Doc III A Section 6.3.4 (01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

4 RESULTS AND DISCUSSION

4.1 Observations

Control group
All controls behaved normally after dosing and were normal during later observations.
Low dose group (100 µg/kg)
After dosing all animals were sedated. At the last observation about five hours after dosing the animals were normal.
Medium dose group (400 µg/kg)
After dosing all animals were sedated and occasionally mild piloerection was found. At the last observation about five hours after dosing the animals were normal or very slightly sedated. They were moving slowly.
High dose group (1600 µg/kg)
The animals were sedated after dosing. Piloerection was found. At the last observation about five hours after dosing the animals were slightly sedated and mild exophthalmos was found in some animals. Next morning the animals were occasionally aggressive.

4.1.1 Clinical signs

Examination and palpations after 28 days.

Control group
No clinical signs.
Low dose group (100 µg/kg)
One male had reddish fur posteriously on back.
Medium dose group (400 µg/kg)
7 males and 6 females had opacity in eye/eyes.
High dose group (1600 µg/kg)
10 males and 9 females had opacity in eye/eyes.

4.1.2 Mortality

No deaths occurred during the study.

4.2 Body weight gain

In male rats, a dose-dependent inhibition of weight development was observed, whereas in female rats the weight development in various dose groups was almost comparable to that of the control group. Sedation, piloerection and exophthalmos were observed as pharmacological effects of the drug.

4.3 Food consumption and compound intake

There was reduced food intake in the high dose group at the beginning of the dosing period in both males and females. At the end of the study there were essentially no differences between dose groups in males or females.

4.4 Ophtalmoscopic examination

Opacity was observed in the cornea at the two highest doses. The reason for this is probably desiccation caused by long-lasting sedation, severe exopthalmos and reduced lacrimal secretion.

Keratitis was also found, probably due to desiccation of the eyes.

4.5 Blood analysis

4.5.1 Haematology

There was a dose dependent decrease in haemoglobin in the males. Packed cell volume was decreased significantly in the medium dose group and the high dose group. The number of red blood cells was decreased in a tendency showing way in the medium and high dose groups of the males. These changes were not seen in the females.

		There was a decrease of lymphocytes in the highest dose group for both males and females. Eosinophiles were increased in a tendency showing way in the high dose group females. This increase does not have toxicological significance. No other changes were observed in the differential count. In the red blood cell indices (MCV, MCH, MCHC), there were no statistically significant differences in any dose group compared to controls. No significant differences were observed in coagulation tests.
4.5.2	Clinical chemistry	Blood glucose values are decreased in tendency showing way in the low and high dose groups of the females. Phosphate ion concentrations were decreased in a tendency showing way in the medium dose group of males and highly significant in the highest dose group. These changes in ionic concentrations were slight and showed statistically significant but they have no toxicological significance. Serum protein concentrations were decreased in all male dose groups highly significantly. Serum urate concentration was decreased in a tendency showing way in the medium dose group of the males. Serum iron concentration was increased in the males in a dose dependent manner: in a tendency showing way in the lowest dose group, significantly in the highest dose group. No differences were found in the females.
4.5.3	Urinalysis	There were no signs of toxicity in urine examinations. Some small but statistically significant differences were observed in the performed haematological and blood chemistry tests. They were not considered signs of toxicity,
4.6	**Sacrifice and pathology**	The histopathological study was performed in all animals. All animals of the control group and the highest dose group were subject to a full histopathological study. The following organs were examined from the animals of the dose groups 100 µg/kg, 400 µg/kg: thymus, heart, lung, liver, right kidney, adrenals, spleen, tested, ovaries, uterus, prostate, epididymis, seminal vesicles, eyes, brain, pituitary, site of injection and possible abnormalities.
4.6.1	Organ weights	Statistically significant differences were observed in the actual and relative weights of organs. Some of these were considered to be mainly due to decreased inhibition if weight gain.
4.6.2	Gross and histopathology	In gross pathology the significant findings were small haemorrhages in the subcutaneous injection site at the highest dose group. No toxicologically significant changes were observed in bone marrow examination.
4.7	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Subacute toxicity study of medetomedine by repeated subcutaneous administration to rats for a minimum of 28 days. The doses used in a four week subcutaneous toxicity study in rats were 100 µg/kg, 400 µg/kg, and 1600 µg/kg,. The number of animals used in the dose groups and the control groups was 10/sex/group.
5.2	**Results and discussion**	In the performed study increased sadation as a function of dose was observed which was farmacologicaly expected. The animals tolerated

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Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

		the administration well and they were in good conditions a day after dosing. The tissue irritation potential of the substance seemed slight. Only in the highest dose group haemorrhages were observed in the subcutaneous injection site. This focal lesion was evidently associated with aggression observed in the highest group. In the ophthalmological examinations, opacity was observed in the cornea at the two highest doses. The reason for this is probably desiccation caused by long-lasting sedation, exophthalmos and reduced lacrimal secretions. Drug –induced changes were not observed in the histopathological studies in either sex at the dose level of 100 µg/kg. The following changes were considered to be related to the administration of the test compound at the levels of 400 µg/kg and 1600 µg/kg. 1. Minimal to slight brown pigmentation observed in the lung in both sexes at the dose levels of 400 µg/kg and 1600 µg/kg. 2. Slightly enlarged zona glomerilosa cells observed in a few male animals at the dose levels of 400 µg/kg and 1600 µg/kg and in one female at the dose level of 1600 µg/kg. 3. Minimal to slight keratitis observed in both sexes at the dose levels of 400 µg/kg and 1600 µg/kg. 4. Slight to moderate atrophy of the prostate observed at the dose levels of 400 µg/kg and 1600 µg/kg. 5. Reduced number of spermatozoa on the testis and epididymis of a few animals at the dose level of 1600 µg/kg. 6. Slight to moderate atrophy of the seminal vesicles in most animals at the dose level of 1600 µg/kg. 7. Haemorrhage and regenerative changes in subcutis at the injection site in both sexes at the dose level of 1600 µg/kg.	
5.3	**Conclusion**	The reason for observed keratitis is probably desiccation of the eyes. The atrophying effects on the development of the male genital organs could be expected on the basis of the pharmacological profile of the substance. The observed slight changes in the lungs and the adrenals cannot be considered toxicologically significant.	
5.3.1	LO(A)EL	400 µg/kg gave rise to histopathological findings.	X
5.3.2	NO(A)EL	Drug –induced changes were not observed in the histopathological studies in either sex at the dose level of 100 µg/kg	X
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Doc III A Section 6.3.4 (01)	Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats
Annex Point IIA VI.6.3	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*25th August 2010*
Results and discussion	*As stated by the Applicant*
Conclusion	LO(A)EL: The LOAEL is actually the low dose group- 0.1 mg/kg bw/day due to the significant reduction in bodyweight gain of 15 and 25% in male and female rats, respectively. NO(A)EL: No NOAEL value can be identified from this study due to the LOAEL value being the lowest dose tested.
Reliability	*1*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3_4 (01)-1. Weight development

parameter changed	Unit	Controls 0			low dose 100 µg/kg			medium dose 400 µg/kg			high dose 1600 µg/kg		
weeks after start of treatment		Week 1	Week 3	Week 4	Week 1	Week 3	Week 4	Week 1	Week 3	Week 4	Week 1	Week 3	Week 4
Weight gain	G												
males		242	320	346	240	306	329	242	289	307	239	254	272
females		195	225	236	192	214	223	196	208	228	197	206	220
Food consumption	g/animal /day												
males		23	25	25	22	25	25	21	23	25	8	21	22
females		17	18	18	15	19	17	14	19	20	5	19	21

* $p < 0,05$

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects
Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_3_4 (01)-2. Results *(specify)* of repeated dose toxicity study

Parameter	Control		100 µg/kg		400 µg/kg		1600 µg/kg		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	10	10	10	10	10	10	10	10	na	na
Mortality	0	0	0	0	0	0	0	0	na	na
clinical signs*	0	0	Sedation	Sedation	Sedation Pilo-erection	Sedation Pilo-erection	Sedation Pilo-Erection Exophthalmos	Sedation Pilo-Erection Exophthalmos	+	+
body weight	-	-	↓	-	↓	-	↓	-	+	-
food consumption	-	-	-	-	-	-	-	-	-	-
Haematology										
hemoglobin	-	-	↓	-	↓	-	↓	-	+	-
packed cell volume	-	-	-	-	↓	-	↓	-	+	-
red blood cells	-	-	-	-	↓	-	↓	-	+	-
reticulocytes	-	-	↑	-	-	-	↑	-	+	-
neutrophils	-	-	-	-	-	-	-	↑	-	+
lymphocytes	-	-	-	-	-	-	↓	↓	+	+
eosinophils	-	-	-	-	-	-	-	-	-	-
coagulation	-	-	-	-	-	-	-	-	-	-
Red blood cell indices	-	-	-	-	-	-	-	-	-	-
Clinical chemistry										
serum sodium ions	-	-	-	-	-	-	-	↓	-	+
potassium ions	-	-	-	-	↓	-	↓	-		
calcium ions	-	-	-	-	-	-	↓	-	+	-
phosphate	-	-	-	-	-	-	↓	-	+	-
serum protein	-	-	↓	-	↓	-	↓	-	+	-
serum urate	-	-	-	-	-	-	-	-	-	-
serum iron	-	-	-	-	↑	-	↑	-	+	-
serum choleterol	-	-	↓	↓	-	-	↑	-	+	-
serum triglycerides	-	-	-	-	-	-	-	↓	-	+
blood glucose	-	-	-	-	-	-	-	-	-	-
serum alkaline phosphatase	-	-	-	-	-	↑	-	↑	-	+
serum ASAT	-	-	-	-	-	-	↑	↑	+	+
serum GGT	-	-	-	-	-	-	-	-	-	-
serum ALAT	-	-	-	-	-	-	-	-	-	-

Parameter	Control		100 µg/kg		400 µg/kg		1600 µg/kg		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Urinalysis										
volume	-	-	na	na	na	na	↓	↓	+	+
pH	-	-	na	na	na	na	↓	-	+	-
osmolarity	-	-	na	na	na	na	↑	↑	+	+
turbidity	-	-	na	na	na	na	-	-	-	-
glucose	-	-	na	na	na	na	↑	↑	+	+
Heart organ weight (relative)	-	-	-	-	-	-	↓	↓		
gross pathology	-	-	inflammation	-	-	-	-	-		
microscopic pathology	-	-	-	trombus	-	-	myocarditis	-	-	-
Lungs organ weight (relative)	-	-	-	-	-	-	-	-		
gross pathology	dark points	foam cells	dark points	dark points	dark points	dark points	dark points	-		
microscopic pathology	granulomatous inflammation	granulomatous inflammation	granulomatous inflammation	granulomatous inflammation perivascular eosinophilic infiltrat-ions	granulomatous inflammation brown pigmentation acute inflammatory cell	granulomatous inflammation focus of foam cells emphysema brown pigmentation	brown pigmentation	emphysema brown pigmentation	-	-

Thymus										
organ weight (relative)	-	-	-	-	-	-	↓	↓		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	-	epithelial tubules	-	epithelial tubules	-	epithelial tubules	-		-	-
Liver										
organ weight (relative)	-	-	-	-	-	↓	-	↓		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	-	minor foci of mononuclear leucocyte	minor foci of mononuclear leucocyte	minor foci of mononuclear leucocyte	minor foci of mononuclear leucocyte	minor foci of mononuclear leucocyte	minor foci of mononuclear leucocyte small focus of necrosis	-	-	-
Kidney										
organ weight (relative)	-	-	-	-	-	-	-	-		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	few degenerative tubili mineral deposits dilated tubili	few degenerative tubili mineralization	few degenerative tubili mineral deposits	mineralization	dilated tubili	mineralization	mineral mineralization degenerative tubili	mineral deposits mineralization	-	-
Spleen										
organ weight (relative)	-	-	-	-	-	-	-	-		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	-	-	increased haematopoesis	-	dilated artery	-	-	-	-	-
Pituitary										
organ weight (relative)	-	-	-	-	-	-	↑	-		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	colloid cysts	colloid cysts	colloid cysts	colloid cysts	colloid cysts	-	colloid cysts	colloid cysts	-	-
Brain										
organ weight (relative)	-	-	-	-	↑	-	↑	↑		
gross pathology	-	-	-	-	-	-	-	-		
microscopic pathology	-	-	-	-	-	-	-	-	-	-

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Annex Point IIA6.3

Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Subacute toxicity study of medetomidine by repeated intramuscular administration to dogs for 28 days, [redacted] (Unpublished)	
1.2	**Data protection**	Yes, data protection is claimed.	
1.2.1	Data owner	[redacted]	
1.2.2			
1.2.3	Criteria for data protection	Data on new [a.s.] for [first approval / authorisation]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, fulfils known requirements of the US Food and Drug Administration	X
2.2	**GLP**	Yes, fulfils known requirements of the US Food and Drug Administration	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine, also known as FB-785, MPV-785. Packed in lacenula ampoules 1 mg/ml. Ampoules labelled: FB-785 1 mg/ml, 20 ml, s. LB217 Composition of ampouls: Methyl parahydroxybenz. Lot 10322 K0.1% Natr.Chloride p.a. lot 10473 0.9% Aqua ad inject 99%	
3.1.1	Lot/Batch number	LB217	
3.1.2	Specification	Medetomidine hydrochloride	
3.1.2.1	**Purity**	[redacted]	
3.1.2.2	**Stability**	No information stated in the study	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	[redacted]	

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Annex Point IIA6.3

Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

3.2.4	Sex	Both sexes
3.2.5	Age/weight at study initiation	1-2 years old, weight 9-15 kg
3.2.6	Number of animals per group	There were 3 male and 3 female dogs in each dose group
3.2.7	Control animals	Yes. Dose group 1.
3.3	**Administration/ Exposure**	Intramuscular administration
3.3.1	Duration of treatment	Minimum 28 days
3.3.2	Frequency of exposure	The animals were dosed 7 days/week for a minimum of 28 days. Dosing was continued to the day of autopsy.
3.3.3	Postexposure period	None
3.3.4	**Oral**	**Not applicable since the administration was intramuscular**
3.3.4.1	**Type**	
3.3.4.2	**Concentration**	
3.3.4.3	**Vehicle**	
3.3.4.4	**Concentration in vehicle**	
3.3.4.5	**Total volume applied**	
3.3.4.6	**Controls**	
3.3.5	**Inhalation**	
3.3.5.1	**Concentrations**	
3.3.5.2	**Particle size**	
3.3.5.3	**Type or preparation of particles**	
3.3.5.4	**Type of exposure**	
3.3.5.5	**Vehicle**	
3.3.5.6	**Concentration in vehicle**	
3.3.5.7	**Duration of exposure**	
3.3.5.8	**Controls**	
3.3.6	**Dermal**	

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Annex Point IIA6.3

Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

3.3.6.1	**Area covered**	
3.3.6.2	**Occlusion**	
3.3.6.3	**Vehicle**	
3.3.6.4	**Concentration in vehicle**	
3.3.6.5	**Total volume applied**	
3.3.6.6	**Duration of exposure**	
3.3.6.7	**Removal of test substance**	
3.3.6.8	**Controls**	
3.3.7	**Intramuscular injection**	
3.3.7.1	**Vehicle**	Extra ampoules containing all agents but the medetomedine. Composition of ampoules: Methyl parahydroxybenz. Lot 10322 K0.1% Natr.Chloride p.a. lot 10473 0.9% Aqua ad inject 99% FB-Placebo, 20 ml, 2s. LB218
3.3.7.2	**Concentration in vehicle**	Dose groups:

DOSE GROUP	DOSE LEVEL
1	0 µg/kg
2	80 µg/kg
3	240 µg/kg
4	400 µg/kg

3.3.7.3	**Total volume applied**	20 ml in each ampoule
3.3.7.4	**Controls**	Dose group 1.
3.4	**Examinations**	
3.4.1	Observations	Yes.
3.4.1.1	**Clinical signs**	Clinical signs were recorded 1-0.5 hours and 3-4 hours after dosing. At least once a week, every animal was examined and palpated and its detailed health status recorded on a clinical observation sheet.
3.4.1.2	**Mortality**	Mortality of the animals was examined on each day of the week once in the morning and once as late as practical. Maximum time between observations was 18 hours.
3.4.2	Body weight	Animal weights were recorded one week before the onset of dosing, on the day dosing started and weekly thereafter until the end of dosing.

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Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

3.4.3	Food consumption	The food consumption was weighed every week.
3.4.4	Water consumption	Yes. Monitored by visual inspection on a weekly basis.
3.4.5	Ophthalmoscopic examination	The eyes were examined before the start of the dosing period and at the end of the study.
3.4.6	Haematology	Blood samples were taken during the pre-test and at the end of the study period from the cephalic vein. The animals were fasted for 18 hours before blood sampling. Water was available to the animals. Blood samples were taken in the morning.
3.4.7	Clinical Chemistry	Blood chemistry measurements were done during the pre-test and at the end of the test. The animals were fasted for 18 hours before blood sampling. Water was available for the animals.
3.4.8	Urinalysis	Urine samples were collected from the control group and the highest dose group at the end of the study period. The samples were collected (18 hours) into specimen vials using metabolism cage.
3.5	**Sacrifice and pathology**	Autopsies were performed at the end of the study and histopathological examinations were performed.
3.5.1	Organ Weights	yes organs: heart, lung, liver, kidney, adrenal, brain, thyroid, pancreas, pituitary, testes/ovaries, prostate/uterus ,brain
3.5.2	Gross and histopathology	yes organs: heart, lung, liver, kidney, adrenal, brain, thyroid, pancreas, pituitary, testes/ovaries, prostate/uterus ,brain
3.5.3	Other examinations	None
3.5.4	Statistics	Statistical program used was BMDP-81. Equality of variance was tested by Levene`s test. If variances are not equal modification of Forsyth was used.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	The dogs were sedated after dosing and in the highest dose group they were still sedated at the last observation in the evening five or six hours after dosing
4.1.1	Clinical signs	In the performed study even the highest dose used (400 µg/kg) was well tolerated. The use of still higher doses in dog toxicity studies is not reasonable as then sedation becomes too long-lasting and the normal vital functions of the dogs are too much deranged because of sedation
4.1.2	Mortality	No mortality was found in any dose group.
4.2	**Body weight gain**	There were no statistically significant differences in weight gain compared to controls. The slight changes are due to individual variations and they have no toxicological significance.
4.3	**Food consumption and compound intake**	There was a significant decrease ($p<0.010$) of food consumption the first week in the highest group 400 µg/kg female dose group compared to control. Reason for this decrease was that the dogs spread their food all over the floor of the cage by the last observation in the evening.

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Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

		During the following weeks the mean food consumption was higher in this group than in the control group. Differences were, however slight, neither statistical nor toxicological significant differences were found.
4.4	**Ophtalmoscopic examination**	No drug-related findings were observed in males. One female animal at the dose level of 240 µg/kg had a minimal horizontal opacity at the central area of both corneas. Similar findings were found in all females at the dose level of 400 µg/kg.
4.5	**Blood analysis**	
4.5.1	Haematology	Thrombin time was decreased in a tendency showing way in the male group of medium dose at the end of the dosing period. No other statistically significant differences were found. In red blood cell indices, the only statistically significant difference found was the increase in MCH of males in the highest dose group before dosing. This increase was not found at the end of the dosing period and it was obviously due to the lowered value of the control male group.
4.5.2	Clinical chemistry	There was no statistically significant difference in serum sodium, potassium, chloride, inorganic phosphate, albumin, urate, cholesterol, triglycerides, total bilirubin or conjugated bilirubin values in any dose group before or after dosing. There were no statistically significant differences in serum enzyme values compared to controls at any dose level.
4.5.3	Urinalysis	There were no statistical significances between these groups in urine volume, pH or microscopy of urine sediment. All control males had protein in the urine. One control female had also protein in urine. No protein was found in urine of the high dose group of males and females. One male in the high dose group had hemi pigments in urine.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	There were no statistically significant differences in any dose group compound compared to controls regarding the organs heart, lungs, liver, kidneys, adrenals, spleen, thyroids, pancreas, pituitary, testis/ovaries, prostate/uterus or brain. The observed differences in weights were slight and they had no toxicological significance.
4.6.2	Gross and histopathology	The histopathological study was performed on all animals. The only drug related changes were seen in the females of the highest dose group. In two animals small cysts were observed in the corneal epithelium of one eye. In all females of the highest dose group level and in one female of the middle dose level minimal horizontal opacity at the central area of the cornea was observed in the ophthalmological studies. The histological changes that are observed in the cornea are possibly due to the drying of the eyes caused by long-lasting repeated sedation. The horizontal position of the opacity that was observed in ophthalmology is strongly indicative of this. The gross pathology found no abnormalities that could be related to administration of the test compound.
4.7	**Other**	None

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Annex Point IIA6.3

Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Toxicity of medetomidine was tested in Beagle dogs by daily intramuscular administration for 28 days. There were 3 male and 3 female dogs in each dose group. The dose levels were 80, 240 and 400 µg/kg. The animals were observed after dosing for possible adverse reactions for about 6 hours. The animals were weighed and their food consumption measured weekly. The heart rate was measured during electrocardiography. Haematological and clinical parameters were measured. Urine was examined at the end of the study. At the end of the study all animals subjected to full histopathological examination. The eyes were examined ophthalmoscopically before the start of the dosing period and at the end of the study.

5.2 Results and discussion

In the performed study, sedation increase was based on dose. This is the desired pharmacological effect of the substance. The animal tolerated the administration well and they were always in good condition a day after the dosing. Vomiting was occasionally observed, which is typical of substances which pharmacologically resemble medetomidine.

The performed investigations revealed only minor changes. Slight decrease was observed in the blood glucose values after the dosing period. Medetomidine and other pharmacologically similar substances are known to increase the blood glucose level after administration. In the present study the blood samples were taken a day after the last dosing. Thus it can be expected that the transient increase in blood glucose levels increases insulin secretion, which again is seen as slight decrease in blood glucose a day after dosing.

The muscle for the injection site was also examined in the histopahtological studies. Some minor degenerative changes were observed. Similar changes were also observed in the control dogs so that they are evidently due to the injection procedure. The tissue irritation potential seems to be very minimal.

The only drug related changes in the histopathological studies were seen in the females of the highest dose group. These findings were small cysts in the corneal epithelium observed in two dogs. The finding correlates well with the minimal horizontal opacity at the central area of the cornea which was found in the ophthalmological studies. The histopathological changes that are found in the cornea are possibly due to the drying of the eyes during long-lasting repeated sedation. The horizontal position of the opacity is strongly indicative of this. No changes were observed in the eyes of the male dogs.

5.3 Conclusion

In the performed study even the highest dose used (400 µg/kg) was well tolerated. The use of still higher doses in dog toxicity studies is not reasonable as then sedation becomes too long-lasting and the normal vital functions of teh dogs are too much deranged because of sedation.

5.3.1 LO(A)EL

None. X

The use of still higher doses in dog toxicity studies is not reasonable as then sedation becomes too long-lasting and the normal vital functions of teh dogs are too much deranged because of sedation.

5.3.2 NO(A)EL

In the performed study even the highest dose used (400 µg/kg) was well

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Repeated dose toxicity - Subacute toxicity of intramuscular administration to dogs

		tolerated.
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2nd September 2010*
Materials and Methods	*GLP: This study does not conform to GLP as it was only introduced as a world standard in 1988. However, the study is extensively documented and well reported.* *As stated by the Applicant*
Results and discussion	*As stated by the Applicant*
Conclusion	*LO(A)EL: Sedation was observed at all doses and as such the LOAEL value for this study is 0.08 mg/kg bw/day* *NO(A)EL: A NOAEL value can not be determined from this study since sedation was observed at all dose levels. A NOAEL of <0.08 mg/kg bw/day can be set.*
Reliability	*As stated by the Applicant*
Acceptability	*acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3_5(01)-1. Results of clinical signs, clinical chemistry, haematology and urinalysis.

Parameter	Control		80 µg/kg		240 µg/kg		400 µg/kg		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	3	3	3	3	3	3	3	3	na	na
Mortality	0	0	0	0	0	0	0	0	na	na
clinical signs*	0	0	Sedation Vomiting	Sedation Vomiting	Sedation Vomiting	Sedation Vomiting	Sedation Vomiting	Sedation Vomiting	+	+
body weight	-	-	-	-	-	-	-	-	-	-
food consumption	-	-	-	-	-	-	-	-	-	-
Haematology										
hemoglobin	-	-	-	-	-	-	-	-	-	-
white blood cells	-	-	-	-	-	-	-	-	-	-
reticulocytes	-	-	-	-	-	-	-	-	-	-
platelets	-	-	-	-	-	-	-	-	-	-
red blood cells	-	-	-	-	-	-	-	-	-	-
Clinical chemistry										
serum sodium	-	-	-	-	-	-	-	-	-	-
potassium	-	-	-	-	-	-	-	-	-	-
chloride	-	-	-	-	-	-	-	-	-	-
phosphate	-	-	-	-	-	-	-	-	-	-
albumin	-	-	-	-	-	-	-	-	-	-
serum protein	-	-	-	-	-	-	-	-	-	-
serum enzymes	-	-	-	-	-	-	-	-	-	-
serum iron	-	-	-	-	-	-	-	-	-	-
cholesterol	-	-	-	-	-	-	-	-	-	-
triglycerides	-	-	-	-	-	-	-	-	-	-
bilirubin	-	-	-	-	-	-	-	-	-	-
blood glucose	-	-	-	-	↓	↓	↓	↓	+	+
serum creatinie	-	-	↓	-	-	↓	↓	↓	+	+
coagulation	-	-	-	-	-	-	-	-	-	-

Parameter	Control		80 µg/kg		240 µg/kg		400 µg/kg		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Urinalysis										
volume	-	-	-	-	-	-	-	-	-	-
pH	-	-	-	-	-	-	-	-	-	-
osmolarity	-	-	-	-	-	-	↓	-	+	-
sediment	-	-	-	-	-	-	-	-	-	-
protein	-	-	-	-	-	-	-	-	-	-
Organ weight (mean and relative)										
heart	-	-	-	-	-	-	-	-		
lungs	-	-	-	-	-	-	-	-		
liver	-	-	-	-	-	-	-	-	-	-
kidneys	-	-	-	-	-	-	-	-	-	-
adrenals	-	-	-	-	-	-	-	-	-	-
spleen	-	-	-	-	-	-	-	-	-	-
thyroids	-	-	-	-	-	-	-	-	-	-
pancreas	-	-	-	-	-	-	-	-	-	-
testes/ovaries	-	-	-	-	-	-	-	-	-	-
prostate/uterus	-	-	-	-	-	-	-	-	-	-
pituitary	-	-	-	-	-	-	-	-	-	-
brain	-	-	-	-	-	-	-	-	-	-
Bone marrow										
lymphocytes	-	-	-	-	-	-	-	-	-	-
monocytes	-	-	-	-	-	-	-	-	-	-
megacaryocytes	-	-	-	-	-	-	-	-	-	-
plasmacells	-	-	-	-	-	-	-	-	-	-
blast cells	-	-	-	-	-	-	-	-	-	-
promyelocytes	-	-	-	-	-	-	-	-	-	-
myelocytes	-	-	-	-	-	-	-	-	-	-
metamyeolcytes	-	-	-	-	-	-	-	-	-	-
band cells	-	-	-	-	-	-	-	-	-	-
segmented neutrophils	-	-	-	-	-	-	-	-	-	-
eosionophils	-	-	-	-	-	-	-	-	-	-
basophils	-	-	-	-	-	-	-	-	-	-

Parameter	Control		80 µg/kg		240 µg/kg		400 µg/kg		Dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]
Ophthalmoscopy										
horizontal opacity	-	-	-	-	-	+	-	+	-	+
Histopathology (drug-related findings only)										
cysts in corneal epithelium	-	-	-	-	-	-	-	+	-	+

Doc III A Section 6.3.6(01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], Study report: MPV:1441 HCl subacute toxicity study by daily subcutaneous administration to rats for 4 weeks., [redacted] (Unpublished)
1.2	**Data protection**	Yes, data protection is claimed.
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	Data on new [a.s.] for [first approval / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. This study was performed to fulfil the known requirements of the US Food and Drug Administration and OECD
2.2	**GLP**	Yes. The toxicology studies have been made at [redacted] and [redacted] operates under GLP. The studies have been made quite a long time ago and these studies reflect the GLP of that time. The fact that medetomidine has been approved as pharmaceutical ingredient for commercial use should be proof of level of practices.
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Levomedetomidine MPV-1441 HCl Levomedetomidine is the nonactive isomer of the racemic substance medetomidine.
3.1.1	Lot/Batch number	8.1.88
3.1.1.1	**Description**	Crystalline powder white to almost white
3.1.1.2	**Purity**	[redacted] %
3.1.1.3	**Stability**	No information stated in the study
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley
3.2.3	Source	[redacted]
3.2.4	Sex	Both sexes
3.2.5	Age/weight at study initiation	Male and female rats. About 28 days when arriving at study site, weight 72-103 g
3.2.6	Number of animals	50 male and 50 female , 10 rats per sex per group

Doc III A Section 6.3.6(01) Annex Point IIA VI.6.3		Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats
	per group	
3.2.7	Control animals	Yes. Dose group 1 (physiological saline)
3.3	**Administration/ Exposure**	Subcutaneous injection
3.3.1	Duration of treatment	At least 4 weeks
3.3.2	Frequency of exposure	Daily treatment
3.3.3	Postexposure period	None, due to sacrifice.
3.3.4	**Oral**	**Not applicable since the administration is subcutaneous**
3.3.4.1	**Type**	
3.3.4.2	**Concentration**	
3.3.4.3	**Vehicle**	
3.3.4.4	**Concentration in vehicle**	
3.3.4.5	**Total volume applied**	
3.3.4.6	**Controls**	
3.3.5	**Inhalation**	**Not applicable since the administration is subcutaneous**
3.3.5.1	**Concentrations**	
3.3.5.2	**Particle size**	
3.3.5.3	**Type or preparation of particles**	
3.3.5.4	**Type of exposure**	
3.3.5.5	**Vehicle**	
3.3.5.6	**Concentration in vehicle**	
3.3.5.7	**Duration of exposure**	
3.3.5.8	**Controls**	
3.3.6	**Dermal**	**Not applicable since the administration is subcutaneous**

Doc III A Section 6.3.6(01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats

3.3.6.1 Area covered

3.3.6.2 Occlusion

3.3.6.3 Vehicle

3.3.6.4 Concentration in vehicle

3.3.6.5 Total volume applied

3.3.6.6 Duration of exposure

3.3.6.7 Removal of test substance

3.3.6.8 Controls

3.3.7 Subcutaneous administration

3.3.7.1 Vehicle Physiological saline (Natrosteril 9mg/ml)

3.3.7.2 Concentration in vehicle Dose groups:

DOSE GROUP	DOSE LEVEL	Concentration µg/ml	Dosing volume ml/kg
1	0	0	1
2	20µg/kg	20 µg/ml	1
3	100µg/kg	100 µg/ml	1
4	500µg/kg	500 µg/ml	1
5	2500µg/kg	2500 µg/ml	1

3.3.7.3 Total volume applied 1 ml/kg

3.3.7.4 Controls Dose group 1, please see 3.3.7.2

3.4 Examinations

3.4.1 Observations

3.4.1.1 Clinical signs Viability, behaviour and clinical signs were observed twice a day.

3.4.1.2 Mortality

3.4.2 Body weight Weekly, first day of the week before dose administration.

3.4.3 Food consumption Weekly

3.4.4 Water consumption Weekly

3.4.5 Ophthalmoscopic examination No

3.4.6 Haematology Five animals per sex group.

3.4.7 Clinical Chemistry Five animals per sex group.

3.4.8 Urinalysis No

Doc III A Section 6.3.6(01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats

3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Adrenals, brain, heart, kidney, liver, lungs, pituitary, spleen, thymus, testes, prostate, uterus, ovaries.
3.5.2	Gross and histopathology	Adrenals, brain, heart, kidney, liver, lungs, pituitary, spleen, thymus, testes, prostate, uterus, ovaries, adrenal, injection site, mesenterial lymph node, vagina and seminal vesicles.
3.5.3	Other examinations	-
3.5.4	Statistics	SAS and BMPD Statistical softwares were used to perform ANOVA, ANCOVA or pairwise comparisons depending on data type.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	Please see section 4.6.2
4.1.1	Clinical signs	No-drug related changes
4.1.2	Mortality	No mortality occurred during the study
4.2	**Body weight gain**	No-drug related changes
4.3	**Food consumption and compound intake**	No-drug related changes
4.4	**Ophtalmoscopic examination**	No information in study
4.5	**Blood analysis**	
4.5.1	Haematology	No-drug related changes
4.5.2	Clinical chemistry	No-drug related changes
4.5.3	Urinalysis	No information in study
4.6	**Sacrifice and pathology**	Clinical observations at terminal sacrifice: no drug related changes. A treatment related increased incidence of haematomas were observed at the injection site, most obviously in the highest treatment group (2500 µg/kg).
4.6.1	Organ weights	No significant changes in absolute and relative organ weight.
4.6.2	Gross and histopathology	In the dose group of 2500 µg/kg drug-related local irritation at the injection site. No histological abnormalities were observed in the other organs or tissues examined. No sex differences were evident.
4.7	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Levo Medetomidine MPV-1441 HCl The test was carried out by subcutaneous injection of 20-2500 µg/kg test

Doc III A Section 6.3.6(01)	Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats
Annex Point IIA VI.6.3	

		substance on a daily basis for at least 4 weeks up to the day of sacrifice. The control dose of physiological saline was administered using the same regimen.	
5.2	**Results and discussion**	Clinical signs, body weight, food consumption, water consumption, haematology, blood chemistry, absolute organ weights, relative organ weights: no significant changes was seen. No mortality occurred during study.	
5.3	**Conclusion**	The dose levels of 20 µg/kg, 100 µg/kg and 500 µg/kg were considered to be no-effect levels. In the high dose group (2500 µg/kg) first minimal toxicological finding was seen. This was the local tissue irritation at the subcutaneous injection site.	
5.3.1	LO(A)EL	2500 µg/kg	X
5.3.2	NO(A)EL	500 µg/kg (up to <2500 µg/kg)	
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

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Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity of subcutaneous administration to rats

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2nd September 2010*
Materials and Methods	*As stated by the applicant*
Results and discussion	*As stated by the applicant*
Conclusion	*The NOAEL identified from this study is the highest concentration tested, 2500 µg/kg bw/day*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3_6 (01)-1. Results of clinical chemistry haematology and urinalysis

Parameter	Control		20 µg/kg		100 µg/kg		500 µg/kg		2500 µg/kg		dose-response+/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	10	10	10	10	10	10	10	10	10	10	na	na
Mortality	0	0	0	0	0	0	0	0	0	0	na	na

clinical signs*	-	-	-	-	-	-	-	-	-	-	-	-
body weight	-	-	-	-	-	-	-	-	-	-	-	-
food consumption	-	-	-	-	-	-	-	-	-	-	-	-
Haematology												
hemoglobin	-	-	-	-	-	-	-	-	-	-	-	-
white blood cells	-	-	-	-	-	-	-	-	-	-	-	-
reticulocytes	-	-	-	-	-	-	-	-	-	-	-	-
platelets	-	-	-	-	-	-	-	-	-	-	-	-
red blood cells	-	-	-	-	-	-	-	-	-	-	-	-
Clinical chemistry												
sodium	-	-	-	-	-	-	-	-	-	-	-	-
potassium	-	-	-	-	-	-	-	-			-	-
chloride	-	-	-	-	-	-	-	-			-	-
calcium	-	-	-	-	-	-	-	-	-	-	-	-
magnesium	-	-	-	-	-	-	-	-	-	-	-	-
phosphate	-	-	-	-	-	-	-	-	-	-	-	-
albumin	-	-	-	-	-	-	-	-	-	-	-	-
urate	-	-	-	-	-	-	-	-	-	-	-	-
urea	-	-	-	-	-	-	-	-	-	-	-	-
serum protein	-	-	-	-	-	-	-	-	-	-	-	-
serum enzymes	-	-	-	-	-	-	-	-	-	-	-	-
serum iron	-	-	-	-	-	-	-	-	-	-	-	-
cholesterol	-	-	-	-	-	-	-	-	-	-	-	-
triglycerides	-	-	-	-	-	-	-	-	-	-	-	-
bilirubin	-	-	-	-	-	-	-	-	-	-	-	-
blood glucose	-	-	-	-	-	-	-	-	-	-	-	-
creatinine kinase	-	-	-	-	-	-	-	-	-	-	-	-
coagulation	-	-	-	-	-	-	-	-	-	-	-	-

Parameter	Control		20 µg/kg		100µg/kg		500 µg/kg		2500 µg/kg		dose-response+/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ weight (mean and relative)												
heart	-	-	-	-	-	-	-	-	-	-	-	-
lungs	-	-	-	-	-	-	-	-	-	-	-	-
liver	-	-	-	-	-	-	-	-	-	-	-	-
kidneys	-	-	-	-	-	-	-	-	-	-	-	-
adrenals	-	-	-	-	-	-	-	-	-	-	-	-
spleen	-	-	-	-	-	-	-	-	-	-	-	-
thymus	-	-	-	-	-	-	-	-	-	-	-	-
testes/ovaries	-	-	-	-	-	-	-	-	-	-	-	-
prostate/uterus	-	-	-	-	-	-	-	-	-	-	-	-
pituitary	-	-	-	-	-	-	-	-	-	-	-	-
brain	-	-	-	-	-	-	-	-	-	-	-	-

Doc III A Section 6.3.7 (01)

Repeated dose toxicity - Subacute toxicity study of intravenous administration to dogs

Annex Point IIA VI.6.3

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], Subacute toxicity study of medetomidine by daily intravenous administration to dogs for 28 days [redacted] (Unpublished)
1.2	**Data protection**	Yes, data protection is claimed.
1.2.1	Data owner	[redacted]
1.2.2		
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first approval / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes. This study was performed to fulfil the known requirements of the US Food and Drug Administration and OECD,
2.2	**GLP**	Yes. The toxicology studies have been made at [redacted] and [redacted] operates under GLP. The studies have been made quite a long time ago and these studies reflect the GLP of that time. The fact that medetomidine has been approved as pharmaceutical ingredient for commercial use should be proof of level of practices
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Levomedetomedine, one of the two isomeric forms of medetomidine, MPV-1441 HCl. Levomedetomidine is considered to be the lesser or nonactive part of the racemic mixture medetomidine HCl.
3.1.1	Lot/Batch number	10056X
3.1.1.1	**Description**	Crystalline powder white to almost white
3.1.1.2	**Purity**	[redacted] %
3.1.1.3	**Stability**	No information stated in the study
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	[redacted]
3.2.4	Sex	Both sexes
3.2.5	Age/weight at study initiation	5-6 months old. Weight of male dogs were 6.5-8.7 kg and weight of female dogs were 6.0 to 8.0 kg.
3.2.6	Number of animals per group	The group size was 3 male and 3 female dogs per group.

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Annex Point IIA VI.6.3	

3.2.7	Control animals	Yes. Dose group 1
3.3	**Administration/ Exposure**	Daily intravenous dosing for a period of 28 days
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	Daily dosing for 4 weeks for groups 1, 2 and 3. Daily for 3 days for male dogs and daily for 2 days for female dogs in group 4.
3.3.3	Postexposure period	None, due to sacrifice.
3.3.4	**Oral**	**Not applicable due to intravenous administration**
3.3.4.1	**Type**	
3.3.4.2	**Concentration**	
3.3.4.3	**Vehicle**	
3.3.4.4	**Concentration in vehicle**	
3.3.4.5	**Total volume applied**	
3.3.4.6	**Controls**	
3.3.5	**Inhalation**	**Not applicable due to intravenous administration**
3.3.5.1	**Concentrations**	
3.3.5.2	**Particle size**	
3.3.5.3	**Type or preparation of particles**	
3.3.5.4	**Type of exposure**	
3.3.5.5	**Vehicle**	
3.3.5.6	**Concentration in vehicle**	
3.3.5.7	**Duration of exposure**	
3.3.5.8	**Controls**	
3.3.6	**Dermal**	**Not applicable due to intravenous administration**

Doc III A Section 6.3.7 (01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity study of intravenous administration to dogs

3.3.6.1	**Area covered**	
3.3.6.2	**Occlusion**	
3.3.6.3	**Vehicle**	
3.3.6.4	**Concentration in vehicle**	
3.3.6.5	**Total volume applied**	
3.3.6.6	**Duration of exposure**	
3.3.6.7	**Removal of test substance**	
3.3.6.8	**Controls**	
3.3.7	**Intravenous injection**	
3.3.7.1	**Vehicle**	Sterile physiological saline (natrosterile 9 mg/ml) batch UKL 68, acted as both control material and vehicle
3.3.7.2	**Concentration in vehicle**	Dose groups:

DOSE GROUP	DOSE LEVEL	Concentration mg/ml	Dosing volume ml/kg
1	0 mg/kg	0 (phys. Saline)	0.25
2	0.4 mg/kg	1.60	0.25
3	2.0 mg/kg	8.00	0.25
4	10.0 mg/kg	40.0	0.25

3.3.7.3	**Total volume applied**	Dosing volume: 0.25 ml/kg
3.3.7.4	**Controls**	Dose group 1, please see 3.3.7.2
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	**Clinical signs**	Clinical observations were made 0.5-1 hours and 3-4 hours after dosing. At least once a week all animals were examined and palpated. Detail health status were recorded.
3.4.1.2	**Mortality**	Mortality was investigated every morning and evening, maximum 18 h between observations.
3.4.2	Body weight	Body weight were recorded one week before dosing, on the day dosing started and weekely until the end of dosing.
3.4.3	Food consumption	Food comsumption were recorded once a week.
3.4.4	Water consumption	No informat5ion available.
3.4.5	Ophthalmoscopic examination	Examination performed before start of study and at the end of the study.

Doc III A Section 6.3.7 (01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity study of intravenous administration to dogs

3.4.6	Haematology	Samples taken before start of study and at the end of the study. Investigated parameters were hematocrit, hemoglobin, red blood cell count, corpuscular haemoglobin, corpuscular volume, whithe blood cell count, platelet count, reticulocyte count, coagulation tests
3.4.7	Clinical Chemistry	Samples taken before start of study and at the end of the study. Investigated parameters were S-Na, S-K, S-Cl, S-Ca, S-trigyl, S-Bil, S-Bil-kj, S-Pi, S-prot, S-alb, S-krea, S-uraat, S-Fe, B-glucos, S-kol, S-afos, S-afat, S-alat, S-LD, S-GGT.
3.4.8	Urinalysis	Urine samples were collected from 3 male and three female animals from the control group and the highest dose group at the end of the study period. Investigated parameters were volume, pH, osmolarity, protein, ketone, glucose, hemoglobine pigments, sediment.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Heart, lung, liver, kidney, adrenal, brain, thyroid, pancreas, pituitary, testes/ovaries, prostate/uterus, brain.
3.5.2	Gross and histopathology	Brain, spinal cord, pituitary, salivary gland, mammary gland, heart, thyroid, thymus, lungs, trachea, esophagus, stomache, small and large intestine, caecum, adrenals, pancreas, liver, gall bladder, kidneys, urinary bladder, aorta, testes/ovaries, prostate/uterus, epididymis, spleen, lymph node, bone with marrow and marrow smear, sciatic nerve and skeletal muscle.
3.5.3	Other examinations	None
3.5.4	Statistics	SAS-DATASET. Mean, standard deviation and one way analysis of variance. Significance were tested with a Bonferroni test.
3.6	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1	**Observations**	Clinical observations at the terminal sacrifice: in the high dose group signs of local irritation (swelling and scars) at the injection site.
4.1.1	Clinical signs	Clinical signs: no drug-related clinical signs in the low and intermediate groups. In the high dose group salivation, tremors, diarrhoea, vocalisation, redness in the eyes, piloerection and aggression transiently after dosing. Later local irritation at the injection sites and reddish urine was seen. The dosing was discontinued in the high dose group after third dosing (males) or second dosing (females).
4.1.2	Mortality	Mortality: no mortality occurred during the study. In the high dose group the dosing was discontinued due to the untoward local irritation combined with untoward central effects including aggressiveness.
4.2	**Body weight gain**	No drug-related changes.
4.3	**Food consumption and compound intake**	No drug-related changes.

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4.4	**Ophtalmoscopic examination**	No drug-related changes.
4.5	**Blood analysis**	
4.5.1	Haematology	No drug-related changes.
4.5.2	Clinical chemistry	No drug-related changes in the low and intermediate dose groups. In the high dose group males serum creatinine and urea values were slightly increased after three days dosing period. The recovery was observed during dosing free period These changes are considered to be possibly drug-related.
4.5.3	Urinalysis	No drug related changes in low or intermediate dose groups. No examination in high dose group.
4.6	**Sacrifice and pathology**	Necropsy findings at terminal sacrifice: no drug-related changes
4.6.1	Organ weights	No significant changes in absolute and relative organ weights.
4.6.2	Gross and histopathology	Dose related findings were seen only at the injection sites. Perivascular fibrosis was seen in all groups including control group. This lesion is related to local trauma caused by repeated injection procedures. The severity of perivascular fibrosis was increased in the intermediate and high dose groups slightly and severely, respectively. In addition, vascular fibrosis and narrowed vascular lumen was seen in all animals in the high dose group.
4.7	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Male and female beagle dogs were intravenous ly injectioned in to a distal forelimb vein. Test concentrations were 0-10 mg/kg. Daily dosing for 4 weeks for groups 1, 2 and 3. Daily for 3 days for male dogs and daily for 2 days for female dogs in group 4. The group's size was 3 male and 3 female dogs per group. Medetomidine (levomedetomedine, one of the two isomeric forms of medetomidine) MPV-1441 HCl were used. Levomedetomidine is considered to be the lesser or nonactive part of the racemic mixture medetomidine HCl.
5.2	**Results and discussion**	Toxicokinetics: after administration, detectable concentrations were measured in all dogs confirming successful drug dosing. No MPV-1441 was detected in control dogs. The highest group mean plasma concentrations in female dogs were 117 ng/ml, 828 ng/ml, and 5200 ng/ml after the first dose in the low, intermediate and high dose groups, respectively. Clinical signs: no drug-related clinical signs in the low and intermediate groups. In the high dose group salivation, tremors, diarrhoea, vocalisation, redness in the eyes, piloerection and aggression transiently after dosing. Later local irritation at the injection sites and reddish urine was seen. The dosing was discontinued in the high dose group after the third dosing (males) or second dosing (females). Mortality: no mortality occurred during the study. In the high dose group the dosing was discontinued due to the untoward local irritation combined with untoward central effects including aggressiveness. Body weight, organ weight, haematology, Ophtalmoscopic examination:

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Repeated dose toxicity - Subacute toxicity study of intravenous administration to dogs

		no drug-related changes.	
5.3	**Conclusion**	The low dose level 0.40 mg/kg) is considered to be a no-effect level. In the intermediate dose group (2.0 mg/kg) the first minimal toxicological findings were seen. These were slight decreased in heart and pulse rate, slight increase in first minimal toxicological findings were seen, These were slight decrease in heart and pulse rate, slight increase in PR-intervals during ECG recording and slight local irritation at the injection site.	
5.3.1	LO(A)EL	2.0 mg/kg	X
5.3.2	NO(A)EL	0.40 mg/kg	X
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

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Repeated dose toxicity - Subacute toxicity study of intravenous administration to dogs

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*2nd September 2010*
Materials and Methods	*As stated by the applicant*
Results and discussion	*As stated by the applicant*
Conclusion	*LO(A)EL: 10 mg/kg bw/day* *NO(A)EL: 2 mg/kg bw/day*
Reliability	*1*
Acceptability	*Acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3_7(01)-1. Results of clinical chemistry haematology and urinalysis

Parameter	Unit	Controls 0			low dose 0.4 mg/kg			medium dose 2.0 mg/kg			high dose 10.0 mg/kg		
		Week 1	Week 3	Week 4	Week 1	Week 3	Week 4	Week 1	Week 3	Week 4	Dosing days 1-3	Dosing days 1-2	Dosing free period 17 days
weight gain (mean)	kg												
males		8.06 ±0.42	9.54±0.44	9.82±0.39	6.91±0.34	8.42±0.74	8.77±0.78	7.84±0.76	8.64±0.78	8.85±0.72	-	-	-
females		7.46±0.61	8.17±0.59	8.58±0.69	7.62±0.40	8.33±0.43	8.56±0.46	7.44±0.05	8.25±0.25	8.53±0.32	-	-	-
Food consumption	group mean (g)												
males		2800	2800	2800	2800	2800	2763	2800	2800	2800	1200		6800
females		2800	2800	2800	2800	2800	2800	2793	2770	2787		800	6800

Table A6_3_7(01)-2. Results *(specify)* of repeated dose toxicity study

Parameter	Control		0.4 mg/kg		2 mg/kg		10 mg/kg		dose-response+/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	3	3	3	3	3	3	3	3	na	na
Mortality	0	0	0	0	0	0	0	0	na	na
clinical signs*	-	-	-	-	-	-	Dosing discon-inued due to aggressio n and local irritation.	Dosing discon-inued due to aggressio n and local irritation.	+	+
body weight	-	-	-	-	-	-	-	-	-	-
food consumption	-	-	-	-	-	-	-	-	-	-
Haematology										
hemoglobin	-	-	+	+	-	-	-	-	-	-
white blood cells	-	-	+	+	-	-	-	-	-	-
reticulocytes	-	-	-	-	-	-	-	-	-	-
packed cell volume	-	-	+	+	-	-	-	-	-	-
lymphocytes	-	-	+	+	-	-	-	-	-	-
esionphils	-	-	-	-	+	+	-	-	-	-
stago prothrobincomplex	-	-	-	-	-	-	+	+	-	-
platelets	-	-	-	-	-	-	-	-	-	-
red blood cells	-	-	-	-	-	-	-	-	-	-
Clinical chemistry										
sodium	-	-	-	-	-	-	-	-	-	-
potassium	-	-	-	-	-	-	-	-	-	-
chloride	-	-	+	+	-	-	-	-	-	-
calcium	-	-	-	-	-	-	-	-	-	-
magnesium	-	-	-	-	+	+	-	-	-	-
phosphorus	-	-	-	-	-	-	+	+	-	-
albumin	-	-	+	+	-	-	-	-	-	-
urate	-	-	-	-	-	-	-	-	-	-
urea	-	-	-	-	-	-	+	-	+	-
serum protein	-	-	-	-	-	-	-	-	-	-
serum enzymes	-	-	-	-	-	-	-	-	-	-
serum iron	-	-	-	-	-	-	-	-	-	-
cholesterol	-	-	-	-	-	-	-	-	-	-
triglycerides	-	-	-	-	-	-	-	-	-	-
bilirubin	-	-	-	-	-	-	-	-	-	-
blood glucose	-	-	+	+	-	-	-	-	-	-

coagulation	-	-	-	-	-	-	-	-	-	-
aspartate aminotransferase	-	-	-	-	+	+	-	-	-	-
alanine aminotransferase	-	-	+	+	-	-	-	-	-	-
lactate dehydrogenase	-	-	-	-	-	-	+	+	-	-
gamma glutamyltransferase	-	-	-	-	-	-	-	-	-	-
creatinie kinase	-	-	-	-	-	-	-	-	-	-
serum creatinine	-	-	-	-	-	-	+	-	+	-
Urinanalysis										
volume	-	-	-	-	-	-	na	na	-	-
osmolarity	-	-	+	+	+	+	na	na	-	-
sediment	-	-	-	-	-	-	na	na	-	-
pH	-	-	-	-	-	-	na	na	-	-
protein	-	-	-	-	-	-	na	na	-	-
glucose	-	-	-	-	-	-	na	na	-	-
ketones	-	-	-	-	-	-	na	na	-	-
blood	-	-	-	-	-	-	na	na	-	-
Bone marrow										
promyelocytes	-	-	-	-	-	-	-	-	-	-
blast cells	-	-	-	-	-	-	-	-	-	-
myelocytes	-	-	+	-	-	-	-	-	-	-
metamyelocytes	-	-	-	-	-	-	-	-	-	-
segmented granulocytes	-	-	-	-	-	-	-	-	-	-
band granulocytes	-	-	-	-	-	-	-	-	-	-
eosinophils	-	-	-	-	-	-	-	-	-	-
basophils	-	-	-	-	-	-	-	-	-	-
monocytes	-	-	-	-	-	-	-	-	-	-
megakaryocytes	-	-	-	-	-	-	-	-	-	-
plasma cells	-	-	-	-	-	-	-	-	-	-
lymphocytes	-	-	-	-	-	-	-	-	-	-

Parameter	Control		20 µg/kg		100µg/kg		500 µg/kg		dose-response+/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ weight (mean and relative)										
heart	-	-	-	-	-	-	-	-	-	-
lungs	-	-	-	-	-	-	-	-	-	-
liver	-	-	-	-	-	-	-	-	-	-
kidneys	-	-	-	-	-	-	-	-	-	-
adrenals	-	-	-	-	-	-	-	-	-	-
spleen	-	-	-	-	-	-	-	-	-	-
thyroids	-	-	-	-	-	-	-	-	-	-
testes/ovaries	-	-	-	-	-	-	-	-	-	-
prostate/uterus	-	-	-	-	-	-	-	-	-	-
pituitary	-	-	-	-	-	-	-	-	-	-
brain	-	-	-	-	-	-	-	-	-	-
epididymes	-	-	-	-	-	-	-	-	-	-

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Official use only

1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes, data protection is claimed.
1.2.1	Data owner	I-Tech AB
1.2.2	Criteria for data protection	Data on new [a.s.] for [first approval / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline 408.
2.2	**GLP**	Yes
2.3	**Deviations**	Minor deviations from OECD guideline 408 occurred but does not affect the validity of the study.

3 MATERIALS AND METHODS

3.1	**Test material**	Medetomidine
3.1.1	Lot/Batch number	1131668
3.1.2	Specification	As given in section 2.
3.1.2.1	**Description**	Molecular weight: 200.28 g/mol Water solubility: 0.20 g/L at pH 7.9 and 25°C
3.1.2.2	**Purity**	[redacted] %
3.1.2.3	**Stability**	Test substance is hydrolytically and photolytically stable.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	[redacted] Sprague-Dawley: SD
3.2.3	Source	[redacted]
3.2.4	Sex	Both sexes
3.2.5	Age/weight at study initiation	8 weeks, weight 150-200 g
3.2.6	Number of animals per group	20 animals per dose group.
3.2.7	Control animals	Yes

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3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	The animals were dosed 7 days/week for 90 days.
3.3.3	Postexposure period	None
3.3.4	Oral	
3.3.4.1	**Type**	Gavage
3.3.4.2	**Concentration**	0, 0.2, 0.4, 1.2, 3.6 mg/kg bodyweight.
3.3.4.3	**Vehicle**	0.5% lactic acid.
3.3.4.4	**Concentration in vehicle**	0, 0.04, 0.08, 0.24 and 0.72 mg/mL.
3.3.4.5	**Total volume applied**	5.0 ml/kg.
3.3.4.6	**Controls**	Yes.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	**Clinical signs**	Clinical signs were observed twice a day (morning and afternoon) except during weekends and public holidays when observations were made once a day. Clinical observations included changes in skin and fur, eyes, mucous membranes, respiration, circulation, autonomic and central nervous system, somatomotor activity and behaviour pattern. Prior to the first exposure and once a week thereafter more detailed clinical observations were made outside the home cage in a standard area. Signs noted were changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (lacrimation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture, response to holding, presence of clonic or tonic movements, stereotypic behaviour (excessive grooming, repetitive circling) or bizarre behaviour (self-mutilation, walking backwards) were also recorded. During study week 13 sensory reactivity to stimuli, grip strength and motor activity were assessed.
3.4.1.2	**Mortality**	Observations of mortality were made when clinical signs were recorded.
3.4.2	Body weight	The rats were weighed prior to the experiment, once a week during the experiment and at necropsy.
3.4.3	Food consumption	Food consumption was determined during the pre-test period and once a week during the study.
3.4.4	Water consumption	Water consumption was determined during the pre-test period and once

		a week during the study.
3.4.5	Ophthalmoscopic examination	Opthalmoscopical inspections of the eyes was performed prior to the fist administration of test substance. Animals dosed with 0 mg/kg and 1.2 mg/kg were examined during the last week of the study. Had changes been detected in the 1.2 mg/kg group the eyes of all animals would have been examined.
3.4.6	Haematology	Blood samples were taken at termination. Number of red blood cells (RBC), haemoglobin, haematocrit (per cent volume of RBC), mean corpuscular volume of RBC, mean corpuscular haemoglobin, mean corpuscular haemoglobin conc., number of platelets, total leucocyte count, reticulocyte count, thromboplastin time and differential leucocyte count were investigated.
3.4.7	Clinical Chemistry	Blood samples were taken at termination. S-ASAT, S-AFOS, S-ALAT, S-K, S-Pi, S-Prot, S-Total Cholesterol, S-Urea, S-Na, S-Ca. S-Cl, S-Alb, S-Gluc and S-Bil were investigated.
3.4.8	Urinalysis	In week 12 animals were placed in metabolism cages for 23 h and urine was collected. Appearance, volume, specific gravity, pH, protein, glucose and blood were measured. An interim urine sample collection and analysis were performed during study week 5 for all surviving animals in the 3.6 mg/kg group due to suspicion of a diabetic state.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes At necropsy, the following organs were weighed of all animals (both sides for paired organs): Liver, kidneys, adrenals, testes (males), epididymides (males), uterus (females), ovaries (females), spleen, thymus, brain (whole brain including cerebellum, excluding olfactory bulbs), heart
3.5.2	Gross and histopathology	At necropsy, the following organs or representative samples of them were taken from all animals into 4 % buffered formalin (eyes were taken into Davidson's solution) (both sides for paired organs): All tissues with macroscopic changes, brain (cerebrum, cerebellum, medulla/pons), eyes, spinal cord (cervical, mid-thoracic and lumbar), pituitary, stomach, small intestine (two samples, one with Peyer's patches), large intestine, liver, kidneys, pancreas, adrenals, spleen, thymus, trachea, oesophagus, thyroid gland, parathyroid glands, salivary glands (submandibular), lungs, aorta, heart, testes (males), ovaries (females), prostate (males), seminal vesicles and coagulating gland (males), epididymides (males), uterus (females), mammary gland (females; with skin), skin (from the chest; males), bladder, lymph nodes (mesenterial and cervical), peripheral nerve (*Nervus ischiadicus*), muscle (*M. gluteus maximus*), bone (distal femur), sternum (including bone marrow). Full histopathological investigation was carried out on the organs and tissues of all animals in the control group and the 1.2 mg/kg dose group. Had significant pathological changes been found in the 1.2 mg/kg group, the histopathological analysis would have been extended to all

		other treatment groups on tissues and organs identified as showing effects in the 1.2 mg/kg group.
3.5.3	Other examinations	Determination of test substance concentration was made during the last study week with blood samples taken from the femoral vein.
3.5.4	Statistics	Continuous variables were summarized by treatment group and sex using descriptive statistics. Categorical and ordinal variables were summarized with frequencies and percentages. All statistical tests were performed as two-sided with 5 % alpha level without any adjustments. Appropriate nonparametric methods were to be used if the normality assumption was not met. Cochran-Mantel-Haenszel statistics were used for categorical and ordinal variables and as a non-parametric alternative for the ANOVA models.
3.6	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Control group
Stress secretions in some animals, females and males, were observed. A fur abnormality was observed in one animal (female).
0.2 mg/kg
Stress secretions in some animals were observed. Less common than in the control group for females but not for males. Sedation following dosing in all animals.
0.4 mg/kg
Stress secretions in some animals were observed. Less common than in the control group for both females and males. Sedation following dosing in all animals. Transient alopaecia were observed in 2 females during week 4-5. Two females had scabs on the cheek during week 12-13. Fur abnormalities in four females during 1-2 weeks.
1.2 mg/kg
Stress secretions more abundant than in the control group. Sedation following dosing in all animals. Broken tooth in one female. Clonic convulsions in two females, start week 7 and onward for one female and isolated to week 11 and 13 for one female.
All females appeared stressed during the sensory reactivity observations made in week 13.
3.6 mg/kg
Stress secretions relatively common during the first week and from week 5 and onward. Sedation following dosing in all animals. Matt eyes were observed in three females during the first week. Slight dental problems, abnormal body position and fur abnormalities were observed in both females and males. Clonic convulsions were seen in all surviving animals from week 6 and onward.
All surviving females and males appeared stressed and showed rushing motor behavior during the sensory reactivity observations made in week 13. Ten animals (female and male) had frightful appearance; two animals (male and female) were hyperreactive to touch and one to noice (female). All seven surviving males had grade 2 piloerection. One male

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		had a swollen nose and three had broken teeth.
4.1.2	Mortality	Three animals died (two females and one male) and three were euthanized (one female and two males) due to weak general condition and loss of weight. The observed convulsions in the 1.2 and 3.6 mg/kg groups were not considered sever enough for termination.
4.2	**Body weight gain**	Weight gain was significantly affected for females in the 0.4 group and upward. Weight gain was significantly affected for males in the 1.2 and 3.6 mg/kg groups.
4.3	**Food consumption and compound intake**	Food consumption was reduced in the 3.6 mg/kg groups during the first week but not evident thereafter. Water consumption was increased in the 1.2 and 3.6 mg/kg groups from week 3 and onward.
4.4	**Ophtalmoscopic examination**	Animals of the control group and the 1.2 mg/kg group were examined during the last week of the study. No changes from the pre-study condition were observed. Thus, further ophthalmoscopical inspection of the animals of the other dosing groups was not performed.
4.5	**Blood analysis**	
4.5.1	Haematology	Control group Slightly elevated haemoglobin concentration in one male and five males had slightly elevated haematocrit values. 0.2 mg/kg Two males had slightly elevated haematocrit values. Mean corpuscular haemoglobin concentration, mean platelet count, mean monocyte counts and thromboplastine time were significantly higher than the control groups (females and males) while the mean basophil counts were significantly reduced. 0.4 mg/kg Two males and one female had elevated haematocrit values. Mean corpuscular haemoglobin, mean platelet count, mean monocyte counts and thromboplastine time were significantly higher than the control groups (females and males) while the mean basophil counts were significantly reduced. 1.2 mg/kg Mean haemoglobin concentration, mean haematocrit values and mean erythrocyte counts were reduced. Mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean platelet count, mean neutrophil count, monocyte counts and thromboplastine time were significantly higher than the control groups (females and males) while the mean basophil and lymphocyte counts were significantly reduced. 3.6 mg/kg Mean haemoglobin concentration, mean haematocrit values and mean erythrocyte counts were reduced. Mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean platelet count, mean neutrophil count, monocyte counts and thromboplastine time were significantly higher than the control groups (females and males) while the mean basophil and lymphocyte counts were significantly reduced.
4.5.2	Clinical chemistry	Control group Normal values. One male had slightly elevated blood haemoglobin concentration and five haematocrit values from males exceeded the

		normal range. 0.2 mg/kg Serum sodium, chloride and phosphorus values were decreased and serum potassium values were increased. Serum total protein, albumin and cholesterol values were decreased. Serum urea and bilirubin values were decreased compared to the control group and alkaline phosphatase levels were increased, but it should be noted that the method to determine serum bilirubin concentrations was changed during the study and a more sensitive method was used for the 0.2 mg/kg dose group than that used for the samples from the other groups. 0.4 mg/kg Serum sodium and chloride values were decreased. 1.2 mg/kg Mean serum glucose value increased. Serum sodium values were decreased. Serum urea, alkaline phosphatase and alanine aminotransferase values were increased. 3.6 mg/kg Mean serum glucose value increased. Serum sodium, potassium, chloride and phosphorus values were increased. Serum total protein and albumin values were decreased. Serum urea, alkaline phosphatase and alanine aminotransferase values were increased.
4.5.3	Urinalysis	Control group Normal values. 0.2 mg/kg Urine volumes were increased by 73 %. Urine pH was significantly increased in female animals. 0.4 mg/kg Elevated concentrations of glucose in urine. 1.2 mg/kg Elevated concentrations of glucose in urine. Urine volumes were decreased by approximately 40%. Urine pH was significantly increased in male animals. Urine appearance was more often turbid and blood was present in urine more often in this group compared to the control group. 3.6 mg/kg Elevated concentrations of glucose in urine. Urine volumes were decreased by approximately 40 %. Urine appearance was more often turbid and blood was present in urine more often in this group compared to the control group.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Control group - 0.2 mg/kg Mean weights of the left and right epididymis, the heart, left and right kidney and the liver were significantly reduced compared to the controls for male animals. No statistically significant differences in mean relative organ weights compared to the control group were observed for females.

In male animals relative liver and left kidney weights were reduced.

0.4 mg/kg

The mean weights of the heart and left and right kidney were significantly reduced compared to the controls for male animals. The mean relative heart and liver weights were significantly increased compared to the controls for female animals. In male animals the relative left kidney weights were reduced.

1.2 mg/kg

The mean weights of the left and right adrenal gland, spleen and thymus were statistically significantly reduced. The mean weight of the brain was statistically significantly increased. In male animals the weights of the left and right epididymis, the heart and left and right kidney were significantly reduced. In the female animals the mean liver weight was significantly increased and the mean weight of the uterus was reduced. The mean relative heart, liver and left and right kidney weights were significantly increased and the relative weights of the left and right adrenal glands were significantly reduced in female animals. In males the relative liver and left testicle weights were significantly increased.

3.6 mg/kg

The mean weights of both adrenal glands and kidneys, spleen and thymus were statistically significantly reduced. In male animals the weights of the left and right epididymis, the heart, liver and left and right testicle were significantly reduced. In the female animals the mean weights of the left and right ovary and the uterus were significantly reduced. The mean relative heart, liver and left and right kidney weights were significantly increased and the relative weight of the left adrenal gland was significantly reduced in female animals. Also the relative left and right ovary weights of this group were significantly reduced. In males the relative liver and left testicle weights were significantly increased.

4.6.2 Histopathology

Control group

Kidney: Sporadic segments of tubular dilatation and intratubular hyaline casts were found in 6 control rats (unilateral in 2 females and 3 males; in one male rat the lesion was bilateral).

Prostate gland: Focal non-specific lymphocytic infiltrations in one male control animal.

0.2 mg/kg and 0.4 mg/kg

Samples from some animals of the 0.4 mg/kg (3 animals) and 0.2 mg/kg (2 animals) dose groups were analyzed histopathologically because of macroscopical findings in necropsy. One female animal of the 0.4 mg/kg group had non-specific findings in the oesophagus and ventricle. One male animal of the 0.4 mg/kg group had reactive lymphatic non-specific findings and non-specific findings in the ileum. Another male animal of this group had non-specific findings in the small intestine and some reactive lymphatic tissue. One male animal of the 0.2 mg/kg group had a sample of fat and muscle tissue with lymphoplasmacytic

infiltration with granulocytes. Another male of this group had a non-specific liver cyst.

1.2 mg/kg

Kidney: Sporadic segments of tubular dilatation and intratubular hyaline casts were found in one treated male animal; the lesion was bilateral.

3.6 mg/kg

No full histopathology performed.

4.7 Other

None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Subchronic toxicity of medetomedine was studied by repeated oral administration to rats for 90 days according to OECD Guideline 408. The doses used were 0, 0.2, 0.4, 1.2 and 3.6 mg/kg bodyweight. The number of animals used in the dose groups and the control groups were 20 per group (10 female and 10 male).

5.2 Results and discussion

Based on the evidence generated by the present study, it appears that the two higher dose levels selected for the study, 1.2 and 3.6 mg/kg, resulted in significant toxicity to the test animals. Three of 20 animals died in the 3.6 mg/kg dose group and three animals of this group were euthanized because of weakness and severe weight loss. Growth rates were very significantly reduced in both females and in males in the 1.2 and 3.6 mg/kg dose groups and many animals showed behavioural signs indicative of toxicity: stress secretions, increased responsivity to sensory stimuli and convulsions. Glucose secretion into urine was increased and blood glucose and HbA1c levels showed dose-related increases. Proteinuria was also commonly observed.

Also the lower dose groups 0.2 and 0.4 mg/kg showed some effects related to exposure to the test substance. Glucose secretion into urine was associated with test substance administration in a dose-related fashion. It is not clear whether increased glucose secretion into urine reflects increased glucose levels in blood, decreased renal thresholds for glucose secretion or a combination of both factors. Fasting blood glucose levels were not increased in the 0.2 mg/kg dose group compared to the controls, and in the 0.4 mg/kg dose group, there was only a small non-significant increase compared to the controls. Glycosylated haemoglobin levels in blood that reflect long-term glucose homeostasis were somewhat increased in the 0.4, 1.2 and 3.6 mg/kg dose groups but not in the 0.2 mg/kg dose group. Urine volumes were increased by 73 % in the 0.2 mg/kg dose group compared to the controls and decreased by 40 % in the 1.2 and 3.6 mg/kg dose groups compared to the controls. The increased urine excretion in the low-dose group reflects the known diuretic effect of medetomidine. The reduced urine output in the high-dose groups may be partly due to impaired kidney function, since proteinuria was more common in the 1.2 and 3.6 mg dose groups than in the controls. Also mean serum urea levels were increased in the 1.2 and 3.6 mg dose groups compared to the controls, by 34 and 28 %.

It may be concluded that a no-observed-effect level (NOEL) of medetomidine exposure was not determined in this 90-day study. If sedation is not considered to be an adverse effect, the 0.2 mg/kg dose

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		may be considered to reflect a no-observed-adverse-effect level (NOAEL) of exposure.	
5.3	**Conclusion**		X
5.3.1	LOAEL	0.4 mg/kg	
5.3.2	NOAEL	0.2 mg/kg	
5.3.3	Other	None	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*20th September*
Materials and Methods	*As stated by the Applicant*
Results and discussion	*As stated by the Applicant*
Conclusion	*LO(A)EL: 0.2 mg/kg bw/day* *NO(A)EL: Not possible to set a NOAEL as the lowest dose tested resulted in sedation.*
Reliability	*1*
Acceptability	*acceptable*
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4_1-1. Results of subchronic dose toxicity study

Parameter	0.2 mg/kg		0.4 mg/kg		1.2 mg/kg		3.6 mg/kg		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined	10	10	10	10	10	10	10	10	na	na
Mortality	0	0	0	0	0	0	3	3	+	+
Clinical signs*	Stress secreation	-	Stress secreation	Transient alopaecia, scabs	Stress secreation	Stress secreation, clonic convulsions, broken tooth		Stress secreation, clonic convulsions, broken tooth, matt eyes, abnormal body position, abnormal fur	+	+
body weight gain	Not affected	Not affected	Not affected	Not affected	↓	↓	↓	↓	+	+
Organ weight	↓	Not affected	↓	Not affected	↓	↓	↓	↓	+	+
food consumption / animal	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
Ophthalmological observations	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
Urine analysis										
volume	↑	↑	Not affected	Not affected	↓	↓	↓	↓	+	+
pH	Not affected	↑	Not affected	Not affected	↑	Not affected	Not affected	Not affected	-	-
protein	Not affected	Not affected	Not affected	Not affected	↑	↑	↑	↑	+	+
glucose	Not affected	Not affected	↑	↑	Not affected	Not affected	Not affected	Not affected	-	-
blood	Not affected	Not affected	Not affected	Not affected	↑	↑	↑	↑	+	+
Haematology										
red blood cells	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	↓	↓	+	+
hemoglobin	Not affected	Not affected	Not affected	Not affected	↓	↓	Not affected	Not affected	-	-
hematocrit	Not affected	Not affected	Not affected	Not affected	↓	↓	↓	↓	+	+
platelets	↑	↑	↑	↑	↑	↑	↑	↑	+	+
leucocytes	↑	↑	↑	↑	↑	↑	↑	↑	+	+

reticulocytes	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
erthocyte count	Not affected	Not affected	Not affected	Not affected	↓	↓	↓	↓	+	+
tromboplastine time	↑	↑	↑	↑	↑	↑	↑	↑	+	+
Clinical chemistry										
aspartat aminotransferas	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
alkaline phosphatase	↑	↑	Not affected	Not affected	↑	↑	↑	↑	+	+
alanin aminotransferas	Not affected	Not affected	Not affected	Not affected	↑	↑	↑	↑	+	+
sodium	↓	↓	↓	↓	↓	↓	↑	↑	-	-
potassium	↑	↑	Not affected	Not affected	Not affected	Not affected	↑	↑	-	-
chloride	↓	↓	↓	↓	Not affected	Not affected	↑	↑	-	-
calcium	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
magnesium	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
phosphorus	↓	↓	Not affected	Not affected	Not affected	Not affected	↑	↑	-	-
albumin	↓	↓	Not affected	Not affected	Not affected	Not affected	↓	↓	-	-
urea	↓	↓	Not affected	Not affected	↑	↑	↑	↑	+	+
serum protein	↓	↓	Not affected	Not affected	Not affected	Not affected	↓	↓	-	-
cholesterol	↓	↓	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
bilirubin	↓	↓	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
blood glucose	Not affected	Not affected	Not affected	Not affected	↑	↑	↑	↑	+	+
Organ weight (relative)										
heart	Not affected	Not affected	Not affected	↑	Not affected	↑	Not affected	↑	-	+
lungs	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
liver	↓	Not affected	Not affected	↑	↑	↑	↑	↑	+	+
kidneys	↓	Not affected	↓	Not affected	Not affected	↑	Not affected	↑	-	+
adrenals	Not affected	Not affected	Not affected	Not affected	Not affected	↓	Not affected	↓	-	+

spleen	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
thymus	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
testes/ovaries	Not affected	Not affected	Not affected	Not affected	↑	Not affected	↑	↓	+	-
prostate/uterus	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
brain	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-
epididymes	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	Not affected	-	-

III A Section 6.4.2 Annex Point VI.6.4	**Subchronic dermal toxicity test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	No data on subchronic dermal toxicity is provided. A 90-days subchronic study of oral toxicity is submitted and the results serve as a base for extrapolation to dermal subchronic exposure.	
Undertaking of intended data submission [ ]	No	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	20th September 2010	
Evaluation of applicant's justification	Applicants justification is acceptable	
Conclusion	Acceptable	
Remarks	None.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		