CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Medetomidine

EC Number: **Not available**

CAS Number: **86347-14-0**

Index Number: **Not available**

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Version number: **1** **Date:** **October 2014**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**Medetomidine**
EC number:	**Not available**
CAS number:	**86347-14-0**
Annex VI Index number:	**Not available**
Degree of purity:	**99.5 % w/w**
Impurities:	**No impurities of relevance to the CLH proposal**

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None available
Current proposal for consideration by RAC	Acute Tox 2; H300 – Fatal if swallowed Acute Tox 2; H330 – Fatal if inhaled STOT SE 3; H336 – May cause drowsiness or dizziness Aquatic Acute 1: H400 – Very toxic to aquatic life (M = 1) Aquatic Chronic 1: H410 – Very toxic to aquatic life with long lasting effects (M= 100)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 2; H300 – Fatal if swallowed Acute Tox 2; H330 – Fatal if inhaled

	STOT SE 3; H336 – May cause drowsiness or dizziness Aquatic Acute 1: H400 – Very toxic to aquatic life (M = 1) Aquatic Chronic 1: H410 – Very toxic to aquatic life with long lasting effects (M= 100)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

2.15.	Organic peroxides	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox 2; H300 – Fatal if swallowed	None	None	
	Acute toxicity - dermal	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Acute Tox 2; H330 – Fatal if inhaled	None	None	
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not classified	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	Not classified	Inconclusive
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Data lacking
3.7.	Reproductive toxicity	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	STOT SE 3; H336 – May cause drowsiness or dizziness	None	None	
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 – Very toxic to aquatic life Aquatic Chronic 1; H410 – Very	Acute M = 1 Chronic M = 100	Not classified	

		toxic to aquatic life with long lasting effects			
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not classified	conclusive but not sufficient for classification

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

Labelling:

Pictogram(s): GHS06, GHS09

Signal word: DANGER
Hazard statements: H300 + H330 – Fatal if swallowed or inhaled
H336 – May cause drowsiness and dizziness
H410 – Very toxic to aquatic life with long lasting effects

Precautionary statements: P statements are not included in Annex VI

Proposed notes assigned to an entry:

None

Table 4: Classification and labelling in accordance with DSD

No longer used

BACKGROUND TO THE CLH PROPOSAL

1.4 History of the previous classification and labelling

There is currently no harmonised classification for the active substance medetomidine in Annex VI of CLP.

At the time of submission, there are no REACH registrations for the substance.

Medetomidine is under review as a biocidal active substance in the scope of Biocidal Products Regulation (EC 528/2012), with the UK as the Rapporteur Member State.

1.5 Short summary of the scientific justification for the CLH proposal

The substance is manufactured as a racemic mixture of two stereoisomers. The active isomer is dexmedetomidine whereas the other isomer, levomedetomidine, is non effective.

Dexmedetomidine is a highly selective α_2 adrenoceptor agonist on presynaptic neurons. The stimulation of these receptors leads to a decrease in norepinephrine release from presynaptic neurons with inhibition of postsynaptic activation, which attenuates CNS (Central Nervous System) excitation, especially in the locus coeruleus of the brain. A similar mode of action (activation of specific neuro-receptors in shell-building organisms leading to an anti-settling effect) is the basis of its biocidal activity as an antifouling agent.

Medetomidine has been found to be acutely toxic via the oral and inhalation routes. Further, given the effects on the CNS, classification with STOT-SE 3; H336 is considered appropriate.

For the environment, available data support classification with Aquatic Acute Category 1, with algae being the most sensitive trophic group. Classification with Aquatic Chronic 1 is also appropriate due to the long term NOEC in fish (based on dry weight and pigmentation). M-factors have been proposed as appropriate

1.6 Current harmonised classification and labelling

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

None currently listed.

1.7 Current self-classification and labelling

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

The current self-classification proposed by the applicant in the biocide application is as follows

SIGNAL WORD:	DANGER
Classification:	Acute Tox 3 Aquatic Chronic 2

H-Statements:	H331 (Toxic if inhaled), H311 (Toxic in contact with skin), H301 (Toxic if swallowed), H411 (Toxic to aquatic life with long lasting effects).

There is currently no entry on the classification and labelling inventory for this substance.

2 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Medetomidine is an active substance within the scope of the Biocidal Products Regulation (Regulation 528/2012). As such, it is subject to harmonised classification and labelling in line with Article 36(2) of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

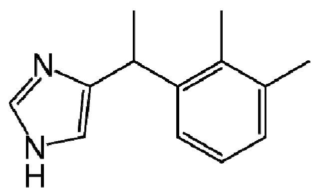
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	Not listed
EC name:	Not listed
CAS number (EC inventory):	86347-14-0
CAS number:	86347-14-0
CAS name:	1H-Imidazole, 5-[1-(2,3-dimethylphenyl)ethyl]-
IUPAC name:	*(RS)*-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole
CLP Annex VI Index number:	Not listed
Molecular formula:	$C_{13}H_{16}N_2$
Molecular weight range:	200.28 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Medetomidine	≥ 99.8%	≥ 99.5% and ≤ 100%	

The substance is manufactured as a racemic mixture of two stereoisomers. The active isomer is dexmedetomidine whereas the other isomer, levomedetomidine, is non effective.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Refer to technical dossier			

No related impurities are present in the active substance at a concentration of 0.1 % or more. Therefore, any impurities are not considered to be of relevance to the classification and labelling proposal.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The substance is manufactured and marketed as a racemic mixture of two stereoisomers. The active isomer is dexmedetomidine whereas the other isomer, levomedetomidine, is non effective.

The toxicology data submitted by the applicant include mainly studies conducted with the racemate. However, certain studies have been conducted on the individual isomers only and these are outlined in the report. There is evidence to indicate that the isomers have different toxicological profiles and that the levo isomer is toxicologically inactive at dose levels at which dexmedetomidine produces toxicologically relevant effects. This is supported by the results of repeated dose toxicity studies conducted with levomedetomidine in rats and dogs (see section 3.5.1.2).

The majority of the toxicological studies submitted were performed with the racemic base. However, there are also a significant number of studies conducted with the racemic HCl salt. From a toxicological point of view, the salt and base forms can be regarded as equivalent. This is because the HCl salt form dissociates in the aqueous environment of the body to release the base. It is noted that at a physiological pH of 7.4, the equilibrium will favour the base form to some extent (see figure 1 below).

It has been confirmed that the medetomidine used in the environmental fate and ecotoxicological tests was manufactured in the same way as the proposed method for the production of commercial medetomidine. Therefore, the racemic form of medetomidine in these studies will be the same as that for commercial production and no further consideration of isomeric issues is required.

As noted above the form of medetomidine present is dependent on the pH. The percentage of medetomidine in its free base form as a function of pH is shown in the following figure.

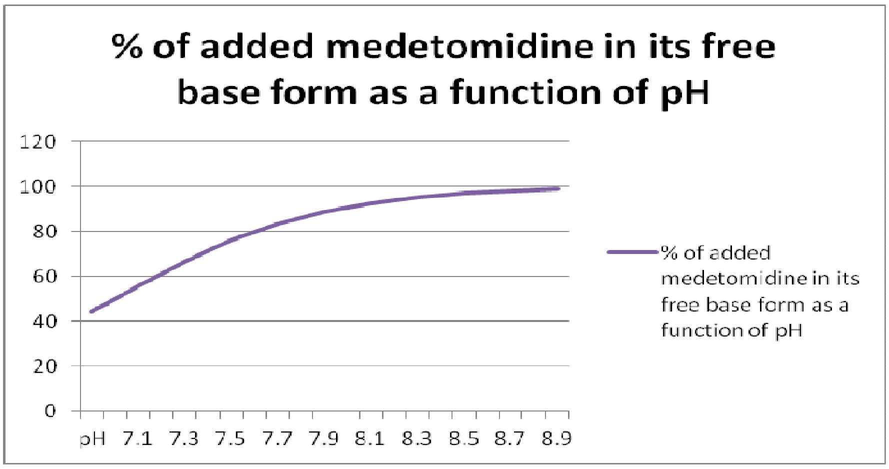


Figure 1 percentage of medetomidine in acid and base form with different pHs

The base and salt ratio used in the studies could vary depending on the pH of the test medium. In some of the fate and ecotoxicological studies, the form was not specified. The pKa of medetomidine is 7.1, so at pH 7.1 the base and salt form of medetomidine will be present in a 50:50 ratio, whereas at pH 9 the concentration of the base form will be approximately 99%. The pH of sea water is considered to be around 8.0, where there is likely to be around 88 % of the base form and 12 % of the acid form (see figure 1). The pH of the ecotoxicological studies undertaken has been considered to ensure they appropriately reflect the conditions of exposure in the environment and the form that medetomidine will occur in. This is discussed further in section 5.4 of this CLH report.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties (Medetomidine – free base form)

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Almost white crystalline powder	Ferrnion Oy (SDS) 2009	GMP not GLP Purity 98-100%
Melting/freezing point	110-116 °C 116.6 °C	Sydney P, 2011 Solvias AF, 2009	EC A1 Metal Block GLP, Purity 100% EC A1 DSC, GMP (not GLP), Purity 100%
Boiling point	Decomposition above ~150 °C 386 °C	Sydney P, 2011 Solvias AF, 2009	EC A2 Siwoloboff GLP, Purity 100% EC A2 DSC, GMP (not GLP), Purity 100%
Relative density	1.113	Solvias AF, 2009	EC A.3 (Helium pycnometry) GMP (not GLP), Purity 100%
Vapour pressure	1.86E-04 Pa at 45.14°C. Estimations from curve 8.3E-6 Pa at 25°C 3.5E-6 Pa at 20°C	Solvias AF 2009	OECD 104 GMP (not GLP), Purity 100%
Surface tension	63.5 mN/m (90% saturated aqueous solution at 20 °C) 68.7, 64.9, 59.8 mN/m	Sydney P, 2011 L. Nilsson, R. Bordes, D. Ostrovskii. 2008	EC A.5, (ring method) OECD 115, GLP (Purity not stated) OECD 115, Ring method (Purity not stated), not GLP
Water solubility	0.186 g/l at pH 7.9 and 20°C 19.8 g/L at pH 5.1 and 25°C 0.20 g/L at pH 7.9 and 25°C 0.16 g/L at pH 9.0 and 25°C 9.75g/l at pH 5 and 10°C 9.86g/l at pH 5 and 20°C 12.1g/l at pH 5 and 30°C 0.353g/l at pH 7 and 10°C 0.425g/l at pH 7 and 20°C 0.489g/l at pH7 and 30°C 0.0834g/l at pH 9 and 10°C 0.153g/l at pH 9 and 20°C 0.189g/l at pH9 and 30°C	Sydney P, 2011 Solvias AF 2009. Pullinger T, 2013	EC A.6, OECD 105, flask method, GLP, Purity 100% EC A.6, OECD 105, flask method, GMP (not GLP), Purity 100% EC A.6, OECD 105, flask method, GLP, Purity 100%
Partition coefficient n-octanol/water	<table><tr><th>pH</th><th>Temperature (°C)</th><th>Log P</th></tr><tr><td>5</td><td>10</td><td>1.1</td></tr><tr><td></td><td>20</td><td>1.2</td></tr><tr><td></td><td>30</td><td>1.3</td></tr><tr><td>7</td><td>10</td><td>2.5</td></tr></table>	Sydney P, 2011 Sydney P, 2014	EC A. 8 (HPLC method) Purity 100% GLP

	20 2.6		
	30 2.6		
	9 10 3.1		
	20 3.1		
	30 3.0		
Flash point	191.3 °C (calculated using Advanced Chemistry Development, ACD software)	L. Nilsson, R. Bordes, D. Ostrovskii. 2008.	Calculated
Flammability	Not highly flammable. From experience in handling and use and consideration of the chemical structure it is not pyrophoric and does not release flammable gases on contact with water.	Sydney P, 2011	EC, A10, GLP, Purity 100%
Explosive properties	From a consideration of the structure, medetomidine is not considered to posses explosive properties	-	-
Self-ignition temperature	No data available	-	-
Oxidising properties	From a consideration of the structure, medetomidine is not considered to posses oxidising properties	-	-
Granulometry	No data available	-	-
Stability in organic solvents and identity of relevant degradation products	No data available	-	-
Dissociation constant	Medetomidine HCl pKa = 7.1 Medetomidine free base pKb = 6.9	M. Mannonan H. Makinen J. Olkarlnen, 2004	OECD 112 Potentiometric titration, GMP not GLP, Purity 99.5%
Viscosity	No data available (solid)	-	-

2 MANUFACTURE AND USES

2.1 Manufacture

Medetomidine is manufactured in the EU.

2.2 Identified uses

Medetomidine is to be used in the EU as an antifouling agent in Product Type 21 of Regulation (EU) No. 528/2011. It is also used within the EU as an anaesthetic in veterinary medicine and an analgesic in human medicine.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Refer to Table 9.

3.1 Physical-Chemical Properties

3.1.1 Summary and discussion of Physical-Chemical Properties

In a standard flammability study (EC A10) medetomidine was found to be not flammable and does not meet the criteria for classification as a flammable solid. Further, experience in handling and use indicates it is not pyrophoric and does not react with water to liberate flammable gases.

From a consideration of the structure, medetomidine is not considered to posses explosive or oxidising properties.

3.1.2 Conclusions on classification and labelling

Not classified – Data conclusive but not sufficient for classification

4 HUMAN HEALTH HAZARD ASSESSMENT

Medetomidine is a synthetic compound used as both a surgical anaesthetic and analgesic in veterinary medicine and sedative in human medicine. The substance is manufactured as a racemic mixture of two stereoisomers: dexmedetomidine and levomedetomidine. The active isomer is dexmedetomidine whereas the other isomer, levomedetomidine, is non effective. Dexmedetomidine is a highly selective α_2 adrenoceptor agonist on presynaptic neurons. The stimulation of these receptors leads to a decrease in norepinephrine release from presynaptic neurons with inhibition of postsynaptic activation, which attenuates CNS (Central Nervous System) excitation, especially in the locus coeruleus of the brain. A similar mode of action (activation of specific neuro-receptors in shell-building organisms leading to an anti-settling effect) is the basis of its biocidal activity as an antifouling agent.

The toxicology data submitted by the applicant include mainly studies conducted with the racemate, but some data on the individual isomers are available, these are highlighted in the report.

The majority of the studies submitted were performed with the racemic base. However, there are also a significant number of studies conducted with the racemic HCl salt, these are again indicated in the report. From a toxicological point of view, the salt and base forms can be regarded as equivalent. This is because the HCl salt form dissociates in the aqueous environment of the body to release the base. It is noted that at a physiological pH of 7.4, the equilibrium will favour the non-protonated base form to some extent.

A significant number of toxicological studies were conducted with batches of medetomidine manufactured using the same process used for the manufacture of the technical material. The purity of these tested batches was ≥ 99%. In some studies, batches manufactured for the pre-clinical and clinical investigations were used. Although no specific information on purity is available, a similarly high purity is expected from these batches because these clinical batches are of pharmacological grade and their method of manufacture is essentially the same as that of the technical material. Overall, therefore, the batches used in the toxicology studies support the technical specification of the material for which Approval is sought.

The grey shading in the summary tables indicates studies of low reliability that contribute little weight to the overall assessment.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The ADME characteristics of medetomidine have been investigated in a number of non-OECD compliant studies in dogs, rats and cats. There are no studies to assess the ADME characteristics of medetomidine via the oral and inhalation routes of exposure. An assumption of 100 % absorption via the oral and inhalation route is supported by the severe systemic toxicity observed in acute inhalation and oral toxicity studies in rats.

The distribution, metabolism and excretion properties of medetomidine in the dog were similar to those in humans (see below). The studies in rats and cats were of insufficient quality to draw any meaningful conclusions.

4.1.2 Human information

The ADME characteristics of medetomidine have been investigated in a number of studies in human volunteers. In addition, the dermal absorption of medetomidine in a paint formulation product was determined to be 0.06 % in a recent study in human volunteers. There are no studies to assess the ADME characteristics of medetomidine via the oral and inhalation routes of exposure.

Following absorption, medetomidine and its metabolites are widely distributed throughout the body and they are anticipated to reach the bone marrow, cross the placenta and be excreted in the breast milk. In humans, following *i.v.* injection, the maximum blood concentration of radioactivity was reached within 10 minutes. Elimination of radioactivity from blood was also rapid with an elimination half-life of approximately 3 hours. In humans, medetomidine was extensively metabolised and rapidly excreted. Comparison of the AUC_{0-24} for dexmedetomidine (3.26 h x ng/mL) with that of the total plasma radioactivity over the same time course indicated that unchanged parent drug accounted for an average of 14.7 % of the total plasma radioactivity. The main metabolite (representing 41.37% of the total plasma radioactivity) was the N-glucuronide conjugate. No parent medetomidine was detected in excreta. The main route of excretion was via the urine. In human volunteers, an average of 95.17 % of the dose was excreted in the urine after 9 days, with approximately 85 % of the dose being recovered within 24 hours post administration. In comparison, approximately 4.08 % of the dose was excreted in the faeces after a period of 9 days post administration. The short half-life and almost complete recovery of the radioactivity in urine and faeces indicate that medetomidine does not accumulate in tissues and organs.

4.1.3 Summary and discussion on toxicokinetics

The ADME characteristics of medetomidine have been investigated in a number of studies in human volunteers, dogs, rats and cats. It is predicted that medetomidine will be extensively absorbed via the oral and inhalation routes (but not the dermal route) and widely distributed. Medetomidine was extensively metabolised and excreted mainly via the urine. There was no evidence of bioaccumulation.

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Acute Oral		
Method	**LD_{50}**	**Observations and remarks**
Non-guideline but similar to OECD 401 (no deviations) GLP Rats, Sprague-Dawley 3 males/ group Dose levels: 0.05, 1.25, 6.25 and 31.25 mg/kg bw/day of medetomidine base 21-day observation period Medetomidine base in saline Hirsimaki (1984a)	LD_{50} > 31.25 mg/kg bw/day	No deaths at any dose Clinical signs of toxicity observed at all dose levels included a dose-dependent increase in the level of sedation. At the top two dose levels a crouched position, piloerection, exopthalmos, shallow respiration and red discharge around the eyes, mouth and nostrils. Exopthalmos was observed in animals dosed 1.25 mg/kg bw 14-days post administration the eyes of animals dosed with 6.25 mg/kg bw and 31.25 mg/kg bw appeared opaque. Hisotpathological analysis of the eyes from the top dose identified subchronic keratitis in both eyes in 2 animals in 1 eye in the 3rd, whereas eyes from the 6.25 mg/kg bw appeared normal. No gross pathological findings were observed in surviving animals
Non-guideline but similar to OECD 401 (no deviations) GLP Mice, NMRI 5 females/group Dose levels: 0.05, 0.25, 1.25, 6.25, 31.25, 156.25, 234.75 and 312.5 mg/kg bw 21-day observation period Medetomidine base in saline Hirsimaki (1984b)	LD_{50} 11 mg/kg bw/day	Deaths: > 6.25 mg/kg bw. 1 death at this dose level, no animal survived at higher dose levels Deaths occurred between days 1-5 Clinical signs of toxicity included sedation (observed at dose levels ≥ 0.25 mg/kg bw), piloerection and exopthalmos (observed at dose levels ≥ 1.25 mg/kg bw), crouched position and convulsions (observed at dose levels ≥ 6.25 mg/kg bw). 3/5 animals treated with 6.25 mg/kg bw developed opaque eyes, although only 1/3 displayed chronic keratitis on histopathological examination. Gross pathological examination of animals that died revealed bright red toes, haemorrhagic lungs, pale liver and gas in gastrointestinal tract. Vacuolation of the liver was also observed in 2/5 top dose animals
Acute Inhalation		
Method	**LC50**	**Observations and remarks**
OECD 403 GLP Rats, Wistar CRL(WI) BR Male & female 5/sex/group 4 hour exposure (nose only) Dose levels: 0.1, 0.2 and 0.5 mg/l aerosol MMAD: 3.43-3.64 µm	LC_{50} 0.14 mg/l (male & female) LC_{50} 0.17 mg/l (male) LC_{50} 0.12 mg/l (female)	Deaths: 1/10, 9/10 and 10/10 low to high dose Deaths occurred between days 1-3 Clinical signs of toxicity were observed at all doses and included: laboured respiration and increased respiratory rate on the day of exposure, lethargy, ataxia, exophthalmos and opacity of the eyes (in all dose groups), hunched posture, red discharge from eyes, eyes partially closed and continuous tremors. In addition, animals in the top dose displayed aggressiveness. Marked bodyweight loss observed in 0.1 mg/L treated animals during the first week. Gross pathological findings in animals killed *in extremis* included red discoloration of the lungs, pale mottling of the

14-day observation period Medetomidine base Nagy (2009)		liver, dark/red thymic discolouration, and enlargement of the stomach, red mottled pancreas and presence of red firm material associated with red mucosal discolouration of urinary bladder. Findings in animals that died during the post-exposure period included red discolouration and/or non-collapsing of the lungs, and bilateral discolouration of the conjunctivae. No gross pathological findings found in survivors.
Acute Dermal		
Method	**LD50**	**Observations and remarks**
OECD 402 GLP Rats, Crl:CD Sprague-Dawley 5 females/group: 30, 60 and 400 mg/kg bw 5/sex: 2000 mg/kg bw 14-day observation period Medetomidine base in corn oil Bull (2010)	LD_{50} > 2000 mg/kg bw	Deaths in 2000 mg/kg bw group: 1 M found dead day 5, another killed *in extremis* day 7, 1 F died day 5 another killed *in extremis* day 6 Clinical signs were observed at all doses and included underactivity, irregular breathing (agonal respiration), brown staining on the head (muzzle, ears and eyes), paws and urogenital region, deep breathing, hunched posture and partially closed eyes. In addition to the above, at the top dose surviving animals also had black faeces, piloerection, distended abdomen, maloccluded teeth, and dilated pupils and splayed hind limbs. Reduced bodyweight gain and bodyweight loss observed from 30 mg/kg bw. Gross pathological examination of animals that died, included congestion of the heart, lungs, and brown/yellow fluid of the duodenum, small and large intestines. 3/5 (f) and 3/3 (m) surviving animals of the mid and top dose group had thickened tissues of the GI tract and 5/5 (f) and 1/3 (m) of the mid and high dose group had gaseous distension of the GI tract. No other gross pathological findings found in surviving animals.

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Via the oral route, data are available from a study in rats and a study in mice. The LD_{50} from the rat study was > 31.25 mg/kg bw, whereas the LD_{50} value derived from the mouse study was 11 mg/kg bw. In accordance with the Guidance on the Application of the CLP Criteria (pg 196), classification is, generally, based on the lowest LD_{50} value from the most sensitive species, unless a robust justification as to why this would not be appropriate can be provided. Although neither study was to guideline, they were conducted at similar times in the same laboratory, suggesting that conditions for each study would have been similar. Consequently, it is proposed to base the classification on the lowest LD_{50} value of 11 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

An inhalation LC_{50} of 0.14 mg/l for 4 hours was derived from a study conducted with rats.

4.2.1.3 Acute toxicity: dermal

A dermal LD_{50} of > 2000 mg/kg bw was derived from a study in rats.

4.2.1.4 Acute toxicity: other routes

In addition to the standard routes of exposure, the applicant provided acute toxicity studies of medetomidine base administered by the intravenous route (Hirsimaki, 1984c), intraperitoneal (Hirsimaki, 1984d) and subcutaneous (Hirsimaki, 1984e) routes of exposure. Mortality occurred only in the intravenous and subcutaneous studies, although the same clinical signs of toxicity were observed across all studies independent of the route of administration. Clinical signs of toxicity included: sedation, exophthalmos, convulsions, piloerection, opacity of the eyes, and red discharge from the mouth, nostrils and eyes. The calculated LD_{50} for the *i.v., i.p.* and *s.c.* routes of administration were 1.8, > 31.25 and 20 mg/kg bw, respectively.

4.2.2 Human information

Table 11: Summary of Human Information

Method	Observations and remarks
Aim: to investigate the dose response relationship for sedation using single and continuous infusion (up to 24 h) of dexmedetomidine in human volunteers A total of 72 volunteers (40 in part 1 and 32 in part 2) healthy adult subjects (32 males and 40 females), aged between 18-45 years. Phase I: single-centred, double-blind, randomized, placebo controlled study. Groups of 6 volunteers/group exposed to either 0.1, 0.3, 0.45, 0.6 and 1.25 ng/ml via intravenous infusion. Placebo group of 10 volunteers received physiological saline At the end, 3 doses were selected for part 2. Part 2 One group received a 12-hour infusion of dexmedetomide HCl to achieve a target concentration of 0.3 ng/ml. Three groups received 24-hour infusions to achieve target steady state blood concentrations of 0.3, 0.6 and 1.2 ng/ml Sedative effects were measured using the VAS-sedation score (individual to rate the level of awareness from 0 [asleep] to 100 [wide wake]); the Critical Flicker Fusion (CFF) threshold (assessed the frequency at which a subject could no longer perceive a light source flickering); Ramsay sedation score (Blind scored: 1 = subject anxious, agitated or restless; 2 = subject cooperative, orientated, and tranquil; 3 = subject responds to commands; 4 = asleep but with brisk response to light glabellar tap or loud auditory stimulus; 5 = asleep, sluggish response to light glabellar tap or auditory stimulus; 6 = asleep, no response) Abbott (1998)	Average plasma concentrations of dexmedetomidine at steady state were similar to the target concentrations. Regardless of the length of infusion, dexmedetomidine-treated subjects, with the possible exception of those treated with 0.1 ng/ml, exhibited greater average sedation than placebo-treated subjects measured by the VAS-sedation scores and by the average percentage time at Ramsay sedation scores of 3, 4 or 5. The dexmedetomidine target concentrations that achieved the most consistent levels of sedation were 0.6 and 1.25 ng/ml. The onset of sedation was rapid (15 – 30 min after the start of the infusion) and the effect was consistently maintained throughout the duration of infusions. Dexmedetomidine –treated subjects and placebo-treated subjects had similar results for the CFF threshold, indicating, in spite of sedation, the dexmedetomidine-treated subjects were easily arousable and maintained cognitive function. Dexmedatomidine was safe and well tolerated. The most commonly noted adverse effects in either part of the study were somnolence and dry mouth, which were described from the 0.3 ng/ml. Bradycardia was noted in two subjects, one at 0.3 ng/ml and one at 1.25 ng/ml. Overall, a human NOAEL of 0.1 ng/ml blood dexmedetomidine was identified, which is equivalent to an external *iv* dose of 0.2 µg/kg bw. This is equivalent to a racemic medetomidine dose of 0.4 µg/kg bw.

Aim: to investigate the effects of a dexmedaetomidine overdose in a perioperative setting Patients accidentally received *i.v.* overdoses of dexmedetomidine, one intraoperatively (192 µg over 20 min) and 2 post operatively (4 µg/kg bw/h rather than 0.4 µg/kg bw/h and 0.5 µg/kg bw/min rather than 0.5 µg/kg bw/h). Jorden (2004)	The only notable sign of toxicity was over-sedation, which resolved within 1 hours of drug discontinuation. No additional clinical signs of toxicity were noted.
Aim: to investigate the pharmacological effects of medetomidine in physiological saline 25, 50, 100 or 120 µg/person (equivalent to 0.4, 0.8, 1.6 and 2 µg/kg bw/h based on bodyweight of 60 kg) were investigated in health male human volunteers via the intravenous route (5 min) in three phase 1 clinical trials Scheinin (1989)	Treatment related in a dose-dependent increase in sedative effects (measured both subjectively and objectively), reduced salivation, decreased blood pressure, heart rate and cardiac output. No additional biological parameters were recorded. Maximal sedative effects were seen at 15-45 min following infusion and disappeared within 4 hours after drug administration.
Aim: to investigate the occurrence of adverse drug reactions in response to *i.v.* infusions of dexmedetomidine in 136 intensive care unit patients in need of short-term use of sedatives Dasta *et al* (2004)	Patients were exposed to an average concentration of dexmedetomidine of 0.32 µg/kg bw/h (range of 0.26-1.4 µg/kg bw/h) for an average time period of 25 hours (range 1-23 hours). Adverse drug reactions were reported in 41 (31.1 %) patients, with 31 (22.7 %) suffering from hypotension.
Aim: to investigate the potential for dexmedetomidine to be used as a sole *i.v.* anaesthetic agent. Ramsey and Luternam, (2004)	The report describes the three patients who presented for surgery with potential airway challenges. These patients were given an initial intravenous loading dose of 1 µg/kg bw dexmedetomidine for 10 minutes followed by a continuous infusion of 5 – 10 µg/kg bw/h for the duration of the surgery. These doses of dexmedetomidine were sufficient to induce anaesthesia without causing respiratory depression.
Extensive literature review of the clinical use of dexmedetomidine Gerlach and Dasta (2007)	Different groups of patients (surgical, paediatric and critically ill patients) were exposed to a range of doses (0.4 – 1 µg/kg bw loading doses followed by continuous infusion of 0.2 – 2.5 µg/kg bw/h) of dexmedetomidine via intravenous infusion for the induction of sedation. Common adverse reactions were hypotension, hypertension and bradycardia

The lead effect following administration of either medetomidine of dexmedetomidine (the active isomer of medetomidine) was sedation. This was observed from doses of 0.6 ng/ml (Abbott, 1998). Sedation was reported to be observed within 15 min of administration with recovery observed between 1 – 4 hours after administration. Other effects observed in the presence of the drug included hypotension; bradycardia; hypertension; reduced salivation; decreased blood pressure, heart rate and cardiac output. From these investigations, an *i.v.* human NOAEL of 0.4 µg/kg bw was identified medetomidine.

4.2.3 Summary and discussion of acute toxicity

See section 4.2.1 and 4.2.2

4.2.4 Comparison with criteria

Via the oral route, an LD_{50} of 11 mg/kg bw meets the criteria for classification as Acute tox 2 (5< ATE ≤ 50 mg/kg).

Via the dermal route, the LD_{50} was > 2000 mg/kg bw, no classification is proposed.

Via the inhalation route, an LC_{50} of 0.15 mg/l meets the criteria of Acute tox 2 (> 0.05 ≤ 0.5 mg/l/ 4h for dusts and mists).

4.2.5 Conclusions on classification and labelling

CLP: Acute Tox 2; H300 Fatal if swallowed and Acute Tox 2; H330 Fatal if inhaled

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

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Sedation and/or related clinical signs (lethargy, under activity) were observed in all species by all routes. Via the oral and inhalation routes, effects in the eyes were also observed. Via the oral route, in both rat and mice, opacity of the eyes was observed from a dose level of 6.25 mg/kg bw. Histopathological examination revealed kerititis in the eyes of all rats in 31.25 mg/kg bw group, but not those dosed 6.25 mg/kg bw group. No mice survived administration with 31.25 mg/kg bw; however, keritis was evident in one (out of 3) surviving animals at 6.25 mg/kg bw. Via the inhalation route, opacity of the eyes was observed at all dose levels (≥ 0.1 mg/L). Keritits was not recorded in surviving animals, although it is not clear whether the eyes were examined microscopically. It is likely the keritits and opacity were a result of desiccation of the cornea as a result of the medetomidine-induced exophthalmos and partially close eyelids. They are therefore considered secondary effects and are not relevant for classification. No effects in the eye were observed in the dermal study.

A number of changes in various organs (including haemorrhagic lungs, pale liver, congestion of the heart and distended abdomen) were observed in decedents or those killed *in extremis*. However, these changes were not considered to represent specific target toxicity. No effects were noted in surviving animals.

4.3.2 Comparison with criteria

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 are classified in **STOT-SE 1 or 2**. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect.

The signs apparent after single oral, dermal and inhalation exposure to medetomidine were indicative of non-specific (or secondary to) general acute toxicity. As there was no clear evidence of specific toxic effects on a target organ or tissue, no classification for specific target organ toxicity (single exposure) 1 or 2 under CLP is proposed.

Classification in **STOT-SE 3** is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

Administration of medetomidine to animals (via any route) led to signs of sedation (≥ 0.05 mg/kg bw via the oral route, 0.1 mg/L via the inhalation route and 30 mg/kg bw via the dermal route). Sedation was observed at much lower doses than those causing lethality. In surviving animals, signs of sedation also appeared to be transient. In humans, a LOAEL of 0.3 ng/ml blood, which is equivalent to an external iv dose of 1.2 µg/kg bw racemic medetomidine was identified. The sedation was again reported to be transient with recovery observed 1 – 4 hours after administration. In both humans and animals, the severity of the effect was reported to increase with dose.

Since signs of sedation were observed in all studies, a simple case for classification as STOT SE 3 can be made.

The LOAEL (expressed as an external iv dose) for this effect in humans is 1.2 µg/kg bw. On this basis, the recommended GCL of 20 % seems inappropriate and consideration to a much lower SCL should be given.

4.3.3 Conclusions on classification and labelling

STOT SE 3: H336: May cause drowsiness or dizziness

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404 GLP Rabbit, New Zealand White (3 females) Semi-occlusive for 4 hours 0.5g mixed with distilled water to form a paste Medetomidine base	Average scores at 24, 48 and 72h Erythema 0,0,0 Oedema 0,0,0	72 hour observation period	Ranta-Panula (2010a)

4.4.1.1 Non-human information

The skin irritation potential of medetomidine has been investigated in a standard guideline study in rabbits. No signs of irritation were observed at any timepoint.

4.4.1.2 Human information

No information available

4.4.1.3 Summary and discussion of skin irritation

The skin irritation potential of medetomidine has been investigated in a standard guideline study in rabbits. No signs of irritation were observed at any timepoint.

4.4.1.4 Comparison with criteria

No signs of irritation were observed; therefore, no classification is proposed.

4.4.1.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405 GLP Rabbit, New Zealand White (3 Males) Males Mixed with sterile water to form a paste Medetomidine base	Average score per animal over 24, 48 and 72 h Cornea: 0, 0, 0 Iris: 0, 0, 0 Conjunctiva Redness: 0, 0.33, 0.33 Oedema: 0, 0, 0	All effects reversible within 7 days	Ranta- Panula (2010b)

4.4.2.1 Non-human information

The eye irritation potential of medetomidine has been investigated in a standard guideline study in rabbits. No effect on the cornea or iris was noted. Slight irritation-redness of the conjunctivae was noted in a single animal at 24 h after administration, although it is possible that this was caused by mechanical irritation because as much as ¼ of the dose was still present in the conjunctival sack. This residual substance was washed from the eye and the symptoms of irritation were resolved by day 7. No oedema was observed.

4.4.2.2 Human information

No information available

4.4.2.3 Summary and discussion of eye irritation

See section 4.4.2.1

4.4.2.4 Comparison with criteria

No effects in the iris or cornea were noted. The scores for erythema of the conjunctivae were less than 2 (value specified in the classification criteria). No oedema was noted. No classification is proposed.

4.4.2.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.2 Human information

No information available

4.4.3.3 Summary and discussion of respiratory tract irritation

This endpoint was not investigated directly; however, no signs of respiratory irritation were observed in the acute inhalation study (see section 4.2).

4.4.3.4 Comparison with criteria

No signs of respiratory tract irritation were observed as outlined in the CLP Regulation.

4.4.3.5 Conclusions on classification and labelling

Not classified; inconclusive

4.5 Corrosivity

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
See table 12			

4.5.1 Non-human information

Medetomidine is not irritating to skin (see section 4.4)

4.5.2 Human information

No information available

4.5.3 Summary and discussion of corrosivity

Medetomidine is not irritating to skin (see section 4.4)

4.5.4 Comparison with criteria

No signs of corrosivity were observed in an *in vivo* skin irritation study.

4.5.5 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15: Summary table of relevant skin sensitisation studies

Species/Method	Doses	No. sensitised/total no.	Result	Reference
Non-guideline and non-GLP test for delayed contact hypersensitivity (draize method; intradermal route of administration Guinea pigs, Crl (HA) BR Hartley Male 10/group (test) 6/ group (controls) Dexmedtomidine HCl	Induction: Intradermal: 10 intradermal injections of 0.06 % (w/v) dexmedetomidine HCl, (first injection volume was 0.1 mL followed by 0.2 mL for the remaining 9) Challenge: Challenge 2 weeks after final injection with 0.06 % (w/v) dexmedetomidine HCL Positive control: 0.05 % (w/v) 1-chloro-2-4-dinitrobenzene	Test: 0/10 Negative Control: 0/6 Positive controls: 3/6	Negative	Hahn (1995)
Local lymph node assay OECD 429 Non-GLP Mouse (strain not provided) 3/group Medetomidine base	Concentrations of 0.1, 0.3, 1 and 4 % medetomidine base in 4:1 mixture of acetone: olive oil	Assay terminated due to severe sedation and anaesthesia of the test animals at all dose levels	Not applicable	Ranta- Panula (2010c)

4.6.1.1 Non-human information

The skin sensitisation potential of dexmedetomidine (the active isomer of medetomidine) has been investigated in a non-standard delayed hypersensitivity study in guinea-pigs (Hahn, 1995). Two weeks after the last induction injection, the animals were challenged with 2 intradermal injections of dexmedetomidine at 0.06 %. Dexmedetomidine did not induce skin sensitisation in any animals tested. Although the positive control gave an appropriate response, as the challenge dose was not maximal, no conclusions can be drawn about medetomidine's skin sensitisation potential at concentrations of > 0.06%.

An OECD compliant local lymph node was initiated with medetomidine, but was terminated due to severe sedation and anaesthesia of the test animals (Ranta-Paula, 2010c).

4.6.1.2 Human information

No information available

4.6.1.3 Summary and discussion of skin sensitisation

There were no signs of sensitisation up to 0.06 % in the one available guinea-pig skin sensitisation study conducted with dexmedotmidine, the active isomer of medetomidine.

4.6.1.4 Comparison with criteria

The sensitisation response from the available study was < 30 % in all guinea-pig maximisation studies. Therefore, no classification is required under the CLP Regulation.

4.6.1.5 Conclusions on classification and labelling

Not classified; inconclusive

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

No data are available.

4.6.2.2 Human information

No data are available.

4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available.

4.6.2.4 Comparison with criteria

No data are available.

4.6.2.5 Conclusions on classification and labelling

Not classified; data lacking

4.7 Repeated dose toxicity

Information on repeated dose toxicity is available via the oral route in a 28-day and a 90-day study in rats. Additional information on the repeated dose toxicity of medetomidine is also available from a subcutaneous 28-day study, an intramuscular study in dogs and a 28-day intravenous study in dogs.

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Dose Levels	Observations and Remarks	Reference
28-day range finding study Non-guideline Non-GLP Oral (gavage) Rat Sprague-Dawley Male, 4/group Clinical signs recorded twice daily, loss of righting reflex and sleeping time recorded on day 1 and 8. Bodyweight gain was recorded daily. Food and water consumption were recorded on days 2 and 23. Gross necropsy performed on all test subjects. Weights of liver, kidneys, adrenals, testes and epididymides and heart were recorded.	0, 2.5, 3.6 and 4.9 mg/kg bw/day medetomidine HCl in 0.9 % NaCl	**4.9 mg/kg bw/day** 75 % mortality (euthanised on days 9 and 10) Sedation, diarrhoea, weakness and hypothermia. Aggressive behaviour noted in a single animal ↓ 140 % reduction in bodyweight gain; ↓ 44 % food consumption Reduced absolute organ weights (heart, liver and kidney) Gross pathological observations: dark contents in the small intestine indicative of internal bleeding. Extreme dehydration Reduced water consumption **2.5 and 3.6 mg/kg bw/day:** No mortality Aggressive behaviour in 2 animals at 3.6 mg/kg bw/day; Sedation and diarrhoea Increased water consumption Reduced absolute organ weights (heart, liver, kidney, testes and epididymis) ↓ 74 and 95 % reduction in bodyweight gain at 2.5 and 3.6 mg/kg bw/day, respectively **No NOAEL derived as range-finding study**	Ranta- Paula (2009)
90-day repeated dose toxicity study Oral (gavage) OECD guideline 408 and GLP Rat: Sprague-Dawley rat 10/sex/group	0, 0.2, 0.4, 1.2, 3.6 mg/kg bw/day Medetomidine base in 0.5 % v/v lactic acid	Sedation was observed at all dose levels; additional clinical signs were observed from a dose level of 1.2 mg/kg bw/day and included piloerection, weakness, locomotor inhibition and convulsions **3.6 mg/kg bw/day:** Mortality 30 % in both sexes (one dead day 3, two dead day 11. Two euthanised day 13 and one euthanized day 66) Increased water consumption Reduced bodyweight gain: 32/37 % (f/m) Reduced absolute organ weights: adrenals, kidneys, spleen, thymus, epididymis, heart, liver, testicles and uterus Haematology: 10 % ↓ haemoglobin (m), 14 % ↓ haematocrit (m), 13 % ↓ erythrocytes, 46+90% ↑ neutrophils (m+f), 14+21% ↓ lymphocytes (m+f), 68+21 % ↑ monocytes (m+f). Clinical chemistry: 35+83 % ↑ serum glucose (m+f), 28	Ranta- Paula (2010)

		% ↑ potassium (f), 18+22 % ↑ phosphate (m+f), 9 + 12 % ↓ albumin (m+f), 90+117 % ↑alkaline phosphatase (m+f), 93+140 % ↑ alanine aminoransferase (m+f) Urinalysis: elevated glucose, ↓ urine volume, proteinurea **1.2 mg/kg bw/day:** Increased water consumption Reduced bodyweight gain: 12/17 % (f/m) Reduced absolute organ weights: adrenals, thymus, epididymis, heart, kidneys and uterus. Haematology: 6 % ↓ haemoglobin (m), 10 % ↓ haematocrit (m), 11 % ↓ erythrocytes (m), 8 % ↓ lymphocytes (m), 76+95 % ↑ monocytes (m+f) Clinical chemistry: 49+57 % ↑alkaline phosphatase (m+f), 47% ↑ alanine aminoransferase (m) Urinalysis: elevated glucose, ↑ urine pH (males), ↓ urine volume, proteinnuria **0.4 mg/kg bw/day:** Reduced absolute organ weight: heart and kidneys in males. Hematology: 44+64 % ↑ monocytes (m+f) Clinical chemistry: 42 % ↑ alkaline phosphatase (f) Urinalysis: elevated glucose **0.2 mg/kg bw/day** Haematology: 50 % ↑ monocytes (f) Clinical chemistry: 31+89 % ↑ alkaline phosphatase (m+f) Reduced absolute organ weights: epididymis, heart, kidneys, liver in males Urinalysis: elevated glucose **A LOAEL of 0.2 mg/kg bw/day was derived from this study**	
28-day repeated dose toxicity Non-guideline (similar to OECD 407) and non-GLP Subcutaneous Rat, Sprague-Dawley 10/sex/group Clinical signs monitored weekly,	0, 0.1, 0.4 and 1.6 mg/kg bw/day Medatomidine HCl in saline	No deaths at any dose level **1.6 mg/kg bw/day:** Sedation, piloerection, exophthalmos Reduced bodyweight gain 69/44 % (m/f) Reduced absolute organ weights: heart, liver, kidney, testis, prostate and seminal vesicle in males, and spleen and thymus in males and females. Pituitary gland in females. Histopathology: Corneal opacity of the eye, brown pigmentation in the lungs (m/f), keratitis of the eye (m/f), atrophy of the prostate and seminal vesicles, ↓ number of spermatozoa, and haemorrhage and regenerative changes at injection site. Haematology: 8% ↓ haemoglobin (m), 14 %↓ haematocrit (m), 7 % ↓ erythrocytes (m), 8-13 % ↓	Hirsimaki (1986a)

bodyweight, food consumption and water consumption determined weekly. Ophthalmic examinations performed prior to start of study and at the end. Hematology, clinical chemistry and urinalysis and weights of several organs wee recorded. A standard range of tissues and organs under went histopathological examination		lymphocytes, 63 % ↑ reticulocytes (m), 64 %↑ neutrophils (f) Clinical Chemistry: 47 % ↑ iron (m), 2.5 + 4 % ↓ sodium (m+f), 12 % ↓ potassium (m), 3 % ↑ chloride (m), 6 % ↓ calcium (m), 14 % ↓ phosphate (m), 10 % ↓ total protein (m), 33 % ↓ triglycerides, 13 % ↓ blood glucose (f), 33 + 66 % ↑ alkaline phosphatase (m+f), 49 + 22 % ↑aspartate aminotransferase (m+f) Urinalysis: 86 + 70 % ↓ total urine volume (m+f), ↓ urine pH (m+f), 286 + 75 %↑ urine osmolality (m+f) **0.4 mg/kg bw/day:** Sedation and piloerection Reduced bodyweight gain 38/22 % (m/f) Reduced absolute organ weight: heart, thymus, liver, kidney and spleen in males; pituitary gland in females. Histopathology: corneal opacity (m+f), brown pigmentation in the lungs (m/f), keratitis of eye (m/f), atrophy of the prostate. Haematology: 5 % ↓ haemoglobin (m), 10 % ↓ haematocrit (m), 6.3 % ↓ erythrocytes (m) Clinical chemistry: 37 % ↑ iron (m), 10 % ↓ potassium (m), 10 % ↓ phosphate (m), 6 % ↓ total protein (m), 54 % ↑ alkaline phosphatase (f), 28 % ↑ aspartate aminotransferase (m) **0.1 mg/kg bw/day**: sedation Reduced bodyweight gain 15/25 % (m/f) Haematology: 5 % ↓ haemoglobin (m), 54 % ↑ reticulocytes (m) Clinical chemistry: 25 % ↑ iron (m), 3 % ↑ chloride (m), 6 % ↓ total protein (m), 24 % ↓ triglycerides (f), 13 % ↓ blood glucose (f), 31 % ↑ alkaline phosphatase (m) **A LOAEL of 0.1 mg/kg bw/day was derived**	
28-days repeated dose study Non-guideline (similar to OECD 407) and non-GLP Intramuscular (daily) Dog, Beagle 3/sex/group Methodology the same as Hirisimaki, 1986a except that clinical signs were monitored daily	0, 0.08, 0.24, 0.4 mg/kg bw/day Medetomidine HCl in saline	No deaths at any dose level All dose levels: No treatment related effects on bodyweight, food consumption, haematology, clinical chemistry, urinalysis, gross necropsy or histopathology Sedation was observed after dosing with severity and recovery time being dose-dependent. Diarrhoea was noted in the mid and high dose groups Corneal opacity was observed in the mid (1/3 females) and high (3/3 females) dose groups **A NOAEL of < 0.8 mg/kg bw/day was derived**	Hirsimaki (1986b)

28-days repeated dose study Subcutaneous Non-guideline (similar of OECD 407) and non-GLP. A standard set of observations/ parameters were measured in line with OECD 407 Rat, Sprague-Dawley 10/sex/group	0, 0.02, 0.1, 0.5, 2.5 mg/kg bw/day *levomedetomidine* HCl in saline	No deaths at any dose level. No adverse effects at any dose level. Local skin irritation at the injection site at the top dose. **A NOAEL of 2.5 mg/kg bw/day levomedetomidine**	Nieminen (1997a)
28-days repeated dose study Intravenous OECD 407 and non-GLP Dog, Beagle 3/sex/group	0, 0.4, 2, 10 mg/kg bw/day *levomedetomidine* HCl in saline	**10 mg/kg bw/day**: study terminated after 2 doses in females and 3 doses in males due to the severity of the clinical signs of toxicity (piloerection, salivation, tremors, diarrhoea, vocalization, redness of the eyes and aggression). **0.4 – 2 mg/kg bw/day**: No treatment related adverse effects. **A NOAEL of 2 mg/kg bw/day levomedetomidine was derived**	Nieminen (1997b)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

The repeat dose oral toxicity has been investigated in a 28-day range-finding experiment in rats and a 90-day study in rats.

In the available 28-day range finding study, effects were observed from the lowest dose level tested (2.5 mg/kg bw/day). At this dose level, effects included sedation, diarrhoea, significant reduction in bodyweight gain and lower absolute organ weights. At higher dose levels (4.9 mg/kg bw/day) additional effects included weakness, hypothermia, and death (3 out of 4 animals were euthanised on days 9 and 10 due to weak condition).

In the 90-day study, effects were observed from the lowest dose (0.2 mg/kg bw/day) and included sedation, reduced absolute organ weights and minor changes in clinical chemistry and haematology parameters. From 1.2 mg/kg bw/day, additional clinical signs (piloerection, weakness, locomotor inhibition, and convulsions) and reduced bodyweight gain was observed. At the highest dose (3.6 mg/kg bw/day), the effects on bodyweight gain, clinical chemistry and haematology parameters worsened and were accompanied by mortality. Deaths were observed on day 3 (one animal) and day 11 (two animals). The pathological findings revealed cerebral haemorrhage and internal blockage in two animals; no adverse findings were observed in the third. Three further animals were killed in extremis as a result of poor condition (general wasting) on day 13 (two animals) and day 66 (one animal). These deaths were considered treatment related.

4.7.1.2 Repeated dose toxicity: inhalation

No other information available

4.7.1.3 Repeated dose toxicity: dermal

No other information available

4.7.1.4 Repeated dose toxicity: other routes

Information on the repeated dose toxicity of medetomidine is available in a subcutaneous 28-day study, an intramuscular study in dogs and a 28-day intravenous study in dogs.

In the 28-day subcutaneous rat study, effects were observed from the lowest dose (0.1 mg/kg bw/day) and included sedation, reduced bodyweight gain and relatively minor changes in haematology and clinical chemistry. The severity of these effects increased with dose. Additional findings included effects in the eye (corneal opacity and keritities of the eye) and piloerection from a dose level of 0.4 mg/kg bw/day. Exophthalmos, atrophy of the prostrate and seminal vesicles was observed at the highest dose (1.6 mg/kg bw/day) but only in the presence of significantly reduced bodyweight gain (69 % in males).

In the 28-day dog intramuscular dog study, sedation was observed at all dose levels from 0.08 mg/kg bw/day with diarrhoea and corneal opacity observed at the mid (0.24 mg/kg bw/day) and top doses (0.4 mg/kg bw/day).

A 28-day rat study (subcutaneous) and a 28-day dog study (intramuscular) have been submitted on the ‘inactive’ isomer of medetomidine. The results of these studies confirm this isomer to be much less active than dexmedetomidine.

4.7.1.5 Human information

No information available

4.7.1.6 Other relevant information

No information available

4.7.1.7 Summary and discussion of repeated dose toxicity

The repeated dose toxicity of medetomidine has been investigated in an oral 28-day and 90-day study in rats and via the subcutaneous and intramuscular routes in rats and dogs, respectively.

The available data on repeated exposure appear to support classification of medetomidine for repeated dose toxicity. In the oral 90-day study, death was observed in both sexes at a dose level of 3.6 mg/kg bw/day. Similar to the acute studies, the deaths occurred after a few days (apart from one death which occurred on day 3, the majority of deaths occurred on or after day 11), as such they are considered likely to be a result of acute exposure. Pathological findings at necropsy revealed cerebral haemorrhage and internal blockage; or general wasting to be the reason for death. Poor condition was also the reason why 3 out of 4 animals were euthanised after day 9 in the oral 28-day study at a dose level of 4.9 mg/kg bw/day. In addition to the deaths observed in the 90-day study, severe sedation and significant adverse effects on clinical chemistry and haematological parameters, bodyweight gain and organ weights were noted. It should be noted that sedation and effects on bodyweight gain were also observed in the acute toxicity studies. As such, although the criteria for repeated dose classification appear to have been met, it is considered that the effects observed are not the consequence of repeated (prolonged) exposure but are in fact acute effects arising from a small number of single exposures.

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

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

See section 4.7.1.7

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

The available data on repeated exposure do appear to support classification of medetomidine for repeated dose toxicity. In the oral 90-day study, death was observed in rats in both sexes from a dose level of 3.6 mg/kg bw/day, the majority from day 11 onwards. In addition to mortality, severe sedation and significant adverse effects on clinical chemistry parameters, bodyweight and organ weights were noted at this dose level. Sedation and effects on bodyweight gain were also observed in the acute toxicity studies. Consequently, whilst the criteria for repeated dose classification appear to have been met, it is considered that the effects observed are not the consequence of repeated (prolonged) exposure, but are in fact acute effects arising from a small number of single exposures, they appear to be the result of acute exposure. As classification for acute toxicity via the oral and inhalation routes is already proposed, it is not proposed to additionally classify for STOT-RE.

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

Not classified; conclusive but not sufficient for classification.

4.9 Germ cell mutagenicity (Mutagenicity)

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

In Vitro Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bacterial reverse mutation (Ames) – plate incorporation OECD guideline 471 (1997) GLP Medetomidine base (in acetone) May, 2011	*S. typhmurium* TA 1535, 1537, 98, 100 and *E.coli* WP2uvrA	Experiment 1: 5-5000 µg/plate; Experiment 2: 5-1500 µg/plate	**Negative with and without S9** Cytotoxicity observed at 1500 and 5000 µg/plate in experiment 1 and 1500 µg/plate (top dose) in experiment 2 Positive controls gave the appropriate responses
In vitro cytogenetics OECD guideline 473 GLP Medetomidine base (in DMSO) Pritchard, 2011	Human lymphocytes	Experiment 1 (3 hour exposure) 56-155 µg/ml with out S9 and 93-259 µg/ml with S9 Experiment 2: 20 – 50 µg/ml without S9 (21 hour exposure) 249-280 µg/ml (3 hour exposure)	**Negative with and without S9** Experiment 1: cytotoxicity (50 % reduction in mitotic index) was observed at 155 µg/ml without S9 and 259 µg/ml with S9. Experiment 2: cytotoxicity was observed at 50 µg/ml without S9 and 280 µg/ml with S9. Positive controls gave the appropriate responses
Bacterial reverse mutation (Ames) – plate incorporation OECD guideline 471 (1997) GLP Dexmedetomidine HCl (in water) Nieminen, 1993	*S. typhmurium* TA 1535, 1537, 1538, 100 and 98	Experiment 1 and 2: 0, 15, 50, 150, 500, 1500 µg/plate	**Negative with and without S9** Experiment 1 and 2: cytotoxicity was observed at 1500 µg/plate Positive controls gave the appropriate responses
In vitro cytogenetics OECD guideline 473 GLP Dexmedetomidine HCl (in water) Nieminen, 1994a	Human lymphocytes	Experiment 1: 6 -94 µg/ml with S9; 100 – 300 µg/ml with S9 Experiment 2: 12.5 – 300 µg/ml without S9; 50 – 350 µg/ml with S9 (18 hr harvest) Experiment 3: 12.5 – 300 µg/ml without S9; 50 – 350 µg/ml with S9 (32 h harvest)	**Negative with and without S9** Cytotoxicity (42-66 %) was observed at the top concentrations Without S9, dexmedetomidine did not cause a significant increase in the number of cells with aberrations (excluding gaps) in any experiment. With S9, there was a statistically significant increase in the number of cells with aberrations (excluding gaps) at the top concentration of 300 µg/ml of the first assay only. As the increase was not repeated in either the second or third test, it is not considered biologically significant. Positive controls gave the appropriate responses

In vitro gene mutation assay in mammalian cells (mouse lymphoma TK assay) OECD Guideline 476 (1984) GLP Dexmedetomidine HCl (in DMSO) Nieminen (1994b)	Mouse Lymphoma L5178Y cells	Preliminary toxicity test: 37-3990 µg/ml ± S9; Experiment 1: 10 – 300 µg/ml ± S9; Experiment 2: 10 – 300 µg/ml ± S9	**Negative with and without S9** In the preliminary experiment, total inhibition of growth was observed at 300 µg/ml ± S9. In the two main experiments cytotoxicity (~ 10 % relative growth) was seen at ≥ 250 µg/ml without S9 and ≥ 200 µg/ml with S9. Positive controls gave the appropriate responses
In vivo Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bone marrow micronucleus test OECD Guideline 474 GLP Intravenous Dexmedetomidine HCl Nieminen, 1997	NMRI mouse 5/sex/dose/sampling time (24 and 48 h post dosing)	40, 100, 250 µg/kg bw Cyclophosphamide 40 µg/kg bw	**Negative** Maximum dose of 250 µg/kg bw set due to severe hypothermia (as a result of sustained sedation) being observed at 500 µg/kg bw in a dose-range finding study. In main study, sedation was observed immediately after dosing at all dose levels. Additionally, mid and top dose animals also displayed piloerection. There was no change in the P/N ratio. Positive controls gave the appropriate responses

4.9.1 Non-human information

4.9.1.1 In vitro data

The genotoxic potential of medetomidine has been investigated *in vitro* in an Ames test and cytogenetics assay. The result of both studies was negative.

The genotoxic potential of the active isomer of medetomidine, dexmedetomidine, has been investigated *in vitro* in an Ames test, cytogenetics Assay and a gene mutation assay. The results of all studies were negative.

4.9.1.2 In vivo data

No information is available on Medetomidine itself; however, information is available from a micronucleus study conducted on the active isomer of Medetomidine, dexmedetomidine. The result of this study was negative. Although there was no change in the P/N ratio, the test substance was judged to have reached the bone marrow.

4.9.2 Human information

No information available.

4.9.3 Other relevant information

No information available

4.9.4 Summary and discussion of mutagenicity

Data on medetomidine and dexmedetomidine indicate that medetomidine is not genotoxic *in vitro* or *in vivo*.

4.9.5 Comparison with criteria

Data indicate that medetomidine is not genotoxic *in vitro* or *in vivo* and does not require classification.

4.9.6 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification.

4.10 Carcinogenicity

No data available

4.10.1 Non-human information

No data available.

4.10.1.1 Carcinogenicity: oral

No information available

4.10.1.2 Carcinogenicity: inhalation

No information available

4.10.1.3 Carcinogenicity: dermal

No information available

4.10.2 Human information

No information available

4.10.3 Other relevant information

No information available

4.10.4 Summary and discussion of carcinogenicity

Not applicable

4.10.5 Comparison with criteria

Not applicable

4.10.6 Conclusions on classification and labelling

Not classified; data lacking

4.11 Toxicity for reproduction

Developmental toxicity studies are available in rabbits by the intravenous route and in rats by the subcutaneous route of administration.

4.11.1 Effects on fertility

Table 19: Summary table of relevant reproductive toxicity studies - Fertility

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Two-generation study FDA guideline similar to OECD 415 Subcutaneous Rat, Sprague-Dawley 24 animals/sex/dose <u>Medetomidine HCL (MPV 785) in saline</u> Male: 60 days exposure premating, during mating and until termination of the study Females: 14 days premating, during mating, throughout pregnancy and lactation until termination of the study. Necropsy performed on parental animals Half of the F0 females in all dose groups were allowed to litter and were sacrificed after weaning. The resulting F1 pups were weighed and examined for sex, abnormalities, survival and	0, 13.3, 40 and 120 µg/kg bw/day	*Parental toxicity* 120 µg/kg bw/day Clinical signs – sedation, piloerection and exophthalmous Males: 14 % ↓ food consumption and 23 % ↓ bodyweight gain. 7 % ↓ testis weight, 17 % ↓ prostate weight, 9.5 % ↓ epididymis Females: 20 % ↓ bodyweight gain during GD 0-20, 45 % ↓ bodyweight gain during lactation (days 0-7), 18 % ↓ placenta weight 40 µg/kg bw/day Clinical signs – sedation and piloerection Males: 13 % ↓ food consumption, 15 % ↓ bodyweight gain, 6.2 % ↓ testis weight, 6 % ↓ epididymis weight Females: 20 % ↓ bodyweight gain during GD 0-20, 49 % ↓ during lactation (days 0-7). 13.3 µg/kg bw/day Clinical signs – sedation and piloerection Males: 9 % ↓ food consumption, 6 % ↓ bodyweight gain, 16 % ↓ prostate weight *Reproductive toxicity* No effects at any dose level *Offspring toxicity* *F1 generation* 120 µg/kg bw/day

postnatal development during lactation. Other half of F0 females were autopsied with Caesarean section on day 20 of gestation. Live foetuses were weighed, sexed and examined for abnormalities (1/3 soft tissue analysis, 2/3 skeletal abnormalities) F1 generation not dosed directly. One male and one female from each F1 litter were mated and pregnant females allowed to litter (F2 generation): sex and number recorded. Sacrificed on day 4 of lactation, no necropsy carried out Hirsimaki (1989)		↑ embryonic deaths (14 vs 5 in controls); 35 % ↓ foetus weight 40 µg/kg bw/day 21 % ↓ foetus weight 13.3 µg/kg bw/day: No treatment related effects *F2 generation* No effects on sex ratio or pup number observed at any does level A LOAEL of 13.3 µg/kg bw/day was derived for parental toxicity; A NOAEL of > 120 µg/kg bw/day was derived for reproductive toxicity and a NOAEL of 13.3 µg/kg bw/day was derived for offspring toxicity

4.11.1.1 Non-human information

The reproductive toxicity of medetomidine has been investigated in a non-standard two-generation study conducted via the subcutaneous route in Sprague-Dawley rats (Hirsimaki, 1989).

In this study, no effects on reproductive toxicity were observed. Parental toxicity in the form of clinical signs (sedation, piloerection, exophthalmos) was observed at all dose levels. Reduced food consumption and an associated decrease in bodyweight gain were observed in F0 males from 13.3 µg/kg bw/day. The reductions in prostate, testis and epididymides weight observed at all dose levels were considered secondary to the reduced bodyweight gain. F0 dams treated with ≥ 40 µg/kg bw/day had significantly reduced bodyweight gain by up to 20 % during GD 0-20 and by up to 49 % during lactation when compared to controls. Although the number of corpora lutea, number of implantation sites, and the number of pre-implantation losses were all comparable to controls, placenta weight was significantly decreased in F0 dams (by 18 %) at the top dose.

In offspring, F1 foetal bodyweight was significantly reduced in a dose dependant manner from a dose of 40 µg/kg bw/day (by up to 35 %), and there was a significant increase in the number of early embryonic deaths in the top dose group (14 vs 5 in controls). There were no effects on the F2 litter parameters of sex ratio and number of pups.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

Table 20: Summary table of relevant reproductive toxicity studies - Development

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Developmental toxicity USA FDA guidelines; Non-GLP but QA statement provided *Intravenous* Rabbit White Russian 12 pregnant females/ dose Dosed GD 6-18 Medetomidine HCl (MPV-785) in 0.9 % NaCl Hirsimaki (1988a)	0, 6, 24, 96 40 µg/kg bw/day	Maternal toxicity: No effect on bodyweight gain or food consumption at any dose level. ≥ 24 µg/kg bw/day: sedation and miosis Developmental toxicity: no treatment related effects at any dose level A NOAEL of 6 µg/kg bw/day for maternal toxicity and > 96 µg/kg bw/day for developmental toxicity
Developmental toxicity Non-guideline but similar to OECD 414 *Subcutaneous injection* Rat Sprague-Dawley 30 pregnant females/dose Dosed GD 6-15 Medetomidine HCl (MPV-785) in saline Bodyweight and clinical signs of toxicity examined daily. On GD 20, necropsies performed on all animals and to foetuses and placenta examined Hirsimaki (1988b)	0, 30, 120, 480 µg/kg bw/day	Maternal toxicity ≥ 120 µg/kg bw/day: sedation, exopthalmous, piloerection, 24 and 38 % ↓ bodyweight gain in mid and top dose group, respectively 9 an 22 % ↓ placental weight at mid and top dose, respectively 30 µg/kg bw/day: sedation Developmental toxicity ≥ 30 µg/kg bw/day: significant reduction in foetal bodyweight in a dose dependant manner (by 10.5, 19 and 35 %, respectively) 480 µg/kg bw/day significant ↑ in the number of early embryonic deaths (1.1 ± 0.4 vs 3.1 ± 2.6). A LOAEL of 30 µg/kg bw/day was derived for maternal and developmental toxicity.

Developmental toxicity Non-guideline, non-GLP and poorly reported *Subcutaneous injection* Rat Sprague-Dawley 8 pregnant females/group Dosed GD 7-19 Dexmedetomidine base in 0.9 % NaCl Tariq (2008)	0, 5, 10, 20 µg/kg bw/day Additional group: single dose 20 µg/kg bw/day on GD 19	*Maternal toxicity* 20 µg/kg bw/day: significant ↓ food consumption ≥ 5 µg/kg bw/day: 4 % ↓ bodyweight gain Sedation of the animals is likely, but this observation was not recorded in this study *Developmental toxicity* ≥ 10 µg/kg bw/day: Significant ↓ in foetal body weight and crown-rump length Due to the limited reporting of the study no NOAEL/LOAEL was derived

4.11.2.1 Non-human information

Developmental toxicity of medetomidine has been investigated in the rabbit via the intravenous route and in rats via the subcutaneous route of administration. There is also limited information from a poorly reported developmental study conducted via the subcutaneous route with dexmedetomidine, the active isomer of medetomidine.

Rabbits

In the rabbit study, maternal toxicity was observed from 24 µg/kg bw/day medetomidine and consisted of sedation and miosis (FDA, 1966). There were no treatment related effects on bodyweight gain or food consumption; however, in the top dose group daily food consumption was reduced compared to the control group from study day 7. No developmental toxicity was observed in this study.

Rats

In a non-guideline developmental study in female Sprague-Dawley rats, medetomidine was administered via the subcutaneous route. Maternal toxicity was observed from a dose level of ≥ 30 µg/kg bw/day and included sedation, piloerection and exopthalmos (Hirsimaki, 1988b). Dams treated with ≥ 120 µg/kg bw/day had significantly reduced body weight gain when compared to controls (by 24 and 38 % in the mid and high dose groups, respectively). Placenta weight was significantly reduced by 9 and 22 % in the mid- and top-dose dams. Foetal bodyweight was significantly reduced in a dose dependent manner from a dose level of 30 µg/kg bw/day and there was a significant increase in the number of early embryonic deaths in the top dose group (1.1 ± 0.4 *vs* 3.1 ± 2.6 for controls *vs* treatment groups, respectively). No malformations or skeletal abnormalities were observed.

In another developmental study, dexmedetomidine was administered to rats via the subcutaneous route (Tariq, 2008). Similar effects were observed in this study as in other studies (food reduction in dams and reduced foetal weights), with no malformations or skeletal abnormalities observed at any dose level. However, due to the small group size and limited examinations, the study is of limited use to inform on the classification of the substance.

4.11.2.2 Human information

No information available

4.11.3 Other relevant information

None

4.11.4 Summary and discussion of reproductive toxicity

Fertility

The reproductive toxicity of medetomidine has been investigated in a two generation study (Hirsimaki, 1989). No effect on fertility was observed in the presence of significant parental toxicity (sedation, piloerection, exophthalmos, reduced food consumption and bodyweight gain).

Developmental toxicity

Information on the developmental toxicity of medetomidine is available from a developmental study in rabbits, one good quality developmental toxicity study in rats and a 2-generation study in rats. In rabbits, no developmental toxicity was observed at any dose level. No malformations or skeletal abnormalities were observed in the rat studies. However, pup deaths were observed at the top dose of both the rat developmental study and the 2-generation study and foetal pup weights were also significantly reduced at lower doses in both studies. Although the deaths and reduced weights were mainly observed in the presence of significant maternal toxicity (sedation, ↓ reduced bodyweight gain) they are not considered secondary to it as they are the same as observed in adult rats following single exposure (see section 4.2). As such, these effects are considered a result of acute toxicity and not a specific developmental effect relevant for classification.

4.11.5 Comparison with criteria

Fertility

No effects were observed in the absence of marked toxicity that provides sufficient evidence to cause a strong suspicion of reduced fertility.

Developmental toxicity

No effects were observed in the absence of marked toxicity that provides sufficient evidence to cause a strong suspicion of causing developmental toxicity.

4.11.6 Conclusions on classification and labelling

Not classified; conclusive but not sufficient for classification.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No additional data

4.12.1.2 Immunotoxicity

No additional data

4.12.1.3 Specific investigations: other studies

No additional data

4.12.1.4 Human information

No additional data

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
OECD 111 GLP	Preliminary hydrolysis study conducted at pH 4, 7 and 9 and at 50°C: <10% hydrolysis after 120 h	Stable to hydrolysis	Sydney, 2011
OECD 3016 GLP	No significant photodegradation was in an aqueous photolysis study	Stable to photolysis	Wehrham, 2009
OECD 301D GLP	0% biodegradation estimated throughout	Not readily biodegradable	Bätscher, 2008
OECD 308 Sediment/Water Study GLP	Whole System degradation DT_{50}: 51.3 days at 20°C	Geomean of two marine sediment/ water systems-	Lewis, 2014

5.1.1 Stability

Abiotic degradation

A hydrolysis study (Sydney, 2011) was carried out to OECD test guideline 111 and to GLP using 100% pure medetomidine. A preliminary test was conducted at pH 4, 7 and 9 and at 50 °C and results showed less than 10 % hydrolysis after 120 hours in all samples. This is considered equivalent to a half-life of greater than one year under environmental conditions and no further testing was performed. Medetomidine is considered to be hydrolytically stable at all environmentally relevant pH and temperatures.

An aqueous photodegradation study (Wehrhan, 2009) was carried out in accordance with OECD guideline 316 and to GLP. Test solutions were prepared in ethanol (p.a) at the concentration 1.09 g/L (pH 8.5) and 0.109 g/L (pH 8.1). Tier 1 of the guideline was conducted at 24 °C and the UV-spectrum of the test item was measured in order to estimate the maximum possible direct photolysis rate constant. Absorption of UV light between 290 and 800 nm was low for the test item and the molar decadic adsorption coefficients were below 10 L $mol^{-1}cm^{-1}$. The test item is therefore assumed to be photolytically stable and neither theoretical nor experimental photolytic half lives were determined.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not available.

5.1.2.2 Screening tests

The ready biodegradation of medetomidine was investigated (Bätscher, 2008) in accordance with OECD guideline 301D (CO_2 closed bottle test; 1992) and GLP. Medetomidine hydrochloride (>99.5 % pure) was used instead of medetomidine due to its higher solubility in water, although concentrations were presented based on medetomidine content. Medetomidine concentration and theoretical oxygen demand (ThOD without nitrification) were 2.4 and 5.1 mg/l , respectively.

In the procedure control, the reference item, sodium benzoate, was degraded by an average 74 % until Day 14 of the test, thus confirming the suitability of the activated sludge. In the toxicity control, containing both medetomidine and the reference item, no inhibitory effect on biodegradation of the reference item was found. Thus, Medetomidine, had no obvious inhibitory effect on the activity of activated sludge microorganisms at the concentration tested. The study was considered to be reliable by the UKCA.

No significant biochemical oxygen demand (BOD) of the test substance was recorded throughout the test period (28 days); per cent biodegradation was estimated to be 0 % throughout. Consequently, medetomidine was found to be not readily biodegradable under the conditions of the test.

5.1.2.3 Simulation tests

Two marine sediment/water studies are reviewed in the biocides CAR for medetomidine but, due to deficiencies, the first study by Jørgensen (2010) was considered unreliable by the UKCA. Although it was apparent that medetomidine was relatively persistent in both test systems in this study and supplemental information was supplied (Jørgensen, 2012 and Rinne, 2012), it was not possible to conclude on a degradation pathway or reliable DT_{50} values.

A second study (Lewis, 2014) on medetomidine (98.1 % radiochemical purity) used two natural marine sediment/water systems (labelled Dyfi Estuary site W1 and W2). The sediment was either clay loam or loamy sand. This study was performed to GLP and followed OECD test guideline 308 and is considered reliable. The sample flasks were incubated in darkness and 20 ^{o}C for 100 days. Samples of water and sediment from duplicate test units at each of six time points were analysed for medetomidine and the formation of metabolites. No individual metabolite was present at > 5 % AR (Applied Radioactivity) in either phase at any time point and therefore the study confirms the absence of major aqueous or sediment phase metabolites. Mineralisation increased slowly through the study and at 100 DAT there was a mean of 4.9 % CO_2 in system W1 and 5.8 % CO_2 in system W2.

The percentage of AR present as medetomidine in the surface water and sediment is summarised in Table 22. The study confirmed that medetomidine dissipated from the water phase *via* a combination of degradation and partitioning to sediment, where further limited degradation occurred.

Table 22: Percentage of applied radioactivity (% AR) recovered as medetomidine from the water and sediment phases

Sampling (days)	Site W1			Site W2		
	Water (% AR)	Sediment (% AR)	System (% AR)	Water (% AR)	Sediment (% AR)	System (% AR)
0	97.4	0.0	97.4	99.6	0.0	99.6
0	95.8	0.0	95.8	99.4	0.0	99.4
7	46.9	32.3	79.2	59.0	23.7	82.7
7	31.4	42.1	73.5	54.5	24.3	78.8
14	33.4	38.3	71.7	42.8	26.8	69.6
14	31.7	41.6	73.3	39.6	27.3	66.9
30	16.6	35.6	52.2	30.4	25.4	55.8
30	17.8	33.2	51.0	30.8	24.0	54.8
59	7.6	28.1	35.7	8.0	18.4	26.4
59	9.2	26.8	36.0	15.0	24.8	39.8
100	5.4	31.0	36.4	5.1	23.6	28.7
100	5.6	31.8	37.4	11.4	30.1	41.5

The data was fitted by the UKCA according to FOCUS kinetic guidance in order to estimate degradation rates in aquatic systems. Visual fitting of the water phase dissipation data using single first order (SFO) kinetics was poor with χ^2 values close to or above 15 %. The data were therefore fitted with first order multi-component (FOMC) kinetics; this fitting was visually good, with χ^2 values below 10 %, parameter confidence intervals were acceptable in both systems. Visual fitting of the whole system data using SFO kinetics was acceptable and χ^2 values were less than 10 %. A summary of the whole system modelling data is presented in Table 23.

Table 23: Kinetic fitting data for medetomidine (water and whole system)

System	Compartment	Model	DT_{50}	Visual fitting	X^2 error (%)
W1	Whole system (degradation)	SFO	54.0	acceptable	9.8
W2			48.8	acceptable	9.7

Conclusion of sediment/water simulation test:

The reliable study by Lewis (2014) on the aerobic aquatic degradation of medetomidine confirms the absence of major aquatic or sediment phase metabolites. For biocidal use of medetomidine, guidance from the Technical Meeting on PT21 assessment (anti-fouling paints) stated that a SFO whole system DT_{50} value from the available aerobic aquatic simulation studies should be used as the kinetic input parameter in a first tier approach for exposure modelling. The geomean DT_{50} value for the whole system is **51.3 days** and this was also used to conservatively represent degradation in the water phase. No sediment DT_{50} value could be obtained from the study due to the absence of a clear decline phase in this compartment. For the purposes of hazard classification under CLP, the geomean degradation DT_{50} for the whole system of 51.3 days (range 48.8 to 54 d) is considered indicative of a lack of rapid degradation in this aquatic test system. Mineralisation (and other losses) was low at ≤ 5.8 %. No fresh water system has been tested, however there is no reason to suggest that it would differ greatly from the marine system and it is considered reasonable to read across.

5.1.3 Summary and discussion of degradation

Medetomidine is considered stable in abiotic hydrolysis and photolysis studies.

The substance is not readily biodegradable. In a simulation study on aerobic aquatic degradation in two marine sediment/water systems (Lewis, 2014), the geomean DT_{50} value for degradation in the whole system was determined to be 51.3 days (range 48.8 to 54 d). A reliable sediment degradation DT_{50} could not be obtained.

No information has been submitted on degradation in soil since the principal biocidal use is in the marine environment only.

For the purposes of hazard classification under CLP, medetomidine does not meet the rapid degradability criterion of >70% degradation in a 28-day period. Therefore, it is considered to be not rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

A GLP compliant adsorption/desorption study was conducted in four contrasting soils and one river sediment according to OECD guideline 106 (Völkel, 2006). Summary results are presented in Table 24 below:

Table 24: Summary of adsorption and desorption results for medetomidine

Property	**Soil I**	**Soil II**	**Soil III**	**Soil IV**	**Soil V**
Classification (USDA)	Loam	Clay loam	Silty clay loam	Sandy loam	Silt loam
Organic carbon [%]	1.28	4.13	2.67	0.78	2.00
pH (1:1 H_2O)	7.37	7.55	5.00	7.29	5.36
K_a (adsorption)	20	50	45	32	45
$K_{a,oc}$	1526	1215	1702	4114	2229
Mean $K_{a,oc}$	2157				
K_d (desorption)	24	78	65	41	74

Sorption was noted to correlate reasonably well with organic carbon content and therefore the derivation of K_{aOC} values appears valid. Since total desorption was less than 75 % of the amount adsorbed, the adsorption cannot be considered reversible according to paragraph 79 of OECD 106.

The study progressed as far as the Tier 2 Screening test stage according to OECD 106. No Tier 3 determination of Freundlich adsorption isotherms was performed, and therefore no conclusion on the influence of concentration on adsorption could be reached. According to the testing strategy in Annex I of OECD 106, since the K_d * (m_{sol}/V_0) was > 0.3 (indirect method) a full Tier 3 test should have been performed. The concentration tested in the Tier 2 screening step stage (0.055 mg/l) was noted to be higher than predicted.

Overall, for the purposes of hazard classification, the available data are considered acceptable and the mean K_{aOC} is 2157 ml/g indicating moderate adsorption.

5.2.2 Volatilisation

The measured vapour pressure of medetomidine was determined using OECD 104 (gas saturation method) to be 1.86 x 10^{-4} Pa at 45.14 °C, with estimations from the curve of 8.3 x 10^{-6} Pa at 25 °C and 3.5 x 10^{-6} Pa at 20 °C (Solvias, 2009, OECD 104 to GMP not GLP).

The Henry's Law Constant was calculated to be 1.00 x 10^{-5} Pa m^3/mol (Solvias and Isaksson, 2009). These values indicate that medetomidine is unlikely to partition significantly from the aqueous environment to the air.

5.2.3 Distribution modelling

Not relevant to this report.

5.3 Aquatic Bioaccumulation

A summary of available information on the bioaccumulation potential of medetomidine is presented below:

Table 25: Summary of relevant information on aquatic bioaccumulation

<table>
<tr><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>EC Directive 92/69/EEC Method A. 8 (HPLC method)
GLP</td><td>Log K_{ow} at 20 °C = 3.1 at pH 9, the substance is in the more hydrophobic base form at pH 9, and this represents a worst case for partitioning to organic matter.
<table>
<tr><th>pH</th><th>Temp. (°C)</th><th>Log P</th></tr>
<tr><td>5</td><td>10</td><td>1.1</td></tr>
<tr><td></td><td>20</td><td>1.2</td></tr>
<tr><td></td><td>30</td><td>1.3</td></tr>
<tr><td>7</td><td>10</td><td>2.5</td></tr>
<tr><td></td><td>20</td><td>2.6</td></tr>
<tr><td></td><td>30</td><td>2.6</td></tr>
<tr><td>9</td><td>10</td><td>3.1</td></tr>
<tr><td></td><td>20</td><td>3.1</td></tr>
<tr><td></td><td>30</td><td>3.0</td></tr>
</table></td><td></td><td>Sydney, 2011, 2014</td></tr>
</table>

Calculation method according to TGD part II, 2003 OECD 305 GLP Sheepshead minnow (*Cyprinodon variegates*)	BCF_{fish} = 86.1 BCF (whole fish) = 1	UK CA calculation	Biocide CAR, doc IIA, 4.1.3.2.1 Sharp and Vaughan, 2012

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

A BCF values for fish was calculated in the biocide CAR by the UKCA using the equation provided by the Technical Guidance Document (EC, 2003) for substances with a log K_{ow} of 2-4:

$$\log BCF_{fish} = 0.85 \text{ x } \log K_{ow} - 0.7$$

The maximum log K_{ow} of 3.1 at pH 9 (20 ^{o}C) is used in this calculation.

The resulting log BCF_{fish} = 0.85 x 3.1 - 0.7
→ log BCF_{fish} = 1.935
→ BCF_{fish} = **86.1**

However, as a measured fish BCF is available (see below) and the log K_{ow} is below 4, this estimate is of limited relevance for hazard classification.

5.3.1.2 Measured bioaccumulation data

Table 26: Measurements of aquatic bioconcentration - marine fish

Guideline/ Test method/test substance	Exposure	Initial concentration of a.s.	Steady-state BCF	Uptake rate constant	Depuration rate constant	Metabolites	Reference
OECD 305. GLP. Medetomidine	Flow-through	3.0 and 30 µg/l	0.8 - 1 (whole fish)	1.8 - 2.5	1.7 - 2.6	Not investigated	Sharp and Vaughan, 2012

A reliable fish bioconcentration study to OECD 305 and GLP was provided using Sheepshead minnow (*Cyprinodon variegatus*). Two concentrations of medetomidine (>99% pure) were used, 3.0 and 30 µg a.s./l and measured concentrations were within 80 to 120 % of nominal concentrations. The pH was 7.9 - 8.1, there were no issues reported over solubility. There was a 7 day uptake phase (until steady state) and 7 day depuration phase. At a steady state the highest whole body BCF was 1.0 at 30 µg/l. The amount of medetomidine that accumulated was low and on transfer to clean water the depuration of accumulated residues was 93-98% after 7 days. Lipid normalisation to 5% lipid content gave steady state BCF values of 0.5 and 0.4 at 3.0 and 30 µg/l, respectively. The kinetic bioconcentration factors (BCF_K) or ratio of the uptake rate constant to the depuration rate constant (k_1/k_2), were calculated to be 1.1 and 0.9 for the 3.0 and 30 µg/L test concentrations, respectively. Lipid normalised kinetic BCFs (BCF_{KL}) of 0.5 and 0.5, growth corrected kinetic BCFs (BCF_{Kg}) of 1.1 and 0.9 and 5% lipid normalised growth corrected kinetic BCFs (BCF_{KLg}) of 0.5 and 0.5 were calculated for the 3.0 and 30 µg/L test concentrations, respectively.

Additional bioaccumulation information on uptake and elimination of medetomidine in *Crangon crangon*, *Mytilus edulis,* periphyton and *Abra nitida* (Hilvarsson, Ohlauson, Blanck and Granmo, 2009 a/b). However, due to the quality of these reports, the UK CA did not considered them to be reliable. This information is available in document IIIA of the CAR at A.7.4.3.3.2 (01) and A.7.4.2 (01). An explanation was provided on the uptake and elimination of medetomidine in periphyton since the steady state BCF in this species of 1195 was higher than in other species. The uptake of medetomidine by periphyton was high compared with other organisms although it could not definitely be stated whether this was due to absorption by the organism or due to adsorption to the large surface area. Nevertheless it was shown that the medetomidine was rapidly eliminated in two phases. The first phase was within 30 minutes to 4 hours whilst the second phase took 8 to 48 hours. This is considered to support the argumentation that medetomidine is adsorbed to the surface rather than absorbed by the organism.

5.3.2 Summary and discussion of aquatic bioaccumulation

The measured maximum log K_{ow} for medetomidine is 3.1 at pH 9 (20 ^{o}C), which represents a worst case for aquatic systems due to the limited ionisation of the substance at this pH. This value is below the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate. Reliable information from a fish bioconcentration study shows medetomidine to have a whole fish BCF of 1.0, which is less than the CLP trigger of ≥ 500. This substance is therefore not bioaccumulative for classification purposes.

5.4 Aquatic toxicity

Medetomidine has two stereoisomers; at relevant concentrations in mammalian studies the active isomer is considered to be dexmedetomidine and levomedetomidine is the toxicologically inactive isomer. There is no evidence to indicate that differential toxicity for the two isomers exists for aquatic organisms, however, the ecotoxicological studies have been conducted with the racemate form of medetomidine, which is the form in which it is manufactured and marketed. Medetomidine is also present in two different forms dependent on pH. The pKa of medetomidine is 7.1 (ref. Table 9) so at pH 7.1 the base and salt form will be present in a 50:50 ratio; at pH 9 the base form is approximately 99% and the acid form is 1%. It has been confirmed that the medetomidine used in the ecotoxicological tests was manufactured in the same way as the proposed method for the production of commercial medetomidine. Therefore, the form of medetomidine used in these studies will be the same as that for commercial production. The base and salt ratio could, however, subsequently change depending upon the pH of the test medium. This would only be a concern if there was a differential toxicity expected between the two forms. The pH of sea water is considered to be around 8.0 and there is likely to be around 88 % of the base form and 12 % of the acid form. The pH of the ecotoxicological studies undertaken has been considered to ensure they appropriately reflect the conditions of exposure in the environment and the form medetomidine that will occur in. No significant issues were found with solubility in the reliable ecotoxicological studies presented.

The ecotoxicological test results for technical medetomidine from both acute and chronic studies are summarised in the following tables and sections. A large number of screening studies are available but generally these do not have analytical verification of concentrations, the method of manufacture of the medetomidine is not always provided and they were not to GLP or strictly according to guideline. Only the valid studies are included in the following table and relevant end points from these studies are discussed in further detail below. Additional information and robust study summaries are available in the biocide CAR.

Unless otherwise stated, all the studies listed were performed on medetomidine in the commercially available racemic form and purity. The endpoint quoted is based on whether measured concentrations are within 80-120 % of nominal concentrations in which case nominal concentrations (n) are used, or if the concentrations measured depart more than this then mean measured concentrations (mm) are reported.

Table 27: Summary of acute and chronic toxicity data for aquatic organism (water exposure)

Guideline/ Test method	**Species**	**Endpoint/ Type of test**	**Exposure**		**Results used for hazard assessment**		**Reference**
			Design	**Duration**	**End point**	**Toxicity (mg/l)**	
OECD 203 GLPe	Zebra fish (*Danio rerio*)	Acute toxicity	*Static*	96 hours	LC_{50}	30 mg/l (mm)	Bätscher. R., 2007a.
No guideline available. GLP	Rainbow trout (*Oncorhyncus mykiss)*	Sub-lethal pigmentation	Static	2 hours	NOEC	0.01 mg/l (n)	Maunder *et al.*, 2012
OECD 210. GLP	Sheepshead minnow, *Cyprinodon variegatus*	Chronic: Hatching frequency, survival, length and weight	Flow-through test system	28 days post-hatch	NOEC	Hatchability: 0.32 mg/l (n) Survival: 0.32 mg/l (n) Length: 0.032 mg/l (n) Dry weight: **0.001** mg/l (n)	Vaughan M, Hutchinson K. 2011
OECD 202 GLP	*Daphnia magna*	Acute immobilisation	*Static*	*48 hours*	EC_{50}	4.5 mg/l (mm)	Bätscher.R., 2007b.
OPPTS 850-1055 GLP	Pacific oyster (*Crassostrea gigas*)	Embryo-larval development	Static	48 hours	EC_{50}	2.5 mg/l (n)	Fox M, Sharpe A., 2012a
ASTM E1563-98 GLP	Sea urchin (*Paracentrotus lividus*)	Embryo-larval development	Static	48 hours	EC_{50}	3.2 mg/l (n)	Fox M, Sharpe A., 2012b
OECD 201. GLP	Green alga (*Scenedesmus* [syn. *Desmodesmus*] *subspicatus*)	Growth inhibition test	Static, non-renewal exposure system	72 hours	NOEC	0.12 mg/l (mm)	Bätscher. R., 2007c
					E_rC_{50}	**0.65** mg/l (mm)	
ISO 10253. GLP	Green alga (*Skeletonema costatum*)	Cell multipli-cation inhibition	Static, non-renewal exposure system	72 hours	NOEC	0.253mg/l (mm)	Maunder R. and Vaughan M., 2011
					ErC_{50}	>0.447 mg/l (mm)	

The key acute/short-term endpoint for aquatic classification purposes is the mean measured 72-hour E_rC_{50} for the green alga *Scenedesmus* [syn. *Desmodesmus*] *subspicatus* of 0.65 mg medetomidine/l. This is supported by the second algal E_rC_{50} of >0.447 mg/l for *Skeletonema costatum* which although a 'greater than' value and not as reliable, is in the same concentration range. The key chronic/long-term endpoint for aquatic classification purposes is the nominal dry weight NOEC for the fish *Cyprinodon variegatus* of 0.001 mg medetomidine/l. These data are discussed further below.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

One reliable static 96-hour acute toxicity study is available (to OECD 203 and GLP) using zebra fish *Danio rerio* (Bätscher, 2007a). Test concentrations of medetomidine were 83-122 % of nominals. Although the report referred to medetomidine hydrochloride being used, the batch details simply referred to medetomidine. The 96-hour LC_{50} was 30 mg/l based on the mean measured concentrations. It is of note that the NOEC in this study was <0.32 mg/l based on effects on fish pigmentation being seen at the lowest concentration tested.

5.4.1.2 Long-term and sub-lethal toxicity to fish

i) Sheepshead minnow early life stage

Vaughan and Hutchinson (2011) investigated the toxicity of medetomidine to early life stages of sheepshead minnow (*Cyprinodon variegates*) according to OECD guideline 210 and GLP. This was a standard flow-through early life stage study undertaken in normal laboratory light conditions (photoperiod 16 hours); effects were reported at 28 days post-hatch. The initial nominal test concentrations used were: 0, 1.0, 3.2, 10, 32, 100 and 320 µg/l. The mean measured test concentrations in the study were 105-120 % of the nominal concentrations and therefore results are based on nominals. The results for each of the main parameters investigated in this key study are summarised in the following table:

Table 28: Summary of chronic toxicity of medetomidine to sheepshead minnow

Nominal test concentration (µg/l)	Hatching frequency (pooled)	Survival	Mean length (mm) (pooled)	Mean weight (mg dw) (pooled)
0	95%	100% 100%	18.95	48.9
1.0	98%	100% 97%	19.6	49.3
3.2	97%	100% 97%	18.95	43.7*
10	95%	97% 100%	18.74	43.4*
32	93%	100% 100%	18.24	39.0*
100	95%	100% 100%	17.43*	33.2*
320	98%	97% 100%	15.43*	23.1*

Results are presented either for the pooled or individual treatment replicates

* Statistically significant difference from control

NOEC values were reported for the following parameters: hatchability: 320 µg/l; survival: 320 µg/l; length: 32 µg/l; dry weight: 1.0 µg/l (0.001 mg/l). Fish of a paler colour were noted at 10 to 320 µg/l but not at lower concentrations. Hence, although a statistically determined NOEC was not provided for fish pigmentation, it was considered to be 3.2 µg a.s./l. It should be noted that an effect on fish colouration is seen at a lower concentration in sheepshead minnow than in rainbow trout. The relevance of pigmentation for hazard classification is discussed further below. Overall the lowest NOEC from this study was a nominal 0.001 mg/l based on dry weight. The study was considered to be reliable.

ii) Rainbow trout sub-lethal effects

The short-term (two hour exposure period) study by Maunder *et al*., 2012 was not conducted to guideline as it was a specifically designed study to examine the sub-lethal effect of medetomidine on fish pigmentation, however it was performed in accordance with GLP and is considered reliable. The study was undertaken in standard laboratory light conditions. The concentrations of medetomidine in this study were 88-100 % of nominals and hence results were based on nominal concentrations. The NOEC of 0.01 mg/l (10 µg/l) was based on a reduction in the pigmentation in fish (affected fish were paler in colour and the grey scale intensity was reduced).

Additional Studies in Fish:

A number of other studies in fish are available. These are reviewed in detail in the biocides CAR, however, due to deficiencies in the methodology and/or reporting of these studies they are not considered to be reliable and they have not been included here.

Many of these non-guideline studies were conducted to investigate the sub-lethal effects of medetomidine on pigmentation in fish. This is of uncertain relevance to hazard classification, however the issue is discussed in some detail in document IIA to the CAR (Section 4.2.5.2). Some studies on pigmentation reported effects as low as 0.0001 mg/l, however the reliability of these data is also questioned.

Conclusion on NOEC for fish:

Overall, it is concluded in the CAR that the use of the dry weight endpoint from the standard fish early life stage (FELS) study also covers any pigmentation effects. This reliable and GLP study by Vaughan and Hutchinson (2011) using sheepshead minnow gave a NOEC of 0.001 mg/l based on fish dry weight. This chronic study does not use the same species as the key acute study (rainbow trout), however, other than pigmentation, no major differences in sensitivity between the species are highlighted in the CAR and sensitivity is expected to be broadly similar over the same time frame. This is discussed further in relation to the chronic M-factor.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 29: Acute toxicity of medetomidine to aquatic invertebrates

Guideline/ test method/ test substance	Species	Endpoint/ Type of test	Exposure		Results			Remarks	Reference
			Design	Duration	EC_0	EC_{50}	EC_{100}		
OECD 202 GLP Medetomidine	*Daphnia magna*	Acute immobilization test	Static	48 hours	3.2 mg/l (at 24-hours; a 48 hour value could not be determined) (mm)	4.5 mg/l (mm)	34 mg/l (mm)	-	Bätscher R., 2007b.
EPA OPPTS 850-1055 GLP Medetomidine	Pacific oyster *Crassostrea gigas*	Embryo-larval development	Static	48 hours	1 mg/l (n)	2.5 mg/l (n)	na	-	Fox M, Sharpe A., 2012a
ASTM E1563-98 GLP Medetomidine	Sea urchin *Paracentrotus lividus*	Embryo-larval development	Static	48 hours	1 mg/l (n)	3.2 mg/l (n)	na		Fox M, Sharpe A., 2012b

n = nominal concentration; mm = mean measured concentration; na = not available.

Three short-term guideline studies that have been performed according to GLP are available on *Daphnia magna*, the pacific oyster (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*). These data are summarised above. For the *Daphnia* study, although the report referred to medetomidine hydrochloride being used, the batch details simply referred to medetomidine. The mean measured concentrations in the study were in the range of 43% (only at the 0.032 mg/l concentration) - 105% of nominals, so results were based on mean measured concentrations. In the oyster study, measured concentrations were between 93-103% of nominal concentrations and so results were based on nominal concentrations. In the echinoderm study the measured concentrations were between 102-111% of nominal concentrations hence results were based on nominal concentrations. The 48-hour EC_{50} values were 4.5 mg/l, 2.5 mg/l and 3.2 mg/l in *Daphnia*, oyster and sea urchin respectively. These studies and endpoints are considered reliable and relevant for acute aquatic hazard classification - the lowest being the EC_{50} of 2.5 mg/l for oyster.

Additional Studies in aquatic invertebrates:

A number of additional short-term studies are available. However, due to deficiencies in methodology and/or reporting, these were not considered by the UKCA to be reliable and they are not included here.

5.4.2.2 Long-term toxicity to aquatic invertebrates

A chronic study on mysid shrimp reproduction and growth to EPA OPPTS 850.1350 (Bjørnestad, 2010) and a study on embryonic development in the mussel, *Mytilus edulis* (Bellas, Granmo and

Ohlauson, 2009) are included in the biocide CAR for medetomidine, however the UKCA considers these studies to be unreliable and so they are not included here. A reliable long-term NOEC for the invertebrate taxonomic group is not available.

5.4.3 Algae and aquatic plants

i) Growth inhibition studies on algae

Table 30: Growth inhibition of medetomidine on algae

Guideline/ test method/test substance	Species	Endpoint/ Type of test	Exposure		Results			Remarks	Reference
			design	duration	NOE_rC	E_bC_{50} or E_yC_{50}[1]	E_rC_{50}		
OECD Guideline 201. GLP. Medetomidine	*Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*)	Growth inhibition test	Static, non-renewal exposure system	72-hours	0.12 mg/l (mm)	E_bC_{50}: 0.34 mg/l (mm)	0.65 mg/l (mm)	-	Bätscher. R., 2007c
ISO 10253 Guideline. GLP. Medetomidine	*Skeletonema costatum*	Cell multipli-cation inhibition	Static, non-renewal exposure system	72-hours	0.253 mg/l (mm)	E_yC50: 0.504 mg/l (mm)	>0.447 mg/l (mm)[2]	-	Maunder R. and Vaughan M., 2011

[1] calculated from the area under the growth curve, E_bC_{50} or E_yC_{50} values are not normally used for hazard classification, instead the E_rC_{50} is preferred.
[2] determined by CA.
mm = mean measured concentration.

Data have been submitted from two algal studies on medetomidine, these followed the respective guidelines without significant deviation and were conducted to GLP; the CA considers them to be reliable.

For the *Scenedesmus* [syn. *Desomodesmus*] study (Bätscher. 2007c), although the report referred to medetomidine hydrochloride being used, the batch details simply referred to medetomidine (>99.5% pure). Initial nominal concentrations were: 0 (control); 0.0032; 0.010; 0.032; 0.10; 0.32; 1.0 and 3.2 mg/l. At the test start the measured concentrations at 0.1 to 3.2 mg/l were between 39-92 % of nominal values. The measured concentrations at the test end were between <0.1 mg/l and 55 % of the nominal concentrations (at the lower nominal concentrations of 0.0032 to 0.32 mg/l the concentrations could not be determined). No reason is given for the low recoveries (adsorption to algal cells is possible), however the results were based on mean measured concentrations.

In the *Skeletonema* study (Maunder and Vaughan, 2011) the initial nominal concentration were: 0 (control); 0.056, 0.1, 0.18, 0.32, 0.56 and 1.0 mg/l. The medetomidine was 99.7 % pure. Measured concentrations were between 76-84 % of nominals and so results were based on mean measured values. The test item had a statistically significant inhibitory effect on the growth (yield and growth rate) after 72 hours at the highest measured concentration of 0.844 mg/l and on growth rate at 0.447 mg/l. Thus, 0.447 mg/l was determined as the 72-hour LOEC for growth rate. The 72-hour NOEC for growth rate was determined to be 0.253 mg/l. An accurate E_rC50 was not calculated but would be >0.477 mg/l.

Overall the lowest algal endpoints for classification purposes are the 72-hour E_rC_{50} of 0.65 medetomidine/l and the NOEC of 0.12 mg/l, both for *Scenedesmus* [syn. *Desmodesmus*] *subspicatus*. An accurate E_rC50 could not be calculated for *Skeletonema costatum* but at >0.477 mg/l is expected to be in a similar concentration range.

ii) Effects on phytotoxicity and structure in aquatic microalgal communities

Short-term non-guideline studies on microalgal communities are summarised in the CAR (Ohlauson etal., 2008 and Ohlauson, 2008 a/b). Community studies are not used for classification purposes however, it is noted that the results from these studies support the NOECs for algae indicated above.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Effects on sediment-dwelling organisms

No acute toxicity data were provided on the toxicity of medetomidine to sediment-dwellers. The available chronic toxicity studies on *Lumbriculus variegatus* (Goodband, 2013) and the marine amphipod *Corophium volutator* (Hutchinson, 2011) summarised in the biocides CAR were sediment-spiked studies which are of uncertain use regarding classification in the aqueous phase. Further studies on the effects of medetomidine on pheromone-induced mate-search behaviour in *Corophium volutator* (Krång and Dahlström, 2006) and burrowing activity in the sea urchin *Brissopsis lyrifera* (Granmo and Ohlauson, 2008) did include exposure via the water phase but were given a reliability score of 4 by the CA and are also of uncertain relevance to classification.

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

Medetomidine is considered not rapidly degradable and not bioaccumulative for classification purposes. No metabolites were present at >5% AR in a sediment/water simulation study at any time point. Therefore, the classification is based on medetomidine ecotoxicity only.

Valid data are available for acute and chronic toxicity in fish and algae, and acute toxicity in invertebrates. Algae are the most acutely sensitive trophic group with a reliable 72-hour mean measured E_rC_{50} of 0.65 mg/l for *Desmodesmus subspicatus* [syn. *Scenedesmus subspicatus*]. Fish are the most chronically sensitive aquatic organisms with a 28-day NOEC of 0.001 mg/l for effects on dry weight in *Cyprinodon variegatus*. As discussed above, this is considered to cover any potential sub-lethal effect on pigmentation. No reliable or relevant chronic invertebrate NOEC is available but there is no impact on the chronic classification as the surrogate approach based on acute invertebrate toxicity would not lead to a higher M-factor.

Based on available acute and chronic data (and because medetomidine is not rapidly degradable) where $L(E)C_{50}$ values are below 1 mg/l, classification with Aquatic Acute 1 is applicable. Where the long-term NOEC is below 0.1 mg/l, classification with Aquatic Chronic 1 is applicable.

An acute M-factor of 1 is applicable based on the algal E_rC_{50} of 0.65mg/l is in the range $0.1 < L(E)C_{50} \leq 1$ mg/l.

A chronic M-factor of 100 is applicable based on the fish NOEC of 0.001 mg/l being in the range $0.0001 < NOEC \leq 0.001$ mg/l for a non-rapidly degradable substance. This is also considered to help cover any differences in sensitivity between the fish species used in acute and chronic tests.

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

Aquatic Acute 1; H400: Very toxic to aquatic life
Acute M-factor = 1
Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects
Chronic M-factor = 100

6. OTHER INFORMATION

None

7. REFERENCES

All references should be viewed as references to the Competent Authority Report – March 2014 – Document IIA – Effects Assessment for the Active Substance – Medetomidine and the relevant sections of Document IIIA – Study Summaries, prepared by the UK for the review of the active substance under Regulation (EU) No 528/2012.

Full References:

CAR Section	Author	Date	Study title
IIIA 3-1	L. Nilsson R. Bordes D. Ostrovskii	2008	Medetomidine: NMR, UV-Vis and IR spectra, Surface Tension, Thermal Stability, Water Solubility and Some Predicted Properties. Chalmers University of Technology, Gothenburg, Sweden. 2008 Not GLP Unpublished
IIIA 3-2	M. Mannonan H. Makinen J. Olkarlnen	2004	European Drug Master File. Medetomidine hydrochloride for veterinary use. Fermion Oy., FIN-02101 Espoo, Finland Non- GLP. Unpublished
IIIA 3-3	A. Solvias	2009	Physical Characterization of Medetomidine Base. Not GLP Unpublished
IIIA 3-4	P. Sydney	2011	Medetomidine Physicochemical Properties Huntingdon Life Sciences GLP Unpublished
IIIA 3-6	T. Pullinger	2013	Medetomidine Aqueous Solubility (pH5, 7 and 9) and effect of temperature (10, 20 and 30°C), 2013 Huntingdon Life sciences GLP Unpublished
IIIA 4/4.1.1.1	Bätscher R	2008	Medetomidine: Ready biodegrability in a closed bottle test. RCC Ltd, Switzerland. Study Number: A00360 GLP / Unpublished
IIIA 4/4.1.1.1.	Jørgensen C.	2010	Aerobic transformation of medetomidine in aquatic sediment. DHI, Denmark, Study Number: 072-0773 GLP/Unpublished
IIIA 4/4.1.1.1.	Jørgensen C.	2012	Aerobic transformation of medetomidine in aquatic sediment. Supplemental study. DHI, Denmark. GLP/Unpublished
IIIA 4/4.1.1.2	Sydney P.	2011	Medetomidine. Abiotic degradation: Hydrolysis as a function of pH. Huntingdon Life Sciences, UK. Study Number. FGT0007 GLP/Unpublished
IIIA 4/4.1.1.2	Wehrhan, A	2009	Aqueous Photolysis. Harlan Laboratories Ltd. Switzerland. Study Number: C39435 GLP/Unpublished
IIIA 4/4.1.1.3	Völkel W	2006.	Adsorption/desorption of [^{14}C]-Medetomidine RCC Ltd, Switzerland. Study Number: A1945 GLP / Unpublished
IIIA 6 – Acute dermal	Bull, A	2010	Medetomidine: Acute Dermal Toxicity to the Rat. Study Number: FGT0001 GLP/Unpublished

CAR Section	Author	Date	Study title
IIIA 6 – Acute inhalation	Nagy K	2009	Acute inhalation toxicity study (nose-only) in the rat. LAB Study Code: 09/056-004P GLP/Unpublished.
IIIA 6 – Acute ip	Hirsimäki P	1984	The acute intraperitoneal toxicity of MPV-785 in rat, study report medet 4/18 pages1-8 GLP / Unpublished
IIIA 6 – Acute iv	Hirsimäki P	1984	The acute intravenous toxicity of MPV-785 in rat, study report medet **4/16** pages1-9 GLP / Unpublished
IIIA 6 – Acute oral	Hirsimäki P	1984	The acute oral toxicity of MPV-785 in rat, Study report department 4/19 pages 1-8 GLP / Unpublished
IIIA 6 – Acute oral	Hirsimäki P	1984	The acute oral toxicity of MPV-785 in mouse, Study report department 4/15 pages 1-10 GLP / Unpublished
IIIA 6 – Acute sc	Hirsimäki P	1984	The acute subcutaneous toxicity of MPV-785 in rat, study report medet **4/17** pages1-9 GLP /Unpublished
IIIA 6 – Acute toxicity	Scheinin H	1989	Pharmacological effects of medetomidine in humans. Acta vet. Scand. Volume **85**, pages 145-147. Not GLP /Published
IIIA 6 – Acute toxicity	Dasta J et al,	2004	Comparing Dexmedetomidine Prescribing Patterns and Safety in the Naturalistic Setting Versus, Ann Pharmacother volume 38 pages 1130-5 Not GLP / published
IIIA 6 – Acute toxicity	Gerlach A	2007	Dexmedetomidine: an updated review. Ann Pharmacother; 41 pages 245-254 No GLP /published
IIIA 6 – Acute toxicity	Abbott	1998	DEXMEDETOMIDINE CLINICAL STUDY REPORT. DEXMEDETOMIDINE DOSE-RANGING STUDY TO EVALUATE THE EFFECTS OF DEXMEDETOMIDINE ON SEDATION. Abbott – 85499 GCP / UNPUBLISHED
IIIA 6 – Acute toxicity	Jorden V	2004	Dexmedetomidine overdose in the perioperative setting, Ann Pharmacother **38** pages 803-7 (published)
IIIA 6 – Dermal abs	Scheinin M	2011	Absorption through skin of medetomidine from antifouling paint – a clinical study in healthy volunteer subjects. Study Number C460 GLP & GCP/ Unpublished.
IIIA 6 – Eye irritation	Ranta-Paula V.	2010	Acute eye irritation/corrosion test with medetomdiine Study number: KEK 0100104-09002 GLP/Unpublished
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8. ANNEXES

None