Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Medetomidine; (*RS*)-4-[1-(2,3-dimethylphenyl)ethyl] -1*H*-imidazole

EC Number: -
CAS Number: 86347-14-0

CLH-O-0000001412-86-85/F

Adopted

4 December 2015

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4 December 2015

CLH-O-0000001412-86-85/F

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

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

Chemical name: **(*RS*)-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole; Medetomidine**

EC number: **not available**

CAS number: **86347-14-0**

The proposal was submitted by the **United Kingdom** and received by RAC on **21 January 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

The United Kingdom has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 February 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **20 March 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Riitta Leinonen**

Co-rapporteur, appointed by RAC: **Zilvinas Uzomeckas**

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

The RAC opinion on the proposed harmonized classification and labelling was reached on **4 December 2015** and was adopted by **consensus** .

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	TBD	(*RS*)-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole; Medetomidine	Not available	86347-14-0	Acute Tox. 2 Acute Tox. 2 STOT SE 3 Aquatic Acute 1 Aquatic Chronic 1	H300 H330 H336 H400 H410	GHS06 GHS09			M = 1 M =100	
RAC opinion	TBD	(*RS*)-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole; Medetomidine	Not available	86347-14-0	Acute Tox. 2 Acute Tox. 2 STOT SE 1 STOT SE 3 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H300 H330 H370 (eye) H336 H372 H400 H410	GHS08 GHS06 GHS09 Dgr	H300 H330 H370 H336 H372 H410		M=1 M=100	
Resulting Annex VI entry if agreed by COM	TBD	(*RS*)-4-[1-(2,3-dimethylphenyl)ethyl]-1*H*-imidazole; Medetomidine	Not available	86347-14-0	Acute Tox. 2 Acute Tox. 2 STOT SE 1 STOT SE 3 STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H300 H330 H370 (eye) H336 H372 H400 H410	GHS08 GHS06 GHS09 Dgr	H300 H330 H370 H336 H372 H410		M=1 M=100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

In a standard flammability study (EC A10), medetomidine was found not to be 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 chemical structure, medetomidine is not considered to posses explosive or oxidising properties.

The Dossier Submitter (DS) concluded that no classification was appropriate; data were conclusive but not sufficient for classification for physical hazards

Comments received during public consultation

One MS commented on the reported flash point for medetomidine (page 15 in the CLH report) that the active substance is a solid and a flash point is not suitable for a solid.

Assessment and comparison with the classification criteria

RAC agrees with the Dossier Submitter's proposal **not to classify medetomidine for physical hazards**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral

Two acute oral toxicity studies (non-guideline but similar to OECD TG 401, no deviations, GLP) in rats and mice with a 21-day observation period were included in the CLH report. Both studies were carried out at the same time in the same laboratory. In the study with male rats (Sprague-Dawley), no deaths were observed at any dose (0.05, 1.25, 6.25 and 31.25 mg/kg bw). The LD_{50} value from this study was > 31.25 mg/kg bw. In the other study, female mice (NMRI) were exposed to 0.05, 0.25, 1.25, 6.25, 31.25, 156.25, 234.75 and 312.5 mg/kg bw of medetomidine. Deaths were observed at dose levels ≥ 6.25 mg/kg bw; one animal (1/5) died at this dose and there were no survivors at higher doses. An LD_{50} value of 11 mg/kg bw was derived from the mouse study. This meets the criteria in the CLP Regulation for classification as Acute Tox 2; H300.

The DS proposed to classify medetomidine for acute toxicity via the oral route as Acute Tox 2; H300: Fatal if swallowed.

Inhalation

One acute inhalation toxicity study (OECD TG 403, GLP) in male and female rats (Wistar CRL(WI)BR) with a 21-day observation period was included in the CLH report. Rats were exposed to 0.1, 0.2 and 0.5 mg/L of medetomidine for 4 hours. Deaths occurred at all dose levels: 10% (1/10), 90% (9/10) and 100% (10/10) of animals died at dose levels of 0.1, 0.2 and 0.5 mg/L, respectively. An LC_{50} value of 0.14 mg/L for 4 hours was derived. This meets the criteria in the CLP Regulation for classification as Acute Tox 2; H330.

The DS proposed to classify medetomidine for acute toxicity via inhalation as Acute Tox 2; H330: Fatal if inhaled.

Dermal

One acute dermal toxicity study (OECD TG 402, GLP) in rats (Crl:CD Sprague-Dawley) was included in the CLH report. The highest dose of medetomidine (2000 mg/kg bw) caused the death of 2/5 rats in both the male and female groups (one died and one was killed in extremis). Lower doses (30, 60 and 400 mg/kg bw) given only to female rats did not cause any deaths. An LD_{50} value > 2000 mg/kg bw was derived. This is higher than the limit values for classification and no classification is warranted.

The DS proposed to not classify medetomidine for acute toxicity via the dermal route.

Other routes

In addition to standard routes of exposure, the results of acute toxicity studies via intravenous (*i.v.*), intraperitoneal (*i.p.*) and subcutaneous (*s.c.*) routes in rats where briefly described in the CLH report. Deaths were observed only following exposure via the *i.v.* (LD_{50} 1.8 mg/kg bw) and *s.c.* (LD_{50} 20 mg/kg bw) routes whereas no deaths occurred via the *i.p.* route (LD_{50} > 31.25 mg/kg bw).

Human information

Medetomidine or dexmedetomidine (the active isomer of medetomidine) have been used in human studies to investigate the dose response relationship for sedation using single or continuous infusion (up to 24 h) of dexmedetomidine, the effects of accidental dexmedetomidine overdose (*i.v.*), the pharmacological effects of medetomidine in physiological saline (*i.v.*), the occurrence of adverse drug reactions in response to *i.v.* infusion of dexmedetomidine, and the potential for dexmedetomidine to be used as a sole *i.v.* anaesthetic agent.

Based on these studies, the lead effect is sedation. Sedation was reported to be observed within 15 min of administration with recovery observed between 1 - 4 hours after administration. Other reported effects of medetomidine or dexmedetomidine include hypotension, bradycardia, hypertension, reduced salivation, decreased heart rate and decreased cardiac output. A NOAEL of 0.4 µg/kg bw was derived for medetomidine in humans.

Comments received during public consultation

One MS agreed to the classification proposal Acute Tox 2; H300: Fatal if swallowed, and H330: Fatal if inhaled.

Assessment and comparison with the classification criteria

Oral

According to CLP, the preferred test species for evaluation of acute toxicity by the oral route is the rat. When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD_{50} value from valid, well-performed tests. Generally the classification for acute toxicity is based on the lowest LD_{50} from the most sensitive species. The classification proposal is based on two acute oral toxicity studies (non-guideline but similar to OECD TG 401 with no deviation, GLP) in rats and mice. The maximum dose (31.25 mg/kg bw) did not cause any deaths in the rat study indicating an LD_{50} > 31.25 mg/kg bw. An LD_{50} of 11 mg/kg, derived from the mouse study with more extensive dose range, meets the criteria for classification as Acute Tox 2 via oral route (5 mg/kg bw < ATE $\leqslant$ 50 mg/kg bw).

RAC agrees with the proposal of the DS to classifiy medetomidine for acute toxicity via the oral route as **Acute Tox 2; H300: Fatal if swallowed**.

Inhalation

Based on one guideline acute inhalation toxicity study (OECD TG 403, GLP), LC_{50} values of 0.14 mg/L (male & female), 0.17 mg/L (male) and 0.12 mg/L (female) for 4 hours were derived. These values meet the criteria for classification as Acute Tox 2 via the inhalation route (0.05 mg/L < ATE $\leqslant$ 0.5 mg/L).

RAC agrees with the proposal of the DS to classify medetomidine for acute toxicity via inhalation route as **Acute Tox 2; H330: Fatal if inhaled**.

Dermal

Based on one guideline acute dermal toxicity study (OECD TG 402, GLP), LD_{50} value > 2000 mg/kg bw was derived. This value does not meet the criteria for acute toxicity classification via the dermal route (ATE $\leqslant$ 2000 mg/kg bw).

RAC agrees with the proposal of the DS to **not classify** medetomidine for acute toxicity via dermal route.

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

Summary of the Dossier Submitter's proposal

Acute toxicity studies revealed medetomidine-induced sedation and/or related clinical signs (lethargy, underactivity) in all studied species via all administration routes at lower dose levels than those causing mortality. Effects in the eyes were also observed following exposure via the oral and inhalation routes. The DS found it likely that the keratitis and opacity in the eyes were a result of desiccation of the cornea as a result of the medetomidine-induced exophthalmos and partially closed eyelids. Therefore, the DS considered they are secondary effects and are not relevant for classification. No effects in the eyes (except partially closed eyes, brown staining and dilated pupils) were observed in the dermal study.

A number of other changes in various organs were observed in decedents or those killed *in extremis*. These included haemorrhagic lungs, pale liver, congestion of the heart and distended abdomen. The DS concluded that these changes are not considered to represent target organ toxicity but are indicative of non-specific (or secondary to) general acute toxicity. These effects were not noted in surviving animals. The DS proposed to not classify medetomidine in STOT-SE category 1 or 2, as there was no clear evidence of specific toxic effects on a target organ or tissue.

As sedation and/or related clinical signs (lethargy, underactivity) were clearly observed in non-human studies in all studied species via all studied routes at clearly lower doses than those causing lethality and the signs of sedation appeared to be transient, the DS proposed to classify medetomidine as STOT-SE 3; H336: May cause drowsiness or dizziness. Medetomidine-induced transient sedation has also been shown in various human studies. Classification as STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

In both humans and animals, the severity of the sedative effect was reported to increase with dose.

Comments received during public consultation

One MS agreed with the proposal for classification as STOT-SE 3; H336: May cause drowsiness or dizziness.

Assessment and comparison with the classification criteria

In an acute oral toxicity study in rats, medetomidine did not cause deaths at any dose (0.05, 1.25, 6.25, 31.25 mg/kg bw). Sedation was observed at all dose levels. Other clinical signs of toxicity were a crouched position, piloerection, exophthalmos, shallow respiration and red discharge around the eyes, mouth and nostrils at the two highest dose levels (6.25 and 31.25 mg/kg bw). The eyes of these animals appeared opaque 14-days post administration. Exophthalmos was also observed at dose level of 1.25 mg/kg bw. Histopathological analysis of the eyes from the animals at the highest dose level identified subchronic keratitis (in both eyes in 2 of 3 animals; and in 1 eye in the 3rd animal). At 6.25 mg/kg bw, the eyes appeared histologically normal.

In the acute oral toxicity study in mice, medetomidine (0.05, 0.25, 1.25, 6.25, 31.25, 156.25, 234.75, 312.5 mg/kg bw) caused deaths at doses ≥6.25 mg/kg bw (1 of 5 animals died at this dose level, no animal survived at higher dose levels). Clinical signs of toxicity included sedation (at doses ≥0.25 mg/kg/bw), piloerection and exophthalmos (at doses ≥1.25 mg/kg bw), crouched position and convulsions (at doses ≥6.25 mg/kg bw). At 6.25 mg/kg bw, 3 of 5 animals developed opaque eyes of which only 1 displayed chronic keratitis. Gross pathological examination of animals that died revealed bright red toes, haemorrhagic lungs, pale liver and gas in the gastrointestinal track. Vacuolisation of the liver was also observed in 2 of 5 animals at the highest dose level.

In an acute inhalation toxicity study in rats, 10, 90 and 100% of animals died at dose levels of 0.1, 0.2, 0.5 mg/L aerosol of medetomidine, respectively. Clinical signs of toxicity were observed at all doses. These included laboured respiration and increased respiratory rate on the day of exposure, lethargy, ataxia, exophthalmos and opacity of the eyes, hunched posture, red discharge from eyes, eyes partially closed and continuous tremors at all dose levels. At the highest dose, animals displayed aggressiveness. Marked bodyweight loss was observed in the animals at the lowest dose (0.1 mg/L aerosol) during the first week. Gross pathological findings in animals killed *in extremis* included red discoloration of the lungs, pale mottling of the liver, dark/red thymic discoloration, and enlargement of the stomach, red mottled pancreas and presence of red firm material associated with red mucosal discoloration of the urinary bladder. Findings in animals that died during the post-exposure period included red discoloration and/or non-collapsing of the lungs, and bilateral discoloration of the conjunctivae. No gross pathological findings were found in survivors.

In an acute dermal toxicity study in rats, medetomidine (30, 60, 400, 2000 mg/kg bw) caused mortality at the highest dose level: 2 rats died (one male and one female) and 2 rats were killed *in extremis* (one male and one female). Clinical signs of toxicity were observed at all dose levels. These 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. At the highest dose, surviving animals also had black faeces, piloerection, distended abdomen, maloccluded teeth, and dilated pupils and splayed hind limbs. At all doses, reduced bodyweight gain and bodyweight loss were observed. Gross pathological examination of animals that died revealed congestion of the heart and lungs, and brown/yellow fluid of the duodenum, small and large intestines. In some surviving animals, thickened tissues and gaseous distension of the GI tract were observed. No other gross pathological findings were found in surviving animals.

The acute toxicity studies via *i.v.*, *i.p.* and *s.c.* routes in rats were only briefly described in the CLH report. Mortality occurred only via *i.v.* and *s.c.* routes. The same clinical signs of toxicity were observed across all studies independent of the exposure route and included sedation, exophthalmos, convulsions, piloerection, opacity of the eyes, and red discharge from the mouth, nostrils and eyes. The CLH report did not contain information on dose levels used at these studies. Therefore, it is unclear what dose levels caused above mentioned clinical signs. Only calculated LD_{50} values were reported for *i.v.*, *i.p.* and *s.c.* administration routes, these being 1.8, >31.25 and 20 mg/kg bw, respectively.

Classifications for STOT-SE and acute toxicity are independent of each other and both may be assigned to a substance if the respective criteria are met. However, care should be taken not to

assign both classifications for the same effect, i.e. a double classification for the same effect has to be avoided. STOT-SE will be considered where there is clear evidence for a specific organ toxicity, especially in absence of lethality.

All the reported acute toxicity studies, regardless of the administration route, indicated that medetomidine causes sedation and/or other related signs (lethargy, underactivity). These effects were observed at much lower doses than those causing lethality. However, the levels of sedation at different dose levels were not precisely described. In surviving animals, signs of sedation also appeared to be transient, according to the CLH report. Data from humans also shows that medetomidine-induced sedation is transient. Human data also indicates that (dex)medetomidine-induced sedation increases dose-dependently. Classification in STOT-SE category 3 covers transient effects occurring after a single dose and includes only narcotic effects and respiratory tract irritation. Narcotic effects may range from slight dizziness to deep unconsciousness. Therefore, the data reported in the CLH report fulfil the classification criteria and clearly warrant classification for medetomidine as STOT-SE 3. This is supported also by the MoA of medetomidine: an α_2-adrenergic agonist known to reduce excitation of the central nervous system. It is used as a surgical anaesthetic and analgesic in veterinary medicine and as a sedative in human medicine.

Eye effects were observed via oral (rat and mouse studies) and inhalation (rat study) routes. In rats, oral administration of medetomidine caused exophthalmos at dose levels ≥ 1.25 mg/kg bw. Histopathological analysis revealed subchronic keratitis at the highest dose (31.25 mg/kg bw). In the oral rat study, medetomidine did not cause mortality at any dose level. After oral administration of medetomidine in mice, exophthalmos and opacity of the eyes were observed at doses ≥ 1.25 and at 6.25 mg/kg bw, respectively. Mortality was observed at ≥ 6.25 mg/kg bw (1 of 5 animals died at this dose, no survivors at higher doses). In acute inhalation study in rats, medetomidine caused exophthalmos and opacity of the eyes, and eyes were partially closed at doses ≥ 0.1 mg/L aerosol. At the lowest dose (0.1 mg/L), 1/10 animals died. In the dermal acute toxicity study, no clear eye effects were observed, only partially closed eyes at all dose levels and dilated pupils at the highest dose (2000 mg/kg bw). In rats, i.p. administration did not cause mortality, but sedation, exopthalmos, opacity of the eyes and red discharge from eyes were observed at all doses. Similar effects but also deaths were observed following exposure via the i.v. or s.c. routes.

If all of the observed eye effects can be regarded as secondary to sedation, no STOT-SE Category 1 or 2 classification is warranted. The DS considered it likely that the keratitis and opacity in the eyes were a result of desiccation of the cornea as a result of the medetomidine-induced exophthalmos and partially closed eyelids. However, the mechanism behind medetomidine-induced exophthalmos is not clear, i.e whether it is secondary to the sedation or whether it is a primary effect. Medetomidine doses causing the eye effects via the oral route without mortality were ≤ 300 mg/kg bw, which is the limit for STOT-SE 1 classification according to guidance value ranges for single-dose exposures. Via the inhalation route the limit for STOT-SE 1 classification according to guidance value ranges for single-dose exposures is ≤ 1.0 mg/L/4h. In the acute inhalation toxicity study, the lowest dose (0.1 mg/L/4h) caused 10% mortality (1 of 10 rats died). RAC is of the opinion that medetomidine-induced eye effects can be regarded as evidence that medetomidine can be presumed to have the potential to produce significant toxicity in humans following single exposure and STOT-SE 1 classification is warranted, because toxic effects were produced in animals at generally low exposure concentrations. If only opacity of the eyes and/or keratitis could be considered to be severe enough for STOT-SE 1 classification, this classification would probably not be needed because these eye effects occurred at the same dose levels as deaths (except in the oral rat study), and are therefore covered by the Acute Tox. 2 classification. However, RAC also regards exophthalmos as a significant toxic effect which is relevant to human health. Therefore, STOT-SE 1 classification is warranted. Exophthalmos was observed at lower dose levels than mortality (except in an inhalation rat study in which 1/10 animals died at the lowest dose level).

A number of other changes in various organs were observed in decedents or those killed *in extremis*. RAC agrees with the conclusion of the DS, that these changes are not considered to

represent target organ toxicity but are indicative of non-specific (or are secondary to) general acute toxicity and do not fulfil the criteria for STOT-SE classification. These effects were not noted in the surviving animals.

RAC agrees with the proposal of the DS to classify medetomidine for specific target organ toxicity - single exposure as **STOT-SE 3; H336: May cause drowsiness or dizziness**. Further to this, RAC concludes that medetomidine should also be classified as **STOT-SE 1; H370 (eye): Causes damage to eyes**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

One skin irritation study (OECD TG 404, GLP) was included in the CLH report. Medetomidine base under semi-occlusive conditions for 4 hours did not cause any signs of irritation in female rabbits (New Zealand White) at any of the time points (24, 48 and 72 h).

No information from humans was available.

The DS proposed to not classify medetomidine for skin corrosion/irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The standard *in vivo* test to study skin irritation (OECD TG 404, GLP) did not reveal any signs of corrosion or irritation during the observation period of 72 h.The average scores for three consecutive days (24, 48 and 72 h) were 0 for each of the three test animals for both erythema and oedema. Therefore, the classification criteria for corrosion/irritation is not fulfilled.

RAC agrees with the proposal of the DS to **not classifiy** medetomidine for skin corrosion or irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

One eye irritation study (OECD TG 405, GLP) in male rabbits (New Zealand White, 3 rabbits) was included in the CLH report. No signs of irritation were observed in the cornea or iris at any of the time points (24, 48 and 72 h), the average score for each test animal being 0. One of the test animals had slight redness in conjunctiva (one was mentioned in the text of the CLH report, but Table 13 in the CLH report indicates this effect in two rabbits). This was probably caused by mechanical irritation because as much as 25% of the dose was still present in the conjunctival sack. It was removed by washing and the symptoms were resolved by day 7. None of the test animals had oedema in the conjunctiva.

The DS proposed to not classify medetomidine for eye corrosion/irritation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The standard *in vivo* test to study eye irritation (OECD TG 405, GLP) did not reveal any signs of corrosion or irritation in cornea or iris during the observation period of 72 h in rabbits.The average scores for three consecutive days (24, 48 and 72 h) in cornea and iris, and also for oedema in conjunctiva, were 0 for each of the three test animals. Classification for eye corrosion or irritation is not warranted, because the average score per animal over 24, 48 and 72 h for conjunctival redness is less than 2.

RAC agrees with the proposal of the DS to **not classifiy** medetomidine for eye corrosion or irritation.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

This hazard class was not covered by the CLH proposal of the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Not applicable.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

A local lymph node assay, LLNA, (OECD TG 429, non-GLP) was initiated with medetomidine (0.1, 0.3, 1 and 4%) in mice (strain not provided). The assay was terminated due to severe sedation and anaesthesia of the test animals at all dose levels and no applicable results were obtained.

A non-guideline test for delayed contact hypersensitivity (non-GLP; Draize method) was utilized to study skin sensitisation potential of dexmedetomidine, the active isomer of medetomidine, in male Crl (HA) BR guinea pigs (10 test animals, 6 negative control animals and 6 positive control animals). Induction was carried out by 10 intradermal injections of 0.06% (w/v) dexmedetomidine HCl. Two weeks after the final induction injection, a challenge was performed by two intradermal injections of 0.06% dexmedetomidine HCl. As a positive control, 0.05% (w/v) 1-chloro-2,4-dinitrobenzene was used. Dexmedetomidine did not induce skin sensitisation in any tested animal. The positive control gave an appropriate response (3 of 6 tested animals were sensitised). As the challenge dose was not maximal, no conclusion can be drawn about skin sensitisation potential of medetomidine at concentrations >0.06%.

No information from humans was available.

There were no signs of sensitisation up to 0.06% in the available guinea-pig skin sensitisation study conducted with dexmedetomidine. The DS proposed that no classification is required under the CLP Regulation.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No applicable results were obtained from the standard guideline study, i.e. mouse LLNA. In a non-standard non-guideline test for delayed contact hypersensitivity of dexmedetomidine in guinea pigs (intradermal induction and challenge), none of the 10 test animals were sensitised. However, it was stated by the DS that the challenge dose used (0.06%) was not maximal. Based on this study, there were no indications of skin sensitisation potential of dexmedetomidine.

There was no human data on the skin sensitisation potential of medetomidine and no data on repeated dose toxicity of medetomidine via the dermal route was available.

Based on the available data, RAC agrees with the proposal of the DS to **not classify** medetomidine for skin sensitisation.

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

Summary of the Dossier Submitter's proposal

Repeated dose toxicity of medetomidine has been investigated in 28-day and 90-day studies via the oral routes in rats. Additional information on the repeated dose toxicity of medetomidine was available from 28-day studies via the subcutaneous route in rats and the intramuscular route in dogs. In addition, 28-day repeated dose studies have been carried out with levomedetomidine, an inactive isomer of medetomidine, via the subcutaneous route in rats and the intravenous route in dogs.

Oral route

In a 28-day range-finding study (non-guideline, non-GLP), medetomidine HCl in 0.9% NaCl (0, 2.5, 3.6, 4.9 mg/kg bw/day) was given via the oral route (gavage) to male Sprague-Dawley rats (4 animals/dose). Medetomidine caused adverse effects at all dose levels. At the highest dose (4.9 mg/kg bw/day), 3 of 4 animals were euthanized (75% mortality). Signs of toxicity included sedation, diarrhoea, weakness and hypothermia. Reduction in bodyweight gain (140%), food consumption (44%) and water consumption (extreme dehydration) was observed. Reductions in absolute heart, liver and kidney weights were observed. Dark contents in the small intestine indicated internal bleeding. Aggressive behaviour was observed in 1 of 4 animals at 4.9 mg/kg bw/day. At lower doses (2.5 and 3.6 mg/kg bw/day) no mortality occurred. Sedation and diarrhoea were observed. Bodyweight gain was reduced by 74% and 95% at 2.5 and 3.6 mg/kg bw/day, respectively. Also, absolute weights of heart, liver, kidney, testes and epididymis were reduced and increased water consumption was noted at the two lowest dose levels. Aggressive behaviour in 2 of 4 animals was observed at 3.6 mg/kg bw/day. No NOAEL was derived.

In a standard 90-day repeated dose toxicity study (OECD TG 408, GLP), medetomidine base in 0.5% v/v lactic acid (0, 0.2, 0.4, 1.2, 3.6 mg/kg bw/day) was given via the oral route (gavage) to Sprague-Dawley rats (10 animals/sex/dose). Sedation was observed at all dose levels. Also other clinical signs, including piloerection, weakness, locomotor inhibition and convulsions, were observed at dose levels of 1.2 and 3.6 mg/kg bw/day. At these dose levels, increased water consumption was observed, despite sedation. Mortality was observed only at the highest dose and it was 30% in both sexes (3/10 animals). Three deaths occurred (one animal died on day 3 and two animals on day 11). The pathological findings revealed cerebral haemorrhage and internal blockage in two animals. No adverse findings were observed in the third animal that died. In addition, three animals were euthanized (two on day 13 and one on day 66) due to poor condition (general wasting). These deaths were considered treatment-related.

Reduced bodyweight gain was observed at dose levels of 1.2 mg/kg bw/day (12% in females and 17% in males) and at 3.6 mg/kg bw/day (32% in females and 37% in males). At 1.2 mg/kg bw/day, absolute weights of adrenals, thymys, heart, kidneys, uterus and epididymis were

reduced. In addition to these, absolute weights of spleen, liver and testicles were reduced at the highest dose. The lowest doses had no effect on bodyweight gain but reductions in absolute weights of epididymis, heart, kidneys and liver were observed at 0.2 mg/kg bw/day, and heart and kidneys at 0.4 mg/kg bw/day in male rats. No histopathological changes were observed at any of the dose levels. Clinical chemistry showed increases in serum alkaline phosphatase at all doses (at 0.4 mg/kg bw/day only in females). The increases at the highest dose were 90% and 117% in males and females, respectively. At the two highest doses, alanine aminotransferase was also elevated (only in males at 1.2 mg/kg bw/day). Elevation of alanine aminotransferase at the highest dose was 93% and 140% for males and females, respectively. In addition, the levels of serum glucose, potassium (only in female rats) and phosphate were increased, and albumin level was decreased at the highest dose level. Urinanalysis revealed elevated glucose at all dose levels. At the two highest doses, urine volume decreased and proteinuria was observed. At 1.2 mg/kg bw/day, urine pH was elevated in male rats. Haematology parameters were also affected. Increases in monocytes were observed at all dose levels (but only in female rats at the lowest dose). Haematology parameters were more severe at the two highest doses. Decreases in haemoglobin, haematocrit, erythrocytes and lymphocytes were observed mainly in male rats (10%, 14%, 13%, 14% at the highest dose, respectively). At the highest dose, neutrophils were increased by 46% in males and by 90% in females.

A LOAEL of 0.2 mg/kg bw/day was derived.

Subcutaneous route

In a non-guideline 28-day repeated dose study (similar to OECD TG 407, non-GLP), medetomidine HCl in saline (0, 0.1, 0.4, 1.6 mg/kg bw/day) was given by the subcutaneous route to Sprague-Dawley rats (10 animals/sex/group). No deaths occurred at any dose level. Sedation was observed at all dose levels. In addition, piloerection (at the two highest dose levels) and exophthalmos (at the highest dose level) were observed. Reduced bodyweight gain was observed at all dose levels: 15% (males) and 25% (females) at 0.1 mg/kg bw/day, 38% (males) and 22% (females) at 0.4 mg/kg bw/day, and 69% (males) and 44% (females) at 1.6 mg/kg bw/day. Reduced bodyweight gain was associated with reduced absolute organ weights at the two highest dose levels. At 0.4 mg/kg bw/day, absolute weights of heart, thymus, liver, kidney and spleen were reduced in males, and pituitary gland in females. At the highest dose level (1.6 mg/kg bw/day), reduced absolute weights of heart, liver, kidney, testis, prostate, seminal vesicle, spleen and thymus were observed in male rats. In female rats, the absolute weights of spleen, thymus and pituitary gland were reduced at this dose level. Histopathological examination revealed corneal opacity, keratitis of the eye and brown pigmentation in the lungs in both sexes. At the highest dose level (1.6 mg/kg bw/day), haemorrhage and regenerative changes at the injection site were observed, and in male rats atrophy of the prostate and seminal vesicles, and reduction in the number of spermatozoa were observed at the highest dose level. Atrophy of the prostate was also observed at 0.4 mg/kg bw/day. Atrophy of the prostate and seminal vesicles, as well as exophthalmos, were observed only in the presence of significantly reduced bodyweight gain. Effects on haematology were observed at all dose levels, but mainly in male rats. Reduction of heamoglobin was observed at all dose levels in male rats: 5% at the lowest dose and 8% at the highest dose. Reticulocytes were increased by 54% and 63% at dose levels of 0.1 and 1.6 mg/kg bw/day, respectively. At the two highest dose levels, haematocrit (10% and 14%) and erythrocytes (6.3% and 7%) were reduced in male rats. In addition, the number of lymphocytes was reduced by 8-13% in male rats at the highest dose. The only haematological parameter affected in female rats was the number of neutrophils, which was increased by 64% at the highest dose level (1.6. mg/kg bw/day). This parameter was not affected in male rats.

Urinalysis revealed reductions in total urine volume (86% in males and 70% in females), reduced urine pH, and increases in urine osmolality (286% in males and 75% in females) at the highest dose level (1.6 mg/kg bw/day). No effects on urinary parameters were reported at lower dose levels.

Various clinical chemistry parameters were affected. Alkaline phosphatase was increased at all dose levels. At the highest dose, the increase was 33% and 66% in males and females, respectively. The increase was 31% (only in males) and 54% (only in females) at 0.1 and 0.4 mg/kg bw/day, respectively. Aspartate aminotransferase increased 28% in male rats at 0.4

mg/kg bw/day, and 49% (in males) and 22% (in females) at 1.6 mg/kg bw/day. Dose-dependent increases (25, 37 and 47%) in iron levels were observed in male rats. Total protein was slightly decreased at all dose levels in male rats. Decreases in potassium (10% and 12%) and phosphate (10% and 14%) were observed in male rats at the two highest dose levels. At the highest dose level, slightly decreased sodium (in males and females) and calcium (in males) levels, and slightly increased chloride (in males) levels were observed. In addition, reduction of triglycerides (33%) and blood glucose (13% in females) was observed.

A LOAEL of 0.1 mg/kg bw/day was derived.

Intramuscular route

In a non-guideline 28-day repeated dose study (similar to OECD TG 407, non-GLP), medetomidine HCl in saline (0, 0.08, 0.24, 0.4 mg/kg bw/day) was given by the intramuscular route to Beagle dogs (3 animals/sex/group). No deaths were observed in this study. At all dose levels, sedation was observed. Severity and recovery time from sedation was dose-dependent. No treatment related effects on bodyweight, food consumption, haematology, clinical chemistry, urinalysis, gross necropsy or histopathology were observed. Diarrhoea was observed at 0.24 and 0.4 mg/kg bw/day. Corneal opacity was observed in female dogs at dose levels of 0.24 (in 1 female dog) and 0.4 mg/kg bw/day (in 3 female dogs). A NOAEL value < 0.08 mg/kg bw/day was derived.

Subcutaneous and intravenous routes (levomedetomidine)

Two 28-day repeated dose studies with levomedetomidine, the inactive isomer of medetomidine, were included in the CLH report. In a non-guideline 28-day repeated dose study (similar to OECD TG 407, non-GLP), levomedetomide HCl in saline (0, 0.02, 0.1, 0.5, 2.5 mg/kg bw/day) was administered via subcutaneous route to Sprague-Dawley rats (10 animals/sex/group). No deaths or adverse effects were observed at any dose level. Local skin irritation at the injection site was observed at the highest dose level. NOAEL of 2.5 mg/kg bw/day for levomedetomidine was derived.

In a standard guideline 28-day repeated dose study (OECD TG 407, non-GLP), levomedetomidine HCl in saline (0, 0.4, 2, 10 mg/kg bw/day) was admistered via intravenous route to Beagle dogs (3 animals/sex/dose). At the highest dose level (10 mg/kg bw/day), severe clinical signs were observed (piloerection, salivation, tremors, diarrhoea, vocalization, redness of the eyes and aggression) and the study was terminated after 2 doses in females and 3 doses in males at this dose level. At the lower dose levels (0.4 and 2 mg/kg bw/day) no treatment-releated adverse effects were observed. A NOAEL of 2 mg/kg bw/day for levomedetomidine was derived.

In the summary of repeated dose toxicity studies, the DS states that available data appear to support classification of medetomidine for repeated dose toxicity. In the oral 90-day repeated dose toxicity study in rats, deaths were observed in both sexes at the highest dose level (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. As a conclusion, the DS stated that although the criteria for repeated dose classification appear to have been met, it is considered that the effects observed are not a consequence of repeated (prolonged) exposure but are in fact acute effects arising from a small number of single exposures. As classification for acute toxicity via the oral and inhalation routes is already proposed, the DS did not propose to additionally classify for STOT-RE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Although the level of sedation at different dose level was not described in the CLH report, it was observed at all dose levels in both repeated dose toxicity studies in which exposure was via the

oral route in rats. It was also an acute effect of medetomidine occurring after a single exposure. Due to this effect, STOT-SE 3 classification was agreed by RAC (see above). The STOT-RE classification should only be assigned where the observed toxicity is not covered more appropriately by another hazard class. Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially acute. This is the case for medetomidine-induced sedation and the classification as STOT-SE 3 is the most appropriate for this effect.

STOT-RE is assigned on the basis of findings of "significant" or "severe toxicity". In this context "significant" means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. "Severe" effects are generally more profound or serious than "significant" effects. The DS concluded that although the criteria for STOT-RE 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. As classification for acute toxicity via the oral and inhalation routes is already proposed, the DS did not propose to additionally classify for STOT-RE. There are, however, some observations indicating that the toxic effects can be seen as an outcome of repeated exposure and cannot be regarded as acute effects.

In an acute oral toxicity study in rats, no deaths were observed (LD > 31.25 mg/kg bw). In the 90-day repeated dose toxicity study, which can be regarded as a key study, an almost ten times lower dose (3.6 mg/kg bw/day) caused 30% mortality. One animal died on day 3 and two animals on day 11. Two animals were euthanized on day 13 and on day 66. Also in the 28-day study in male rats via the oral route, 75% mortality was observed at the highest dose level (4.9. mg/kg bw/day); three of four animals were euthanized on days 9 and 10. The mortality in these cases seems to result from repeated exposure to medetomidine at doses lower than those not causing mortality in an acute oral toxicity study. The same rat strain (Sprague-Dawley) was used both in acute oral and repeated dose toxicity studies (28-day and 90-day). The acute oral toxicity study was carried out only in male rats. In the 90-day repeated dose toxicity study, both sexes were used. In this study, male rats seemed to be more sensitive to toxic effects of medetomidine at lower doses than those causing mortality. This is seen in more pronounced effects on bodyweight gain, haematology and clinical chemistry parameters in males compared to females. In male rats, a reduction in absolute organ weights (e.g. heart and kidney) in the absence of an effect on bodyweight gain was also observed at the lowest doses (0.2 and 0.4 mg/kg bw). Other signs of toxicity included the reduction in absolute organ weights, but only in the presence of reduced bodyweight gain at the two highest dose levels (1.2 and 3.6 mg/kg bw/day). In acute toxicity studies, bodyweight loss was observed only via the inhalation and dermal routes, but not via the oral route in rats. In addition, changes observed in urinalysis and clinical chemistry parameters indicated toxicity of medetomidine after repeated exposure. However, if all the observed signs of toxicity can be regarded as secondary to medetomidine-induced sedation, STOT-RE classification is not needed as STOT-SE 3 classification is already proposed. If not, the criteria for STOT-RE classification are fulfilled based on the clear dose-dependent effects on bodyweight gain and mortality (which occurred at lower doses than those not causing deaths in acute toxicity studies).This is also supported by the effects found in haematology, clinical chemistry and urinanalysis.

The levels of sedation at different dose levels are not clearly described in the CLH report. However, it is possible to assess the levels of sedation to some extent based on other observations in repeated dose toxicity studies. In a 28-day oral study, at the highest dose level (4.9 mg/kg bw/day) 75% mortality was observed. At this dose level, a clear reduction in bw gain and food consumption was observed, and also extreme dehydration and reduced water consumption were reported. This may be due to severe sedation. At lower doses (2.5 and 3.6 mg/kg bw/day), on the other hand, no mortality was observed. Although a clear reduction in body weight gain was observed, an effect on food consumption was not reported. Increased water consumption was also reported. This suggests that the level of sedation was not severe enough to prevent the animals from eating and drinking. It may also be an indication that the decrease in body weight gain is a direct sign of toxicity and is not secondary to sedation. Similarly, in the 90-day repeated dose toxicity study, despite sedation and decreases in body weight gain at two highest dose levels, no effects on food consumption were reported in the CLH report. This is again an indication that

sedation was not so severe that it prevented animals from eating. This is supported by the observation that increased water consumption was observed at the two highest dose levels. The highest dose level also caused 30% mortality in the 90-day repeated toxicity study.

In addition to the central nervous system (sedation), medetomidine-induced toxicity on other target organ(s) after repeated exposure is equivocal. In the 90-day repeated toxicity study, increased water consumption was observed at the two highest dose levels (1.2 and 3.6 mg/kg bw/day). At these dose levels, urinanalysis revealed, however, a reduction in urine volume. At the same time, proteinuria was observed. These effects may be an indication of medetomidine-induced adverse renal effects. Absolute weights of kidneys were reduced in the presence of reduction in bodyweight gain at the highest doses (1.2 and 3.6 mg/kg bw/day), but also in the absence of an effect on bodyweight gain in male rats, at the lowest doses (0.2 and 0.4 mg/kg bw/day). No histopathological changes in kidneys were reported. Clinical chemistry revealed increase in the levels of alkaline phosphatase already at the lowest dose level (0.2 mg/kg bw/day). The alkaline phosphatase increase was more pronounced at the dose levels of 1.2 and 3.6 mg/kg bw/day. At these dose levels, increases in alanine aminotransferase were also observed. Dose-dependent elevations in these enzymes may indicate liver damage. A reduction in albumin levels was observed at the highest dose level and it may be a consequence of liver damage but also an indication of malnutrition. Absolute liver weight was reduced only in the presence of a reduction in bodyweight gain at dose level of 3.6 mg/kg bw/day. No histopathological changes in liver were reported. Based on the effects observed in erythrocytes, haemoglobin and reticulocytes, anaemia may result from repeated exposure to medetomidine.

The highest dose levels causing the most significant toxicity in the 28- and 90-day studies were 4.9 and 3.6 mg/kg bw/day via the oral route in rats, respectively. These are within the range of guidance values in the CLP Regulation for the oral route in rats to assist with a STOT-RE classification. Guidance values via oral route in rats for Category 1 are ≤ 10 mg/kg bw/day (90-day study) and ≤ 30 mg/kg bw/day (28-day study). Based on the guidance values and the observed effects at generally low doses, RAC considers that STOT-RE 1 classification is justified.

Effects not supporting STOT-RE classification include changes in organ weights with no evidence of organ dysfunction, small changes in bodyweight gain or clinical observations that do not by themselves indicate 'significant' toxicity, or small changes in clinical chemisty, haematology or urinary analysis parameters when such changes or effects are of doubtful or minimal toxicological importance. A STOT-RE classification is supported by the following medetomidine-induced effects after repeated exposure: mortality and significant effect on bodyweight gain, and by the evidence of organ dysfunction supported by urinalysis and/or clinical chemistry in the presence of reduced absolute organ weights. However, based on the data presented in the CLH report, it is not possible to clearly identify specific target organ(s) other than the central nervous system after repeated exposure to medetomidine.

RAC disagrees with the proposal of the DS to not classify medetomidine for specific target organ toxicity - repeated exposure (STOT-RE). RAC is of the opinion that medetomidine should be classified for specific target organ toxicity - repeated exposure as **STOT-RE 1; H372: Causes damage to organs through prolonged or repeated exposure**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Several standard guideline-compliant *in vitro* studies and one guideline-compliant *in vivo* mutagenicity study were summarised in the CLH report. All of these studies gave negative results with or without metabolic activation (S9).

Two Ames tests (OECD TG 471, GLP) with several *S. typhimurium* strains and one *E.coli* strain showed negative results with and without metabolic activation (S9). An Ames test with medetomidine base in acetone (5 - 5000 µg/plate) gave negative results in *S. typhimurium*

(strains TA 1535, 98, 100) and *E.coli* (WP2uvrA) both with and without S9. Cytotoxicity was observed at the two highest doses (1500 and 5000 µg/plate). Similarly, dexmedetomidine HCl in water (0, 15, 50, 150, 500 and 1500 µg/plate) gave negative results in *S. typhimurium* (strains TA 1535, 1537, 1538, 100, 98). Cytotoxicity was caused by the highest dose (1500 µg/plate). Positive controls gave appropriate results in the Ames tests.

Medetomidine base (in DMSO) and also dexmedetomidine HCl (in water) gave negative results in two *in vitro* cytogenetics (choromosomal aberration) tests (OECD TG 471, GLP) in human lymphocytes with or without metabolic activation (S9). Medetomidine was tested in two experiments and caused cytotoxicity at the highest concentrations used in the experiments, i.e. in experiment 1 (3 h) at 155 µg/mL (without S9) and 259 µg/mL (with S9), and in experiment 2 (3 or 21 h) at 50 µg/mL (without S9, 21 h) and 280 µg/mL (with S9, 3 h). Dexmedetomidine was tested in three experiments. In experiment 1, 6-94 µg/mL and 100-300 µg/mL concentrations of dexmedetomidine with S9 were used. In experiment 2 (18 h harvest) and 3 (32 h harvest), concentrations were 12.5-300 µg/mL without S9 and 50-350 µg/mL with S9. In experiment 1, a statistically significant increase in the number of cells with aberrations (excluding gaps) was observed at the highest concentration (300 µg/mL) with S9. This was not observed in experiments 2 and 3. Therefore, the increased number of aberrations in experiment 1 was not considered biologically significant. Dexmedetomidine did not significantly increase the number of aberrations (excluding gaps) without S9 in any of the three experiments. Positive controls gave appropriate results in cytogenetics studies.

Dexmedetomidine HCl gave negative results in *in vitro* gene mutation assay (OECD TG 476, GLP) in mouse lymphoma L5178Y cells both with and without metabolic activation (S9). A preliminary toxicity test was carried out with 37-3990 µg/mL dexmedetomidine with and without S9. In this test, total inhibition of growth was observed at 300 µg/mL with and without S9. In the main experiments 1 and 2, dexmedetomidine concentrations were 10-300 µg/mL with and without S9. Cytotoxicity was seen at ≥ 250 µg/mL without S9 and ≥ 200 µg/mL with S9. Positive controls gave appropriate results.

One *in vivo* bone marrow micronucleus test (OECD TG 474, GLP) was included in the CLH report. In this study, dexmedetomidine (40, 100, 250 µg/kg bw) was administered to NMRI mice (5 animals/sex/dose/sampling time) via the intravenous route. Sampling times were 24 and 48 h post dosing. Maximum dose of 250 µg/kg bw was set due to severe hypothermia as a result of sustained sedation observed at 500 µg/kg bw in a dose-range finding study. In the main study, sedation was observed immediately after dosing at all dose levels. Piloerection was observed also in animals at the two highest doses. There was no change in the P/N ratio. Positive controls (40 µg/kg bw of cyclophosphamide) gave appropriate responses.

In summary, medetomidine, and also its active isomer dexmedetomidine, gave negative results in *in vitro* genotoxicity studies (Ames test and cytogenetics assay). Dexmedetomidine was also negative in the mammalian gene mutation assay. The *in vivo* micronucleus study has only been conducted on dexmedetomidine. The result of this study was negative. Although there was no change in the P/N ratio, dexmedetomidine was judged to have reached the bone marrow. The DS proposed to not classify medetomidine for germ cell mutagenicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Medetomidine has not shown any genotoxic potential in several standard guideline-compliant *in vitro* studies. Ames tests with several *S. typhimurium* strains and one *E.coli* strain showed negative results both with and without metabolic activation (S9). In addition, a negative outcome was observed in the cytogenetics study in human lymphocytes with and without metabolic activation (S9). Similar negative results have been observed in the Ames test and cytogenetics studies with dexmedetomidine. Dexmedetomidine was also negative in the *in vitro* gene mutation assay. Furthermore, dexmedetomidine was negative in the *in vivo* bone marrow micronucleus test.

Various mutagenicity studies with negative outcome indicate that the classification criteria for germ cell mutagenicity are not fulfilled.

RAC agrees with the proposal of the DS to **not classifiy medetomidine for germ cell mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

This hazard class was not covered by the CLH proposal of the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Not applicable.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The reproductive toxicity of medetomidine (0, 13.3, 40 and 120 µg/kg bw/day) has been investigated in a non-standard two generation study (FDA guideline similar to OECD TG 415) via the subcutaneous route in Sprague-Dawley rats. No effects related to reproductive toxicity (including fertility) were observed. Clinical signs of parental (male and female) toxicity were observed; at all doses sedation and piloerection, and at the highest dose also exophthalmos. In F0 males, reduced food consumption was observed at all dose levels and it was associated with reduced bodyweight gain. At the highest dose level (120 µg/kg bw/day), prostate and epididymidis weights were reduced. The reduction of testis and epididymidis weights were observed at 40 µg/kg bw/day. At the lowest dose level (13.3 µg/kg bw/day) prostate weight was reduced. These effects were considered secondary to reduced bodyweight gain. In F0 dams, bodyweight gain was reduced during gestation (days 0-20) and lactation (days 0-7) at dose levels ≥ 40 µg/kg bw/day. Placenta weight was reduced by 18% at the highest dose. No effects on the number of corpora lutea, implantation sites and pre-implantation sites were observed. In the F1 generation, embryonic deaths were increased (14 vs. 5 in control) at the highest dose. Foetal weight was reduced by 21% and 35% at 40 and 120 µg/kg bw/day, respectively. No treatment-related effects were observed at the lowest dose level. F1 generation was not dosed directly. One male and one female from each F1 litter were mated and pregnant females were allowed to litter (F2 generation). In the F2 generation, no effects on sex ratio or pup number were observed at any dose level.

No human information is available.

In summary, no effect of medetomidine on fertility was observed in the presence of significant parental toxicity including sedation, piloerection, exophthalmos, reduced food consumption and bodyweight gain.

Development

Developmental toxicity of medetomidine has been studied in rabbits via the intravenous route and in rats via subcutaneous injection. There is also one poorly reported study on the developmental effects of dexmedetomidine, the active isomer of medetomidine, in rats via subcutaneous injection. No human information is available.

In the study with White Russian rabbits (compliant with USA FDA guidelines, non GLP), medetomidine (0, 6, 24, 40, 96 µg/kg bw/day) was adminstered intravenously to pregnant females (12 animals/dose) during gestation days 6-18. Maternal toxicity was observed as sedation and miosis at dose levels ≥ 24 µg/kg bw/day. No effects on maternal bodyweight gain and food consumption were observed. No developmental effects were observed at any dose level.

In the study with Sprague-Dawley rats (non-guideline, similar to OECD TG 414), medetomidine (0, 30, 120, 480 µg/kg bw/day) was given by subcutaneous injection to pregnant females (30 animals/dose) during gestation days 6-15. Maternal toxicity (sedation, exopthamalamos, piloerection, reduced bodyweight gain) was observed at doses ≥ 120 µg/kg bw/day. In addition, placental weight was reduced by 9 and 22% at 120 and 480 µg/kg bw/day, respectively. At the lowest dose, only sedation was observed in pregnant females. Reduction of foetal bodyweight by 10.5%, 19% and 35% was observed at 30, 120, 480 µg/kg bw/day, respectively. At the highest dose, a significant increase in the number of early embryonic deaths was observed. No malformations or skeletal abnormalities were observed. A LOAEL of 30 µg/kg bw/day was dervived for maternal and developmental toxicity.

In another study with Sprague-Dawley rats (non-guideline, non-GLP and poorly reported) dexmedetomidine (0, 5, 10, 20 µg/kg bw/day) was given by subcutaneous injection to pregnant females (8 animals/dose) during gestation days 7-19. At all doses, bodyweight gain was reduced. At the highest dose, a significant reduction in food consumption was observed. Sedation is also considered likely, but it was not recorded in this study. A significant reduction in foetal bodyweight and crown-rump length was observed at doses ≥ 10 µg/kg bw/day. No malformations or skeletal abnormalities were observed at any dose level. Due to the limitations in reporting of the study no NOAEL or LOAEL was derived.

In summary, no developmental toxicity was observed at any dose level in rabbits (i.v. administration). No malformations or skeletal abnormalities were observed in the rat studies (two developmental studies and one 2-generation study). However, pup deaths were observed at the top dose of the good-quality rat developmental study (480 µg/kg bw/day via s.c. administration) and the 2-generation study in rats (120 µg/kg bw/day via s.c. administration). In both of these studies, pup weights were also reduced at lower doses. The deaths and reduced bodyweights of the pups were mainly observed in the presence of significant maternal toxicity (sedation and reduced bodyweight gain). The DS considered that the effects observed in pups are not secondary to maternal toxicity as these effects are also observed in adult rats following a single exposure. As such, the DS considered these effects in pups were a result of acute toxicity and not specific developmental effects relevant for classification for reproductive toxicity.

No effects on fertility or development were observed in the absence of marked parental toxicity and therefore there was not sufficient evidence to cause a strong suspicion that medetomidine reduced fertility or caused developmental toxicity. The DS proposed to not classify medetomidine for reproductive toxicity.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The observations in the 2-generation study in rats that medetomidine has no effects on fertility, even in the presence of significant parental toxicity, indicate that the classification criteria for reproductive toxicity (fertility) are not fulfilled. Medetomidine did not have any developmental

effects in the rabbit study in the absence or presence of maternal toxicity. In the good-quality rat study, developmental toxicity (a significant dose-dependent reduction in foetal bodyweight gain and an increase in early embryonic deaths at the highest dose) was only observed in the presence of maternal toxicity (sedation at the lowest dose, and more pronounced toxicity at higher doses). RAC considers that the observed foetal effects are secondary to maternal toxicity. Therefore, the classification criteria for reproductive toxicity (development) are not fulfilled.

RAC agrees with the proposal of the DS to **not classifiy medetomidine for reproductive toxicity.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

This hazard class was not covered by the CLH proposal of the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Not applicable.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Medetomidine has not previously been reviewed for harmonised classification and labelling and does not have an existing entry in Annex VI of CLP.,Considering theavailable data, the dossier submitter (DS) proposed an environmental hazard classification as Aquatic Acute 1 (H400) with an M-f actor of 1 based on acute aquatic toxicity to the alga *Desmodesmus subspicatus* (72-h ErC_{50} = 0.65 mg/L), and Aquatic Chronic 1 (H410) with an M-factor of 100, based on chronic aquatic toxicity to the fish *Cyprinodon variegatus* (28-d NOEC = 0.001 mg/L) and a lack of rapid degradation.

In the CLH report the DS clarified that the substance is manufactured as a racemic mixture of two stereoisomers. The active isomer is dexmedetomidine whereas the other isomer, levomedetomidine, is non effective. Medetomidine used in the environmental fate and ecotoxicological tests was manufactured in the same way as the commercial medetomidine. Therefore and because the racemic form of medetomidine in these studies is the same as that for commercial production, the DS concluded that no further consideration of isomeric issues is required.

Degradation

The DS considered medetomidine to be hydrolytically stable at all environmentally relevant pH values and temperatures. A preliminary test was conducted at pH 4, 7 and 9 and at 50 °C and the results showed less than 10 % hydrolysis after 120 hours in all samples (Sydney, 2011). This was considered equivalent to a half-life of greater than one year under environmental conditions and no further testing was performed.

The DS assumed that medetomidine, based on an aqueous photodegradation study (Wehrhan, 2009) (OECD TG 316, GLP), was photolytically stable and neither theoretical nor experimental photolytic half-lives were determined.

The ready biodegradation of medetomidine was investigated by a screening test (Bätscher, 2008) (OECD TG 301D, GLP). Medetomidine had no obvious inhibitory effect on the activity of activated sludge microorganisms at the concentration tested. No significant biochemical oxygen demand (BOD) of the test substance was recorded throughout the test period (28 days); the percent biodegradation was estimated to be 0 % throughout. Consequently, the DS considered that medetomidine was found to be not readily biodegradable under the conditions of the screening test.

A marine water/sediment study (Lewis, 2014) on medetomidine (98.1 % radiochemical purity) that used two natural marine water/sediment systems (W1 and W2) was performed, which followed GLP and was conducted according to OECD TG 308. The DS considered the geomean DT_{50} value for the whole system of 51.3 days (range 48.8 – 54 days) being indicative of a lack of rapid degradation in this aquatic test system (temperature was 20 ^{o} C). Based on the lack of hydrolysis and whole system degradation half-lives > 16 days in aquatic simulation tests with limited mineralisation (≤5.8 %), the DS proposed to not consider medetomidine as rapidly degradable.

Aquatic Bioaccumulation

The measured maximum log K_{ow} for medetomidine was 3.1 at pH 9 (20 ^{0}C), which represents a worst case for aquatic systems due to the limited ionisation of the substance at this pH (Sydney, 2011, 2014). This value is below the CLP log K_{ow} trigger value of ≥ 4 intended to identify substances with a potential to bioaccumulate.

However, a reliable study on BCF (Sharp and Vaughan, 2012) (OECD TG 305, GLP) was provided using Sheepshead minnow *(Cyprinodon variegatus)*. Two concentrations of medetomidine (>99% pure) were used, 3.0 and 30 µg a.s./L (pH 7.9 - 8.1), respectively. The measured concentrations were within 80 to 120 % of nominal concentrations. At a steady state the highest whole body BCF was 1.0 L/kg (at 30 µg/L), which was lower than the trigger value under CLP. Therefore, the DS proposed not to consider medetomidine as bioaccumulative.

Aquatic Toxicity

Medetomidine is used as an antifouling agent. Activation of specific neuronal receptors in shell-building organisms leading to an anti-settling effect is the basis of its biocidal activity. The ecotoxicological test results for medetomidine from both acute and chronic studies are summarised in the following table and sections. Only the valid studies are included in the following table and relevant endpoints from these studies are discussed in further detail below.

Test organism / guideline, test method	**Short-term result (endpoint)**	**Long-term result (endpoint)**
Zebra fish (*Danio rerio*) /OECD TG 203, GLP	96-h LC_{50} = 30 mg/L (Acute toxicity)	
Rainbow trout (*Oncorhyncus mykiss*) / No guideline, GLP	-	2 hours NOEC = 0.01 mg/L (Sub-lethal pigmentation)
Sheepshead minnow *Cyprinodon variegatus* / OECD TG 210, GLP	-	28-d NOEC = (Hatchability): 0.32 mg/L (Survival): 0.32 mg/L (Length): 0.032 mg/L **(Dry weight): 0.001 mg/L**
Daphnia magna / OECD TG 202, GLP	48-h EC_{50} = 4.5 mg/L (Acute immobilisation)	-
Pacific oyster (*Crassostrea gigas*) / OPPTS 850-1055, GLP	48-h EC_{50} = 2.5 mg/L (Embryo-larval development)	-

Test organism / guideline, test method	Short-term result (endpoint)	Long-term result (endpoint)
Sea urchin (*Paracentrotus lividus*) / ASTM E1563-98, GLP	48-h EC_{50} = 3.2 mg/L (Embryo-larval development)	-
Green alga (*Desmodesmus* [syn.*Scenedesmus*] *subspicatus*) / OECD TG 201, GLP	**72-h E_rC_{50} = 0.65 mg/L** (Growth inhibition test)	72-h NOEC = 0.12 mg/L (Growth inhibition test)
Green alga (*Skeletonema costatum*) / ISO 10253, GLP	**72-h E_rC_{50} > 0.447 mg/L** (cell multiplication inhibition)	72-h NOEC = 0.253 mg/L (cell multiplication inhibition)

There are no reliable aquatic chronic toxicity studies available for invertebrates. However, the DS concluded that given the relative acute insensitivity of invertebrates compared with algae this would not lead to a higher aquatic chronic classification or M-factor.

The DS identified algae as the most sensitive trophic group in acute aquatic toxicity studies and based the classification on the 72-h E_rC_{50} of 0.65 mg/L (growth inhibition) for the green alga *D. subspicatus*. This is supported by the acute aquatic toxicity to a second green alga, *Skeletonema costatum*, with an E_rC_{50} of > 0.447 mg/L (based on cell multiplication inhibition), which although a "greater than" value and not as reliable, is in the same concentration range. The most sensitive species in chronic aquatic toxicity studies is Sheepshead minnow (*C. variegatus*) with an 28-d NOEC of 0.001 mg/L (based on dry weight).

Comments received during public consultation

Four Member State Competent Authorities (MSCA) submitted comments on the environmental part of the DS's proposal, of which two of them agreed with the DS's proposal to classify medetomidine as Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=100) without any notes.

One MS agreed with the proposed classification but noted that since no reliable aquatic chronic toxicity studies were available for invertebrates, the surrogate approach should be explored, although they mentioned that this would not result in a more stringent outcome than if the classification was based on the lowest NOEC. The DS replied that use of the surrogate approach for long-term hazard classification was considered. However, given the relative acute insensitivity of invertebrates compared to algae, this would not lead to a higher chronic M-factor.

Another MS neither agreed nor disagreed with the classification proposal but pointed to the slightly incorrect data provided in the CLH report for one water/sediment simulation study. In its reply the DS confirmed recently agreed slight changes to environmental fate endpoints in the finalised CAR, but also stressed that these changes would not affect the 'not rapidly degradable' proposal for medetomidine or the resulting environmental classification and/or M-factors.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal by the DS that medetomidine is hydrolytically and photolytically stable at environmentally relevant pH. RAC further agrees that the substance is not readily biodegradable based on 0% biodegradation in an OECD TG 301D test and on the geomean DT_{50} value for degradation in the whole system (DT_{50} of 50.2 days). RAC agrees with the DS's proposal that medetomidine does not meet the criteria for being rapidly degradable in the environment.

Aquatic Bioaccumulation

The measured maximum log K_{ow} for medetomidine is 3.1 at pH 9 (20 ^{0}C), which is less than the CLP log K_{ow} trigger value of ≥ 4. In addition, 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 value of ≥ 500. RAC agrees with the DS's conclusion that the substance is not bioaccumulative.

Aquatic Toxicity

Reliable acute aquatic toxicity studies are available for fish, aquatic invertebrates and algae. RAC notes that there are no reliable data for chronic aquatic toxicity data for aquatic invertebrates. However, RAC agrees with the DS that this has no impact on the chronic classification as the surrogate approach based on acute invertebrate toxicity would not lead to a higher M-factor.

Acute toxicity
RAC agrees with the DS that the green alga *D. subspicatus* 72-hour mean measured E_rC_{50}=0.65 mg/L based on growth inhibition test is the lowest acute (short-term) result to be used for aquatic acute classification purposes.

Chronic toxicity
RAC agrees with the DS that the fish Sheepshead minnow *(C. variegatus)* 28-d mean measured NOEC = 0.001 mg/L based on dry weight is the lowest chronic (long-term) result to be used for aquatic long-term classification purposes.

Conclusion on classification

Medetomidine is considered not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC is of the opinion that medetomidine should be classified as follows:

Aquatic Acute 1 based on a 72-hour E_rC_{50}=0.65 mg/L for *D. subspicatus*. As 0.1 < L(E)C_{50} ≤ 1 mg/L, the **acute M-factor is 1**, as proposed by the DS**.**

Aquatic Chronic 1 based on a 28-d NOEC of 0.001 mg/L for Sheepshead minnow *(C. variegatus)*. As 0.0001 < NOEC ≤ 0.001 mg/L, the **chronic M-factor is 100**, as proposed by the DS.

ANNEXES:

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

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