil provided by the applicant as more robust than the NOEC.
b) The applicant proposed NOEC = 122.4 mg/kg soil dw, However, the RMS considered that the 18.5% effect on reproduction at this treatment level may be regarded as biologically relevant although not statistically significant. It is not clear how the study author estimated EC10 to be > 122.4 mg/kg soil dw, since no ECx analysis was provided.
c) NOEC = highest tested concentration

In addition to the standard laboratory tests, a higher-tier field study with the representative formulation is available. The field study was conducted on grassland and therefore its representativeness for the proposed use of Quinoclamine in nursery stock plants is uncertain. On the other hand, the relevant test guideline explicitly recommends grassland as the preferred study site for testing earthworms in the field, since earthworm density and diversity in grassland are “generally higher and more stable than on arable land which makes it easier to detect significant effects on earthworm populations” (ISO 11268-3 updated by Kula et al. 2006). Hence, considering the community structure of earthworms, the RMS proposes that the available field study results may be considered as suitable also for the risk assessment in nursery stock plants. However, considering the lack of verification of

exposure levels in the soil as well as the less optimal timing of the study, the reliability of these higher-tier data may need further discussion.

2.9.4.2 Effects on other soil macro organisms

The relevant available data on toxicity to other soil macro organisms are summarised in the table below, with endpoints selected for the risk assessment marked in bold.

Table 2.9.4.2-1. Summary of relevant information on toxicity to other soil macro organisms

Species	Test substance	Application method / OM	Time scale	End point	Toxicity (mg a.s./kg soil dw)	Remarks	Reference
Laboratory studies							
Folsomia candida	Mogeton 50 % WG	Test item incorporated into the soil / 5% peat	Chronic, 28 days	$NOEC_{mortality}$ $NOEC_{reproduction}$ **EC_{10}** EC_{20} EC_{50}	514 159 **3.63** 70.4 n.d.	 a a	Rathke 2016c Report 160405DH / ICR16877
Folsomia candida	Metabolite M6 Phthalic acid	Test item incorporated into the soil / 5% peat	Chronic, 28 days	**$NOEC_{mortality}$** $NOEC_{reproduction}$ EC_{10}, EC_{20}, EC_{50}	**68.0** 122.4 > 122.4		Friedrich 2016c Report 16 10 48 129 S
Folsomia candida	Metabolite M10 2-(Carboxy-carbonyl) benzoic acid	Test item incorporated into the soil / 5% peat	Chronic, 28 days	**NOEC** EC_{10}, EC_{20}, EC_{50}	**61.2** > 61.2		Friedrich 2016d Report 16 10 48 130 S
Hypoaspis aculeifer	Mogeton 50 % WG	Test item incorporated into the soil / 5% peat	Chronic, 14 days	$NOEC_{mortality}$ $NOEC_{reproduction}$ **EC_{10}** EC_{20} EC_{50}	514 87.9 **70.9** 180 n.d.	 a a	Rathke 2016d Report 160405DH / IHL16877
Hypoaspis aculeifer	Metabolite M6 Phthalic acid	Test item incorporated into the soil / 5% peat	Chronic, 14 days	**NOEC** EC_{10}, EC_{20}, EC_{50}	**122.4** > 122.4		Schulz 2016a Report 16 10 48 131 S
Hypoaspis aculeifer	Metabolite M10 2-(Carboxy-carbonyl) benzoic acid	Test item incorporated into the soil / 5% peat	Chronic, 14 days	**NOEC** EC_{10}, EC_{20}, EC_{50}	**61.2** > 61.2		Schulz 2016b Report 16 10 48 132 S
Field studies							
Collembola and Acarina	Mogeton 50 % WG	Field study in Germany / Grassland/ 3.66% OM / Application in May / Boom sprayer	12.5 months	**$NOEC_{abundance}$**	**3.75 kg a.s./ha** (2.08 mg a.s./kg soil dw, initial measured, at 10 cm depth)	grassland	Henkes and Henkes 2017 Report 1640054

a) Large 95% confidence intervals indicate that the EC10 and EC20 are uncertain

In addition to the standard laboratory tests, a higher-tier field study with the representative formulation is available. The field study was conducted on grassland and therefore its representativeness for the proposed use of Quinoclamine in nursery stock plants is uncertain. However, since the concentrations of test substance in the soil

were satisfactorily verified on the day after application in the field, the RMS proposes that the NOEC from the study can be considered in the risk assessment together with the calculated PECsoil, also for nursery stock plants. This reasoning is further evaluated in the risk assessment in Volume 3, Annex B.9 on the representative formulation, section B.9.8.

2.9.5 Summary of effects on soil nitrogen transformation

The available data on effects on nitrogen transformation are summarised in the table below.

Table 2.9.5-1. Summary of relevant information on effects on nitrogen transformation.

Measurement parameter	Test substance	Time scale	No effects (< 25 % difference to control) up to	Remarks	Reference
Nitrogen transformation	Quinoclamine	28 days 57 days	5 mg a.s./kg soil dw 25 mg a.s./kg soil dw		van der Kolk 2002 Report 1052.011.747 (In DAR 2007)

2.9.6 Summary of effects on terrestrial non-target higher plants

The available data on toxicity to terrestrial non-target higher plants are summarised in the table below, with endpoints selected for the risk assessment marked in bold.

Table 2.9.6-1. Summary of relevant information on effects on terrestrial non-target higher plants.

Test type	Test substance	Species	Most sensitive species	Toxicity (kg a.s./ha)	Remarks	Reference
Vegetative vigour (n=6)	Quinoclamine	Monocot *Avena sativa* *Zea mays* *Allium cepa* Dicot *Trifolium pratense* *Daucus carota* *Brassica napus*	*Brassica napus* (rape)	NOEC = 0.435 **ER50 ~ 0.87**	Previously EU-agreed endpoint	Fiebig 2000 Report 990914SS (TNW71151) (In DAR 2007)
Vegetative vigour (n = 6)	Mogeton 50% WG	Monocot *Avena sativa* *Allium cepa* Dicot *Brassica napus* *Daucus carota* *Cucumis sativus* *Pisum sativum*	*Pisum sativum* (pea)	**ER50 ≥ 3.75**	Limit test; NOEC could not be determined	Friedrich 2015a Report 15 10 48 002 P
Seedling emergence (n = 6)	Mogeton 50% WG	Monocot *Avena sativa* *Allium cepa* Dicot *Brassica napus* *Daucus carota* *Cucumis sativus* *Pisum sativum*	*Allium cepa*	**ER50 ≥ 3.75**	Limit test; NOEC could not be determined	Friedrich 2015b Report 15 10 48 001 P

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No further studies on terrestrial organisms are available.

2.9.8 Summary of effects on biological methods for sewage treatment

The available data on effects on sewage treatment are summarised in the table below.

Table 2.9.8-1: Summary of relevant information on effects on biological methods for sewage treatment.

Test type/organism	Endpoint	Reference
Activated sludge	3-h EC_{50} >180 mg a.s./L	Mattock 2002; Report 619/146-D2149 (In DAR 2007)

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Summary of product exposure and risk assessment for birds and mammals

A risk assessment for birds and mammals was conducted according to the Guidance Document on Risk Assessment for Birds and Mammals (EFSA 2009/1438).

Birds

Tier 1

Based on first tier calculations, the acute risk to birds is acceptable in all uses at the lower application rates (1.875 and 1.44 kg a.s./ha). However, at the highest application rate (3.75 kg a.s./ha), unacceptable acute risks were identified both in golf greens and in plant nurseries. Moreover, unacceptable long-term risks were identified in all uses. For the uses on golf greens, concern was mainly raised for large herbivorous birds (acute and long-term risks) and for small insectivorous birds (long-term risks). For the uses in nursery stock plants, concern was raised for small insectivorous birds feeding on foliar arthropods (long-term risks), which is only relevant for the proposed use with tractor mounted downward spraying at 1.44 kg a.s./ha.

Higher tier

To address the identified risks to birds, the applicant also submitted a refined risk assessment. Based on this assessment, for the intended uses of Mogeton TOP on golf greens, the RMS proposes that acceptable risk to birds can be concluded, when taking into account all the available higher-tier information and reasoning about low attractiveness of golf greens as foraging habitats for birds.

Regarding the proposed uses in nursery stock plants, acceptable use can be concluded for hand-held application directly to the substrate surface, while concern remains for outdoor tractor mounted downward spraying of plants at 1.44 kg a.s./ha. Following this use, an unacceptable long-term risk to small insectivorous birds feeding on foliar arthropods was identified in the first-tier risk assessment. This risk was not further discussed by the applicant.

Mammals

Tier 1

Based on first tier calculations, unacceptable acute and long-term risks to mammals were identified in all proposed uses. For the uses on golf greens, concern was mainly raised for large herbivorous mammals, small herbivorous mammals (acute and long-term risks), and small insectivorous mammals (long-term risk). For the uses in nursery stock plants, concern was raised for small herbivorous mammals, small omnivorous mammals (acute and long-term risks), and small insectivorous mammals (long-term risk).

Higher tier

To address the identified risks to mammals, the applicant also submitted a refined risk assessment. Based on this assessment, for the intended uses of Mogeton TOP on golf greens, the RMS proposes that acceptable risk to mammals can be concluded, when taking into account all the available higher-tier information and reasoning about low attractiveness of golf greens as foraging habitats for mammals.

Also in nursery stock plants, low attractiveness of the treated area as foraging habitat to most mammals can be assumed based on the available data. However, the wood mouse (*Apodemus sylvaticus*) was identified as a relevant focal species, requiring a refined numerical risk assessment. As some input parameters (including the selected reproductive endpoint) proposed for this refined risk assessment may need further revisions following the peer review of this RAR, no conclusion regarding the risk to small mammals in plant nurseries can be drawn at this stage.

2.9.9.2 Summary of product exposure and risk assessment for aquatic organisms

A risk assessment for aquatic organisms was performed according to the EFSA Aquatic Guidance Document (EFSA 2013/3290).

Quinoclamine

Tier 1

A first tier risk assessment was performed considering surface water and sediment PEC simulations for the active substance based on FOCUS Steps 3-4, but with some modifications of the standard assumptions in the model which were in general accepted by the RMS (see Volume 3, Annex B.8 on the representative formulation).

Golf greens: For hand-held application on golf-greens acceptable aquatic risk was demonstrated at Tier 1 when considering a 20 meter vegetated buffer zone together with 95% spray drift reduction by nozzles. For tractor mounted application, no safe use could be concluded at Tier 1.

Tree nurseries: Acceptable risks without mitigation measures could be concluded for all proposed uses in greenhouse and walk-in tunnel. Following outdoor application in tree nurseries, acceptable risk was also

demonstrated at Tier 1 for all relevant FOCUS drainage (D) scenarios (considering a 20 meter vegetated buffer zone together with 95% spray drift reduction by nozzles), but the run-off (R) scenarios were not acceptable.

Higher tier

The applicant proposed that a few additional FOCUS scenarios could be demonstrated as acceptable, when considering the available higher tier data (geomean acute fish LC50 and chronic pulse exposure studies for fish and *Daphnia*) together with a visual graphical evaluation of PECsw peaks simulated in FOCUS Step 4 (20 meter vegetated buffer zone together with 95% spray drift reduction by nozzles). The geomean approach for the acute risk assessment was tentatively accepted by the RMS, whereas the pulse exposure approach for the long term risk assessment will need further consideration. Both pulse exposure studies as such were considered to be well performed. However, there is no clear evidence that the most sensitive life stage was exposed during the fish test. Further, it should be noted that the associated refinement based on high-resolution analysis of FOCUS surface water peaks is generally not accepted within the Northern Zone. Other MS are invited to express their views during the peer review.

Metabolites

For the relevant metabolites in surface water and sediment, acceptable surface water and sediment PEC simulations were only available based on FOCUS Steps 1-2.

The risk to surface water and sediment organisms from the following relevant photolytic degradation products were assessed at Tier 1: M6 (Phthalic acid), M9 (Phthalamic acid), M10 (2-Oxalyl-benzoic acid), M11 (2-Amino-oxalyl-benzoic acid), 2-Carboxy-benzaldehyde and 'Unknown 2'. None of these degradation products are considered to retain the toxophore of Quinoclamine. Therefore, when toxicity data were lacking the metabolites were assumed to be equally toxic as Quinoclamine. Based on FOCUS Step 2, acceptable risk from the metabolites M9, M10 and M11 could be concluded for all uses (except for metabolite M11 in plant nurseries in southern Europe at the highest application rate of 3.75 kg a.s/ha). ***On the other hand, the metabolites M6, 2-Carboxybenzaldehyde and 'Unknown 2' failed the risk assessment based on FOCUS Step 2 for all proposed representative uses, which needs to be further addressed.***

In addition a risk assessment was performed for sediment dwelling organisms exposed to AN (2-amino-1,4-naphthoquinone), a degradation product of Quinoclamine which is relevant only in sediment. ***Also from this metabolite unacceptable risks were identified for most uses, based on FOCUS Step 2. This needs to be further addressed.***

2.9.9.3 Summary of product exposure and risk assessment for arthropods

Bees

A risk assessment for bees was conducted both according to the EFSA Bee Guidance Document (EFSA Journal 2013;11(7):3295) and the EPPO (2010) scheme (OEPP/EPPO Bulletin 40: 323-331, Chapter 10.

Acceptable acute risks to adult bees could be concluded at the initial screening step, both via contact and oral exposure (EFSA 2013; EPPO 2010). Moreover, a low risk for effects on hypopharyngeal glands of honeybees was demonstrated (EFSA 2013). The risk to honeybees via water consumption was also assessed as acceptable according to EFSA (2013).

Unacceptable chronic risks to adult honeybees and larvae was identified at the initial screening step and these risks were further investigated at Tier 1 according to EFSA (2013). It was concluded that the chronic risk to adult honeybees and larvae exposed to the treated crop was still unacceptable, whereas other routes of exposure (weeds in the treated field, field margin, adjacent crop and following year on a permanent crop or on a succeeding crop for annual crops) did not cause unacceptable risk to honeybees.

It should be noted that both tree nurseries and golf greens are probably less attractive to bees. Golf greens are meticulously cultivated areas (year-round) where presence of flowering weeds at any time is highly unlikely. With regard to outdoor tree nurseries, GAP treatment is restricted to wooden plants, of which very few are likely to be flowering during application. Furthermore, treatment of flowering crop is already excluded in the proposed GAP. Finally, with the exception of tractor-drawn application (1.44 kg ai/ha), contamination of the flower blossom is highly unlikely due to targeted application to the substrate in the planting pot, with weed growth prevented by the soil covering.

Nevertheless, the applicant proposed the following **label restrictions** in order to address the identified risk to foraging honeybees (chronic adult and larvae) within the treated field:

Lawns, nursery potted plants: *Dangerous to bees. To protect bees and other pollinating insects do not apply on flowering crops. Do not use where bees are actively foraging.*

Other arthropods

Tier 1

A risk assessment was performed according to the Guidance document on terrestrial ecotoxicology (SANCO/10329/2002 rev 2 final, 17 October 2002) and the Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi et al. 2000).

The available standard laboratory study results as well as the Tier 1 exposure calculations indicated that no unacceptable effects are to be expected from the application of Quinoclamine according to GAP. However, since results from extended laboratory tests were also available, a higher tier risk assessment is presented below.

Higher tier

Based on the available extended laboratory data for the predatory mite *Typhlodromus pyri* a risk was indicated for non-target arthropods within the treated field, since the calculated in-field exposure exceeded the laboratory ER50 for reproduction. However, in an aged residue study with *Typhlodromus pyri*, an initially reduced reproduction until day 14 was followed by a recovered reproduction within the next 14 days when continuously exposed to the aged residues on the plants. After the 28 days test period no significant effects on reproduction were observed.

Based on the assessment above, it was concluded that acceptable risks to non-target arthropods following the proposed uses of Quinoclamine can be expected.

2.9.9.4 Summary of product exposure and risk assessment for non-target soil meso- andmacrofauna

A risk assessment for soil-dwelling organisms was performed according to the Guidance document on terrestrial ecotoxicology (SANCO/10329/2002 rev 2 final, 17 October 2002). Where no toxicity data were available, relevant degradation products were assumed to be 10 times more toxic than the active substance. At a late stage of the evaluation, the applicant proposed that toxicity of the metabolites should be estimated based the approach given in the more recent guidance document for aquatic risk assessment (EFSA 2013). The RMS considers this approach as reasonable, at least as a weight of evidence, but did not revise the risk assessment at this stage. Opinions from other MS would be welcome during the peer review.

Tier 1

For the proposed uses of Quinoclamine on golf greens, acceptable risk to earthworms and other macro organisms could be concluded based on Tier 1 laboratory data and also based on additional higher tier field data (grassland).

Based on a Tier 1 risk assessment for nursery stock plants, an unacceptable risk was indicated at the higher application rates for earthworms and springtails (*Folsomia candida*) but not for predatory mites (*Hypoaspis aculeifer*). Acceptable risks to all non-target soil meso- and macrofauna in nursery stock plants could be concluded at Tier 1 following the lowest proposed application rate of 0.81 kg a.s./ha.

Higher tier

For a higher tier assessment in nursery stock plants, results from two available field studies with earthworms and other macro organisms were available. The representativeness of these studies for the uses in nursery stock plants has been discussed in Volume 3, Annex B.9 on the representative formulation, section B.9.8.2, but may need to be further discussed considering that both studies were conducted on grassland and that the earthworm field study had a less optimal application timing and that also no analytical verification of the test item concentrations in the soil was performed in this study If the available higher tier data are considered, though, an acceptable risk to non-target soil meso- and macrofauna following all proposed representative uses of Quinoclamine in nursery stock plants can be concluded.

2.9.9.5 Summary of product exposure and risk assessment for soil nitrogen transformation

The maximum concentrations with no effect (less than 25% on soil nitrogen transformation within maximum 100 days) were compared to the maximum calculated concentration in soil considering the proposed application rates on golf greens and in nursery stock plants respectively. Where no toxicity data were available, relevant degradation products were assumed to be 10 times more toxic than the active substance.

A low risk for effects on soil nitrogen transformation following the proposed uses of Quinoclamine was concluded.

2.9.9.6 Summary of product exposure and risk assessment for terrestrial non-target higher plants

A risk assessment for terrestrial non-target higher plants was performed according to the Guidance document on terrestrial ecotoxicology (SANCO/10329/2002 rev 2 final, 17 October 2002).

The results demonstrated that exposure to non-target higher plants is acceptable without drift reduction technique even at the standard buffer distance of 1 m, when the proposed representative use pattern for Quinoclamine (formulated as Mogeton TOP, i.e. Mogeton 50% WG) is followed.

2.9.9.7 Summary of product exposure and risk assessment for biological methods for sewage treatment

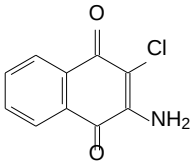
Although effects of 11 – 17% compared to the control were seen at the lowest concentration (10 mg/L) in the available study, it is not considered likely that the recommended use of Quinoclamine will result in contamination of sewage treatment plants at concentration levels that would cause severe effects. Therefore, the risk for harmful effects on biological methods of sewage treatment is considered to be acceptable.

2.10 Proposed harmonised classification and labelling according to the CLP criteria

2.10.1 Identity of the substance

2.10.1.1 Name and other identifiers of the substance

Table 2.10.1.1-1. Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	**2-amino-3-chloro-1,4-naphthoquinone** **2-amino-3-chloro-1,4-naphthalenedione**
Other names (usual name, trade name, abbreviation)	Quinoclamine, ACN, ACNQ, K-1616, Mogeton
ISO common name (if available and appropriate)	Quinoclamine
EC number (if available and appropriate)	220-529-2
EC name (if available and appropriate)	2-amino-3-chloro-1,4-naphthoquinone
CAS number (if available)	2797-51-5
Other identity code (if available)	CIPAC no 648
Molecular formula	$C_{10}H_6ClNO_2$
Structural formula	
SMILES notation (if available)	O=C2c1ccccc1C(=O)C(Cl)=C2N
Molecular weight or molecular weight range	207.6
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity 96.5 %

2.10.1.2 Composition of the substance

Table 2.10.1.2-1. Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
Quinoclamine CAS No. 2797-51-5 EC-No. 220-529-2	Min. 96.5%	-	There are five aggregated notifications with a total of 75 notifiers. The main notification comprising 46 notifiers has the following proposal: Acute Tox. 4 Skin Sens. 1 Eye Irrit. 2 Acute Tox. 3 Repr. 2 STOT SE 2 Aquatic Acute 1

			Aquatic Chronic 1 The other minor notifications have in most cases less severe classification proposals. However, STOT RE 2 is also added in two instances (a total of 5 notifiers).

Table 2.10.1.2-2. Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Dichlone CAS-No. 117-80-6 EC-No. 204-210-5	Max 1.5 %	Acute Tox. 4 H302 Skin. Irrit. 2 H315 Eye Irrit. 2 H319 Aquatic Acute 1 H400 Aquatic Chronic 1 H410	There are eight aggregated notifications with a total of 191 notifiers. The main notification (66 notifiers) has the same proposal as the harmonised classification except for the addition of Skin Sens. 1 (H317). The other minor notifications has less sever classification proposal except in one instance where Skin Sens. 2 is also added (2 notifers).	No

Table 2.10.1.2-3. Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Quinoclamine contains no additives	-	-	-	-	-

Table 2.10.1.2-4. Test substances (non-confidential information)

Study title	Batch No	Purity
(14C)-Quinoclamine: Absorption, distribution, metabolism and excretion in the rat. Covance Lab. Ltd., North Yorkshire, England Report No. 619/102-D1145 Report date: 2002-12-23 GLP, not published	8086	99%
Quinoclamine: Acute oral toxicity study in the rat Covance Lab. Ltd., North Yorkshire, England Report No. 619/141-D6144 2002-02-28 GLP, unpublished	8086	99%

Study title	Batch No	Purity
Acute oral toxicity of Quinoclamine in rats Public Interest Incorporated Foundation Biosafety Research Center (BSRC) 582-2, Shioshinden, Iwata, Shizuoka437-1213, Japan Report No. G427 /154-768 Report date: 2016-01-18 GLP, unpubulished	3061	98.3%
Quinoclamine: Acute dermal toxicity study in the rat Covance Lab. Ltd., North Yorkshire, England Report No. 619/143-D6144 Report date: 2002-01-28 GLP, unpublished	8086	99%
Acute inhalation toxicity in rats – 4 Hour exposure Huntingdon Research, Cambridgeshire, England Report No. TYC 3786432 Report date: 1986-08-13 GLP, unpublished	5063	98.1%
Primary skin irritation study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. 105/8509 Report date: 1985-11-22 GLP, unpublished	5063	98.1%
Primary eye irritation study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. 106/8509 Report date: 1985-11-27 GLP, unpublished	5063	98.1%
Quinoclamine: Skin sensitisation study in the guinea pig Covance Lab. Ltd., North Yorkshire, England Report No. 619/119-D6144 Report date: 2000-12-22 Non-GLP, unpublished	8086	99%
Evaluation of in vitro phototoxicity of Quinoclamine technical in 3T3 fibroblasts using the Neutral Red uptake assay WIL Research Europe, The Netherlands Report No. 508771 Report date: 2015-11-17 GLP, unpublished	3061	98.3
Quinoclamine: 28 day oral (dietary administration) toxicity study in the rat. Covance Lab. Ltd., North Yorkshire, England Report No. 619/148 Report date: 2002-09-06 GLP, unpublished	8086	99%
Quinoclamine: 28 day oral (capsule administration) toxicity study in the dog Covance Lab. Ltd., North Yorkshire, England Report No. 619/149 Report date: 2002-07-04 GLP, unpublished	8086	99%
90 Days subacute toxicological test of Mogeton on rat Tokyo Women's Medical College, Department of Hygiene II Report No. – Report date: – Non-GLP, unpublished	No data	No data
Quinoclamine: 13 week oral (dietary administration) toxicity study in the rat Covance Lab. Ltd., North Yorkshire, England Report No. 0619/132 Report date: 2003-03-28 GLP, unpublished	8086	99%
Quinoclamine: 13 week oral (capsule administration) toxicity study in the dog Covance Lab. Ltd., North Yorkshire, England Report No. 0619/134 Report date: 2002-11-12 GLP, unpublished	8086	99%

Study title	Batch No	Purity
Quinoclamine: 28 day (dermal administration) toxicity study in the rat Covance Lab. Ltd., North Yorkshire, England Report No. 619/133 Report date: 2002-09-30 GLP, unpublished	8086	99%
Quinoclamine: Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and one Tryptophan-requiring strain of Escherichia coli. Covance Laboratories Ltd., North Yorkshire, UK Report No. 619/103-D6171 Report date: 2002-01-29 GLP, unpublished	8086	99%
Metaphase analysis of human lymphocytes treated with ACN technical Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. 238/8702 Report date: 1987-09-29 GLP, unpublished	5063	98.1%
Tests for gene mutations resistant to Quabain in L5178Y mouse lymphoma cells treated with ACN technical Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. 239/8702/A Report date: 1989-08-01 No GLP, unpublished	5063	98.1%
Mouse micronucleus test on ACN technical Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. M/MMN/1582 Report date: 1987-09-01 No GLP, unpublished	5063	98.1%
Quinoclamine: Measurement of unscheduled DNA Synthesis in rat liver using an in vivo/in vitro procedure Corning Hazleton (Europe), North Yorkshire HG3 1PY, England Report No. 619/5-1052 Report date: 1996-11-01 GLP, unpublished	5081	97.6%
ACN-technical 104 week (dietary) combined chronic toxicity and carcinogenicity study in the rat Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/7/90 Report date: 1991-08-30 No GLP, not published	2010	98.3%
ACN Technical, 80-week (dietary) carcinogenicity study in the mouse Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/56/93 1993-11-17 GLP, not published	8055	98.5%
Two year dietary toxicity study in dogs Hazleton Laboratories America Inc, Virginia 22180, USA Report No. 854-110 1976-02-18 No GLP, unpublished	K-1616	98.5%
A two generation reproduction study in rats Hazleton Laboratories America Inc, Virginia 22180, USA Report No. 854-111 Report date: 1975-05-30 No GLP, not published	K-1616	No data
Rat Teratology Range Finding Study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/2/86 Report date: 1986-06-01 No GLP, not published	5063	98.1%

Study title	Batch No	Purity
ACN (Technical), Rat Teratology Study Toxicol Laboratories Ltd, Ledbury, Herefordshire HR8 1LH, England Report No. AKJ/4/86 Report date: 1986-10-01 No GLP, not published	5063	98.1%
Quinoclamine: Oral (Gavage) Range-finding study of prenatal toxicity in the rat Covance Laboratories Ltd., North Yorkshire, UK Report No. 619/123-D6154 Report date: 2002-08-19 GLP, not published	8086	99%
Qunoclamine: Oral (Gavage) prenatal developmental toxicity study in the rat Covance Laboratories Ltd., Harrogate, UK Report No. 619/94-D6154 Report date: 2002-08-19 GLP, not published	8086	99%
ACN (Technical), Rabbit Teratology range finding study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/1/86 Report date: 1986-06-01 No GLP, not published	5063	98.1%
Addendum to rabbit teratology range finding study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/1A/89 Report date: 1989-07-28 No GLP, not published	5063	98.1%
ACN (Technical), Rabbit Teratology Study Toxicol Laboratories Ltd, Ledbury, Herefordshire, HR8 1LH, England Report No. AKJ/3/86 Report date: 1986-10-01 No GLP, not published	5063	98.1%
Quinoclamine: Oral (Gavage) Range-finding study of prenatal toxicity in the rabbit Covance Laboratories Ltd., North Yorkshire, UK Report No. 619/122-D6154 Report date: 2002-08-19 GLP, not published	8086	99%
Quinoclamine: Oral (Gavage) prenatal developmental toxicity study in the rabbit Covance Laboratories Ltd., North Yorkshire, UK Report No. 619/155-D6154 2002-08-19 GLP, not published	8086	99%
Quinoclamine: Dermal embryo-foetal development study Corning Hazleton, D-48163 Münster, Germany Report No. 1312-1416-001 Report date: 1996-11-08 GLP, not published	4036	97.7%
Avian single-dose oral LD50 of Mogeton in bobwhite quail Hazleton Laboratories America Report No. 6028-602 Report date: 1986-12-03 GLP, unpublished	5063	98.1%
5-day dietary toxicity study in bobwhite quail with Quinoclamine technical. + Amendments 1+2 Notox Safety & Environmental Research, The Netherlands Report No. 318869 Report date: 2001-12-14 GLP, unpublished	8086	99.0%
Avian Dietary Toxicity Test of Quinoclamine Technical on Mallard Duck (Anas platyrhynchos). Lab International Research Center Hungary Report No. 05/896-113TÖ Report date: 2005-08-24 GLP, unpublished	1096	99.8%

Study title	Batch No	Purity
Reproduction study in bobwhite quail with Quinoclamine technical (by dietary admixture). Notox Safety & Environmental Research, The Netherlands Report No. 318915 Report date: 2002-09-20 GLP, not published	8086	99%
Acute toxicity fish test (OECD 203) Quinoclamine, Salmo gairdneri. + Lenz (1991) Determination of Quinoclamine in combination with the acute toxicity fish test Salmo gairdneri. Biochem GmbH. Report No. 912043117 Report date: 1991-10-31 GLP, unpublished	1060	98.5%
Acute toxicity fish test (OECD 203) Quinoclamine, Brachydanio rerio. + Lenz (1991) Determination of Quinoclamine in combination with the acute toxicity fish test Brachydanio rerio. Biochem GmbH. Report No. 912043562 Report date: 1991-12-17 GLP, unpublished	1060	98.5%
Rainbow trout (Oncorhynchus mykiss), early life stage toxicity test, flow through conditions, test item Quinoclamine Fraunhofer Institute IME, Schmallenberg, Germany Report No. AGK-001/4-43/E Report date: 2015-07-08 GLP, unpublished Document also used as KCA 4.1.2/14	3061	98.3%
Prolonged toxicity fish test (OECD Guideline 204) 21-day study Quinoclamine, Salmo gairdneri + Lenz (1991) Determination of Quinoclamine in combination with the prolonged toxicity fish test Salmo gairdneri. Biochem GmbH. Report No. 912043117B Report date: 1991-11-19 GLP, unpublished	1060	98.5%
Rainbow Trout (Oncorhynchus mykiss), Early Life Stage Toxicity Test, Flow through conditions	3061	98.3%
Acute toxicity in Daphnia magna, test article: Quinoclamin IBR Forschungs GmbH Report No. 80-91-1397-10-91 Report date: 1991-12-10 GLP, unpublished	1060	98.5%
21 d Reproduction test in Daphnia magna IBR Forschungs GmbH, Walsrode, Germany Report No. 83-00-0992/00-94 Report date: 1994-08-25 GLP, not published	1060	98.5%
Effects on larvae of Chironomus riparius in a water-sediment system according to OECD Guideline (Draft 1998) and BBA Guideline (Proposal 1995) Quinoclamin (tech.) BioChem agrar, Cunnersdorf, Germany Report No. 99 10 48 113 Report date: 2000-11-14 GLP, not published	8033	98.1%
Analytical Part: Effects on larvae of Chironomus riparius in a water-sediment system according to OECD Guideline (Draft 1998) and BBA Guideline (Proposal 1995) Quinoclamin (tech.) Dr. Specht & Partner, Hamburg, Germany Report No. 99 10 48 113 – BIO-0003 Report date: 2000-06-22 GLP, not published	P-18	99.9%

Study title	Batch No	Purity
Algae growth inhibition test – test article: Quinoclamin IBR, Walsrode, Germany Report No. 80-91-0045/05-93 Report date: 1994-02-28 GLP, not published	01/1090	No data
Algae (Navicula pelliculosa) growth inhibition test following OECD 201 (1984) and US EPA OPPTS 850.5400 'Algal toxicity, Tiers I and II' (Public Draft 1996). Quinoclamin (tech.) BioChem agrar, Cunnersdorf, Germany Report No. 99 10 48 121 Report date: 2000-11-15 GLP, not published	8033	98.1%
Analytical part: Algae (Navicula pelliculosa) growth inhibition test following OECD 201 (1984) and US EPA OPPTS 850.5400 'Algal toxicity, Tiers I and II' (Public Draft 1996). Quinoclamin (tech.) Specht & Partner, Cunnersdorf, Germany Report No. BIO-0008 Report date: 2000-10-31 GLP, not published	P-18	99.9%
Effects of Quinoclamine technical on Myriophyllum spicatum in a growth inhibition test under semi-static test conditions BioChem agrar, Gerichshain, Germany Report No. 14 10 48 008 W Report date: 2015-02-17 GLP, not published Document also used as KCA 4.1.2/16	3061	98.3%
Lemna minor growth inhibition test following OECD ringtest guideline (version 1997) and US EPA OPPTS 850.4400 'Aquatic plant toxicity test using Lemna spp., Tiers I and II' (Public Draft 1996). Quinoclamin (tech.). BioChem agrar, Germany Report No. 99 10 48 122 Report date: 2000-11-14 GLP, not published	8033	98.10%
Analytical part: Lemna minor growth inhibition test following OECD ringtest guideline (version 1997) and US EPA OPPTS 850.4400 'Aquatic plant toxicity test using Lemna spp., Tiers I and II' (Public Draft 1996). Quinoclamin (tech.). Specht & Partpner, Hamburg, Germany Report No. BIO-005 Report date: 2000-06-13 GLP, not published	P-18	99.90%
Quinoclamine technical: Sublethal toxicity of Quinoclamine technical to the earthworm Eisenia fetida in artificial soil with 5% peat BioChem agrar, Gerichshain, Germany Report No. 09 10 48 067S Report date: 2009-10-19 GLP, not published	7064	99.28%
Quinoclamine: Determination of effects on soil microflora activity Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland Report No. 1052.011.747 Report date: 2002-10-29 GLP, not published	8086	99.0%
Quinoclamine techn. Terrestrial plants toxicity, Vegetative vigor, Tier II Dr. U. Noack – Laboratorium für Angewandte Biologie, Sarstedt, Germany Report No. 990914SS (TNW71151) Report date: 2000-10-31 GLP, not published	8033	98.10%
Quinoclamine: Determination of inhibition of respiration of activated sludge Covance Laboratories Ltd., North Yorkshire, UK Report No. 619/146-D2149 Report date: 2002-10-02 GLP, not published	8086	99.0%

2.10.2 Proposed harmonized classification and labelling

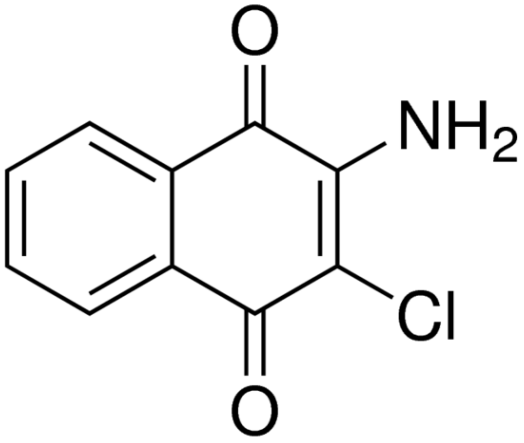
2.10.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	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, kidneys) H400 H410	GHS07 GHS08 GHS09 Wng	H351 H361d H302 H319 H317 H373 (blood system, kidneys) H410		oral: ATE = 500 mg/kg bw M=10 M=10	

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.

2.10.2.2 Additional hazard statements / labelling

Table 2.10.2.2-1. Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Conclusive but not sufficient for classification	Yes
Self-reactive substances	Data lacking	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data (experience in handling) is conclusive but not sufficient for classification.	Yes

Self-heating substances	Data lacking	No
Substances which in contact with water emit flammable gases	Data (experience in handling) is conclusive but not sufficient for classification.	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data lacking	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not applicable	No
Acute toxicity via oral route	Harmonised classification proposed	Yes
Acute toxicity via dermal route	Conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data inconclusive	Yes
Skin corrosion/irritation	Conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Harmonised classification proposed	Yes
Germ cell mutagenicity	Data inconclusive	Yes
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Harmonised classification proposed	Yes
Specific target organ toxicity-single exposure	Conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Data lacking	No

2.10.3 History of the previous classification and labelling

Quinoclamine is not yet subject to harmonised classification.

2.10.4 Identified uses

Quinoclamine is an active substance used in plant protection products which is currently re-evaluated under Regulation 1107/2009. It is used as an herbicide/algaecide for post-emergence control of common mosses and algae occurring on golf greens and of liverwort occurring on the substrate of nursery stock plants.

2.10.5 Data sources

Quinoclamine was included in Annex I of EU Council Directive 91/414/EEC on 1st January 2009 (Commission Directive 2008/66/EC of 30 June 2008), and was subsequently approved under Regulation (EC) No. 1107/2009 (repealing Council Directive 91/414/EEC) via Commission Implementing Regulation (EU) No. 540/2011 of 25th May 2011.

Quinoclamine is currently being re-evaluated under the following regulations for renewal of approval as an active substance in plant protection products:

- REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
- COMMISSION IMPLEMENTING REGULATION (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

The data presented in this dossier have been submitted by one applicant (Agro-Kanesho Co. Ltd.) as part of the renewal process. Some of the data were submitted and evaluated during the first approval while other data was submitted for the first time for the purpose of renewal of approval.

2.11 Relevance of metabolites in groundwater

2.11.1 STEP 1: Exclusion of degradation products of no concern

No metabolites were excluded for this reason.

2.11.2 STEP 2: Quantification of potential groundwater contamination

PECgw for the representative uses on golf greens and nurseries were available for quinoclamine and products observed in study on photochemical transformation in soil (Adam, 2016a): M6, M9, M10 and M11.

PECgw for the parent compound and the transformation products were ≤ 0.000 for all scenarios.

2.11.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.11.3.1 STEP 3, Stage 1: screening for biological activity

Not required.

2.11.3.2 STEP 3, Stage 2: screening for genotoxicity

Not required.

2.11.3.3 STEP 3, Stage 3: screening for toxicity

Not required.

2.11.4 STEP 4: Exposure assessment – threshold of concern approach

Not required.

2.11.5 STEP 5: Refined risk assessment

Not required.

2.11.6 Overall conclusion

Due to the very low PECgw an assessment of the relevance of the transformation products M6, M9, M10 and M11 was not necessary.

2.12 Consideration of isomeric composition in the risk assessment

Not relevant as Quinoclamine does not consist of any stereoisomers.

2.12.1 Identity and physical chemical properties

Not relevant, see 2.12.

2.12.2 Methods of analysis

Not relevant, see 2.12.

2.12.3 Mammalian toxicity

Not relevant, see 2.12.

2.12.4 Operator, Worker, Bystander and Resident exposure

Not relevant, see 2.12.

2.12.5 Residues and Consumer risk assessment

Not relevant, see 2.12.

2.12.6 Environmental fate

Not relevant, see 2.12.

2.12.7 Ecotoxicology

Not relevant, see 2.12.

2.13 Residue definition

2.13.1 Definition of residues for exposure/risk assessment

Food of plant origin: Not relevant for the representative use.

Food of animal origin: Not relevant for the representative use.

Soil: Quinoclamine, M6, M9, M10 and M11.

Groundwater: Quinoclamine, M6, M9, M10 and M11.

Surface water: Quinoclamine, M6, M9, M10, M11, 2-carboxybenzaldehyde and Unknown 2.

Sediment: Quinoclamine, M6, M9, M10, M11 and metabolite AN.

Air: Quinoclamine

2.13.2 Definition of residues for monitoring

Food of plant origin: Not relevant for the representative use.

Food of animal origin: Not relevant for the representative use.

Soil: Quinoclamine

Groundwater: Quinoclamine

Surface water: Quinoclamine

Sediment: Quinoclamine

Air: Quinoclamine

2.14 Effect of water treatment processes on the nature of residues present in surface water

During check of completeness of the dossier the RMS asked the applicant to "address the effect of water treatment processes on the nature of residues present in surface water when surface water is abstracted for drinking water. Probably in the first instance, a consideration of the processes of ozonation and chlorination would appear appropriate. If an argumentation is made that concentrations at the point of abstraction for drinking water purposes will be low, this argumentation should cover metabolites predicted to be in surface water, as well as the active substance. Should this consideration indicate that novel compounds might be expected to be formed from water treatment, then the risk to human or animal health through the consumption of drinking water containing them should be addressed."

In response, the applicant provided data to show that concentrations of quinoclamine and its metabolites and transformation products will be low at the point of abstraction for drinking water. Available PECsw at which no risk to aquatic organisms was identified were used as a starting point. Corresponding PECgw were calculated using the software Exposit from the German UBA. The model assumes dilution of PECsw in running water and then calculation of PECgw in a bank infiltration step. Maximum PECgw at the drinking water abstraction sites were calculated to ≤ 0.0028 µg/L. The RMS is unfamiliar with the model EXPOSIT ver 3.01 but according to information at the BVL web-site the model is used for national authorisation in Germany to estimate exposure in groundwater and surface water. It is not an agreed model at the EU level.

The applicant apparently misunderstood the request. There was no attempt to consider the potential effect of water treatment on the nature of possible residues in water, such as the potential for formation of genotoxic compounds. Hence, the submitted study does not address the concern mentioned in Regulation (EC) 1107/2009 art. 4.3 b.
A data gap is therefore identified, but in the absence of guidance at the EU level it may be less useful to request further data.

Level 3

3 Proposed decision with respect to the application

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

APPENDICES

Appendix 1 Guidance documents used in this assessment

Candolfi M.P., Barrett K.L., Campbell P.J., Forster R., Grandy N., Huet M-C., Lewis G., Oomen P. A., Schmuck R. and Vogt H., 2000. Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop, Wageningen, 21-23 March 2000.

ECHA (European Chemicals Agency), 2017. Guidance on the Application of the CLP Criteria; Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0, July 2017.

EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals. EFSA Journal 2009; 7(12):1438.

EFSA (European Food Safety Authority), 2012. Guidance Document on Dermal Absorption. EFSA Journal 2012; 10(4):2665.

EFSA (European Food Safety Authority), 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera, Bombus spp*. and solitary bees). EFSA Journal 2013;11(7):3295.

EFSA (European Food Safety Authority), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290.

EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014, 12(10):3874.

EPPO/OEPP, 2010. Environmental risk assessment scheme for plant protection products; Chapter 10: Honeybees (PP 3/10(3)). Bulletin OEPP/EPPO Bulletin 40: 323-331.

European Commission, 2000. Residues: Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414. SANCO/3029/99 - rev. 4, 11 July 2000.

European Commission, 2001. Guidance for the setting of an acute reference dose (ARfD). 7199/VI/99 - rev 5, 5 July 2001.

European Commission, 2002. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 - rev.2 final, 17 October 2002.

European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000 - rev. 10, 25 February 2003.

European Commission, 2006. Draft Guidance for the setting and application of acceptable operator exposure levels (AOELs) SANCO/7531 - rev.10, 7 July 2006.

European Commission, 2009. Guidance Document on the Assessment of the Equivalence of Technical Materials of Substances Regulated under Regulation (EC) No 1107/2009. SANCO/10597/2003 - rev. 10.1, 13 July 2012.

European Commission, 2010. Guidance document on residue analytical methods. SANCO/825/00 - rev. 8.1, 16 November 2010.

FOCUS, 1997. Soil persistence models and EU registration. SANCO/7617/VI/96.

FOCUS, 2000. FOCUS Groundwater Scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000-rev.2. 202 pp, as updated by the Generic Guidance for FOCUS groundwater scenarios, version 1.1 dated April 2002.

FOCUS, 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp., as updated by the Generic Guidance for FOCUS surface water scenarios, version 1.1 dated March 2012.

FOCUS, 2006. Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp, as updated by the Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1, 2014.

FOCUS, 2007. Landscape and Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations. Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2005 v2.0. 169 pp.

FOCUS, 2014. Generic guidance for Tier 1 FOCUS ground water assessments, version 2.2. FOCUS groundwater scenarios working group

FOCUS, 2014. Generic guidance for FOCUS Surface water scenarios. Version 1.3.

Appendix 2 Reference list