Doc III A sections A6.1.8(01) **Acute Subcutaneous Toxicity in Rat**

Annex Point IIA 6.1

Table A6_1_8(01)-1. Table for Acute Subcutaneous Toxicity (modify if necessary)

Dose [unit]	*Number of dead / number of investigated*	*For observations, please see section Results*
10 µg/kg	0/3	-
50 µg/kg	0/3	-
250 µg/kg	0/3	-
1.25 mg/kg	0/3	-
6.25 mg/kg	0/3	-
31.25 mg/kg	2/3	-
LD_{50} value	20 mg/kg	

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Introduction to metabolism in mammals (dog)

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			Official use only
		1 REFERENCE	
1.1	**Reference**	Mayer M, Machinist J.1997. Metabolism and Disposion of [3H] Dexmedetomidine HCl following subcutaneous and intravenous administration to dogs. **Abbott-85499 Drug metabolism Report No 19.** Pharmacology Reviews, FDA, Centre for Drug Evaluation and Research, Division of anaesthetic critical care and addiction drug products. NDA 21-038, volume 1-4. Study No: R&D 97/291 (published)	
1.2	**Data protection**	Yes, data protection is claimed.	
1.2.1	Data owner	[redacted]	
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**	No	
2.2	**GLP**	Yes	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomedine HCl, with tritium on the bridge methyl group, was synthesised by Amersham and the dexmedetomidine isomer was separated at Abbott by chiral chromatography. Lot 50498-ST-223; 80Ci/mmol. Unlabeled dexmedetomidine Lot 295260-0-AX, was added to the labelled dexmedetomidine HCl to provide a solution of 20 µCi/ml and 0.018 mg salt/ml. Normal saline was used as solvent.	X
3.1.1	Lot/Batch number	Dexmedetomidine Lot 50498-ST-223; 80Ci/mmol. Unlabeled dexmedetomidine Lot 295260-0-AX	
3.1.2	Specification	As given in section 2	X
3.1.2.1	**Description**	Medetomedine HCl , with tritium on the bridge methyl group, was synthesised by Amersham and the dexmedetomidine isomer was separated at Abbott by chiral chromatography.	
3.1.2.2	**Purity**	No information	
3.1.2.3	**Stability**	No information	
3.1.2.4	**Radiolabelling**	Medetomedine HCl , with tritium on the bridge methyl group	
3.2	**Test Animals**		
3.2.1	Species	Dog	

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3.2.2	Strain	Beagle	
3.2.3	Source	[redacted]	
3.2.4	Sex	Both sexes	
3.2.5	Age/weight at study initiation	0.5 to 3 years of age, 8-12 kg	
3.2.6	Number of animals per group	2 beagle dogs/ sex	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dogs were injected with labelled dexmedetomidine iv and 2 weeks later sc. The urine and faeces were extracted for labelled metabolites and an enzymatic hydrolysis of the urine sample to evaluate glucoronides and sulfate conjugates. The injections were in the cephalic vein, 0.02 mg/kg of labelled dexmedetomidine, and approximately 50 µCi/mmol. The blood was sampled and the urine and faeces were collected for 5 days.	
3.3.1	Preparation of test site	No information	
3.3.2	Concentration of test substance	0.02 mg/kg of labelled dexmedetomidine , approximately 50 µCi iv.	
3.3.3	Specific activity of test substance	50 µCi	
3.3.4	Volume applied	0.25 mL/kg	
3.3.5	Size of test site	No information	
3.3.6	Exposure period	Dogs were injected with labelled dexmedetomidine iv and 2 weeks later sc.	
3.3.7	Sampling time	The blood samples after dexmedetomidine administration were at 0, 0.1, 0.25, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 48, 72, 96, 120 hour post-dosing. The urine was collected in 0-24 hr, 24-48 hr and was pooled by volume for a representative 0-48 hour sample. The faeces samples for 0-24 hours and 24-28 hours were extracted and analyzed. An average of 63% of the radioactivity was extracted.	**X**
3.3.8	Samples	Urine, faeces, blood samples.	**X**

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Introduction to metabolism in mammals (dog)

4 RESULTS AND DISCUSSION

The tritiated water was comprised less than 1% of the total tritium label but the percent of the radioactivity in the plasma constituting tritiated water increased with time and by 120 hours post dosing, it was 74% of the plasma radioactivity.

There were no apparent sex-related differences in metabolic profile. A comparison of AUC_{0-120} was 110.4 ng Eq.hr/ml after sc administration and 101.8 ng Eq.hr/ml after iv administration, supporting good bioavailability. The peak plasma levels of labelled dexmedetomidine occurred about 2 hours after sc administration (4.39 ng Eq/ml) and the peak level of radioactivity occurred with 6 hours of sc dosing (8.98 ng/Eq/ml) Radioactivity was excreted mainly in the urine (>80%) by both routes of administration and in both sexes. The faecal amounted to about 13%.

The following tables were taken from the submission (V42/PG 384, 386) and present the metabolite distribution:

Urine Metabolite Distribution

Urine Component	Intravenous	Subcutaneous
COOH	13.21	11.77
SO_3OH	13.12	15.25
G-OH	10.81	8.06
OH	1.32	2.21
D-1	9.00	9.88
D-2	7.52	6.76
D-3	6.12	6.60
D-4	6.31	6.29
D-6	1.91	2.46
Dex	0.00	0.43
Others	9.65	10.80
% Dose Excreted	78.97	80.51

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Plasma Metabolite Distribution % AUC ^{3}H

Plasma Component*	Intravenous	Subcutaneous
Total ^{3}H	100.00	100.00
Dex	21.47	24.48
COOH	7.09	5.89
SO_3OH	10.21	9.87
G-OH	11.88	7.64
OH	1.43	1.28
D-2	4.54	3.81
D-4	9.68	7.73
D-6	5.57	5.22
D-7	1.75	4.45
Others	26.38	29.65
*Corrected for tritiated water		

4.1 Toxic effects, clinical signs No effects

4.2 Dermal irritation No effects

4.3 Recovery of labelled compound See table above.

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Introduction to metabolism in mammals

5 APPLICANT'S SUMMARY AND CONCLUSION

The bioavailability of tritiated dexmedetomidine, in dogs, by sc injection was comparable to iv dosing and there was no sex differences observed in metabolic profiles. The exposures to the parent drug accounted for 21% to 25% of the AUC of total radioactivity. The plasma metabolites included carboxyl and hydroxyl metabolites with the glucoronides and sulfate conjugates of the latter.

5.1	**Materials and methods**	Dexmedetomidine HCl following subcutaneous and intravenous administration to dogs.	
5.2	**Results and discussion**	The tritiated water comprised less than 1% of the tritium label but the percent of the radioactivity in the plasma constituting tritriated water increasing with time and by 120 hours postdosing, it was 74% of the plasma radioactivity. There were no apparent sex-related differences in metabolic profile. A comparison of AUC 0-120 was110.4 ng Eq.hr/ml after sc administration and 101.8 ng Eq.hr/ml after iv administration, supporting good bioavailability. The peak levels of labelled dexmedetomidine occurred about 2 hours after sc administration (4.9 ng Eq.hr/ml) ad the peak level of radioactivity occurred after 6 hours of sc dosing (8.89 ng Eq.hr/ml) Radioactivity was excreted mainly in the urine (>80%) by both routes of administration and in both sexes. The faecal excretion amounted to about 13 %.	
5.3	**Conclusion**	There were no apparent sex-related differences in metabolic profile. A comparison of AUC 0-120 was110.4 ng Eq.hr/ml after sc administration and 101.8 ng Eq.hr/ml after iv administration.	
5.3.1	Reliability	1	**X**
5.3.2	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	*27/7/10*
Guidelines and Quality Assurance	*GLP: There is no GLP certificate attached to the study report*
Materials and Methods	***Specification**: No information on specification provided in section 2. Unsure which section this referring to.* ***Test material**: The final solution is 20 µCi/mL and 0.080 mg salt/mL and **NOT** 0.018 mg salt/mL as stated by the applicant.* ***Sampling time**: The correct sampling times were 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 2, 96 and 120 hours.* ***Sampling time**: The urine and faeces were collected daily for 5 days, with the 24 hour urine being collected over dry ice. The 0-24 hour next urine samples were clarified by centrifugation and analysed by HPLC. In addition, 0-24 and 24-48 hour urine was pooled for each dog to give representative 0-48 hour samples. Faecal samples from 0-24 and 24-48 hours were pooled prior to analysis.* ***Samples**: Plasma was also sampled and analysed.*
Results and discussion	*As described by the Applicant*
Conclusion	*As described by the Applicant*
Reliability	*2- due to lack of GLP certificate.*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
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	

Doc III A section 6.2.2(01)

Annex Point VI.6.2

Basic pharmacokinetics of medetomidine in rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Basic pharmacokinetics of medetomidine in rat. [redacted] (unpublished)	
1.2	**Data protection**	Yes, data protection is claimed	
1.2.1	Data owner	[redacted]	
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**	No – the objective was to get basic information about pharmcokinetics of medetomidine after a single dose – and there are no guidelines for such an overview.	
2.2	**GLP**	Yes, [redacted] works according to GLP and the substance was approved by the FDA.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Tritiated medetomidine synthesised from medetomidine HCl and dissolved in methanol solution	
3.1.1	Lot/Batch number	Not available	
3.1.2	Specification	As given in section 2	X
3.1.2.1	**Description**	Tritiated medetomidine in methanol solution. Final solution contained 80 µg/ml medetomidine HCl and had a radioactivity of 188 µCi/ml.	
3.1.2.2	**Purity**	Purity of crude product had specific activity of 7.3 Ci/mmol , over 98% purity by TLC analycer	
3.1.2.3	**Stability**	No information	
3.1.2.4	**Radiolabelling**	Tritium labelled medetomidine. Purity of crude product had specific activity of 7.3 Ci/mmol ,	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley	
3.2.3	Source	No information	
3.2.4	Sex	Both	
3.2.5	Age/weight at study initiation	270±67 g, no information regarding age available	
3.2.6	Number of animals per group	6 animals per group, in total 72 animals	

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Basic pharmacokinetics of medetomidine in rat

Annex Point VI.6.2

3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Subcutaneous
3.3.1	Preparation of test site	No direct preparation of test site stated in the study. Blood was taken from anestesized animals with cardiopuncture and tissue samples.
3.3.2	Concentration of test substance	3H-medetomidine 80 µg/kg or 188 µCi/kg)
3.3.3	Specific activity of test substance	188 µCi/kg
3.3.4	Volume applied	No information
3.3.5	Size of test site	No information
3.3.6	Exposure period	6, 24 hours or other
3.3.7	Sampling time	0.083, 0.167, 0.33, 0.67, 1.33, 3.0, 5.0, 8.0, 14, 24, 48, 72 h
3.3.8	Samples	Blood and tissue samples, faeces and urine samples
		4 RESULTS AND DISCUSSION
4.1	**Toxic effects, clinical signs**	No effect
4.2	**Dermal irritation**	No effects.
4.3	**Recovery of labelled compound**	No information
4.4	**Percutaneous absorption**	No information
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The disposition of medetomidine in the rat after a single subcutaneous dose was studied using the tritium labelled drug.

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Basic pharmacokinetics of medetomidine in rat

Annex Point VI.6.2

5.2	**Results and discussion**	Medetomidine appeared to be rapidly absorbed and distributed. Half-lives for absorption and distribution in plasma were 0.06 h and 0.12 h, respectively. The drug penetrated the blood-brain barrier to reach its site of action. It was also eliminated rather rapidly from the CNS; elimination half-life from brains was about 1.5 h which is nearly the same as the half-life for elimination from plasma, 1.6 h. Elimination of total radioactivity from plasma was slower, half-life 5.2 h, indicating the presence of some metabolites. These could not be identified from plasma, however. Nearly half of the dose was extracted during the first day and an additional 11% during the next two days. The main part of the excretion, 40% of the dose, was in the urine. A considerable portion, about 20% of the dose, was also recovererd in the feces. One major metabolite mostly in its glucoronide conjugate form was observed in urine while only traces of the parent compound were present. Thus biotransformation is the transferred route of elimination. Concentrations of the drug in tissues were clearly higher than in plasma and that in adrenals remained high for the first day. No irreversible cumulation, however, was observed.	
5.3	**Conclusion**	The main part of the excretion, 40% of the dose, was in the urine. A considerable portion, about 20% of the dose, as also recovered in thee faeces.	
5.3.1	Reliability	1	**X**
5.3.2	Deficiencies	No	

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Basic pharmacokinetics of medetomidine in rat

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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	*11/8/2010*
Guidelines and Quality Assurance	*GLP compliance is stated but a valid GLP certificate is not attached to the study report.*
Materials and Methods	***Specification**: no information on specification is provided in section 2. Unsure what section this is referring to.*
Results and discussion	*As stated by the Applicant.*
Conclusion	*As stated by the applicant.*
Reliability	*2- lack of GLP certificate.*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
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_2_2(01)-1. Table for Basic pharmacokinetics of medetomedine in rat

	Half-lives (h)	Half-lives (h)	Half-lives (h)		
	Absorpiton	Distribution	Elimination	Total clearance ml/min kg	Distribution volume l/kg
Unchanges drug in plasma	0.06	0.12	1.6	No measurable	No measurable
Total activity in plasma	0.04	0.08	5.2	2.7	1.2
Liver	-	-	6.6	-	-
kidneys	-	-	4.1	-	-
lungs	-	-	6.4	-	-
brains	-	-	1.5*	-	-
adrenals	-	-	20.9	-	-
*During the first 3 hours					

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Annex Point IIA VI.6.2

Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs

			Official use only
		1 REFERENCE	
1.1	**Reference**	Kuusela E, 2004. Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs. Academic dissertation. Department of clinical veterinary sciences, University of Helsinki, Finland. Pages 1-69. (Published)	
1.2	**Data protection**	No	
1.2.1	Data owner	Public domain	
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**	No	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine (Domitor), dexmedetomidine and levomedetomidine. All drugs were in HCl form.	
3.1.1	Lot/Batch number	No information available from the study	
3.1.2	Specification	No information available from the study	
3.1.2.1	**Description**	Injection fluid 1mg/ml 1 ml contains: medetomidine HCl 1 mg, 1 ml contains: Medetomidine Hydrochloride 1 mg, Metylparahydroxybensoat, propylparahydroxybensoat, natriumchloride, water for injection to 1 ml.	
3.1.2.2	**Purity**	No information available from the study	
3.1.2.3	**Stability**	No information available from the study	
3.1.2.4	**Radiolabelling**	None	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Beagle	
3.2.3	Source	No information available from the study	
3.2.4	Sex	Both	
3.2.5	Age/weight at study initiation	No information available	**X**
3.2.6	Number of animals per group	6 dogs in each experiment,	

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Annex Point IIA VI.6.2

Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs

3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Injection	
3.3.1	Preparation of test site	No information available	
3.3.2	Concentration of test substance	1mg/ml	X
3.3.3	Specific activity of test substance	None	
3.3.4	Volume applied	**Study 1**: each dog was studied 6 times on separate days. Medetomidine 40 µg/kg , dexmedetomidine 20 µg/kg, dexmedetomidine 10 µg/kg, levomedetomidne 20 µg/kg, levomedetomidine 10 µg/kg and saline placebo were administered as an iv bolus dose. **Study 2**: each dog was studied three times on separate days. The dogs were administered a low dose of levomedetomidine 10 µg/kg as an iv bolus, followed by infusion at a dose of 25 µg/kg/h, a high dose of levomedetomidine 80 µg/kg, followed by infusion at a dose of 200 µg/kg/h, and saline, followed by saline infusion. The infusions were continued for 120 minutes, and at 60 minutes, 10 µg/kg of dexmedetomidine was administered as an iv bolus. **Study 3:** each dog was studied 6 times on separate days. Medetomidine 0.4 µg/kg , dexmedetomidine 0.2 µg/kg, 2 µg/kg and 20 µg/kg were administered as an iv bolus dose preceding a light level of propofol/isoflurane anaesthesia. **Study 4 and 5**: each dog was studied 6 times on separate days. Dexmedetomidine 10 µg/kg was administered intramuscular preceding a light level of propofol/isoflurane (end tidal 1.0%; twice) or propol infusion 200 µg/kg/min twice) anaesthesia or premedication alone (twice).	
3.3.5	Size of test site	No information available	
3.3.6	Exposure period	Please see 3.3.4	
3.3.7	Sampling time	Please see 3.3.4	X
3.3.8	Samples	No information available	
		4 RESULTS AND DISCUSSION	
4.1	**Toxic effects, clinical signs**	Scoring of sedation, respiratory depression and slow recovery, ventricular arrhythmias	
4.2	**Dermal irritation**	No information available	
4.3	**Recovery of labelled compound**	None	
4.4	**Percutaneous absorption**	Not applicable	

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Annex Point IIA VI.6.2

Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effects of dexmedetomdidine were studied in healthy laboratory beagles. Dexmedetomidine is the active isomere of medetomidine. The clinical effects and pharamcokinetics of medetomidine, dexmedetomidine and levomedetomidine were compared. Medetomidine (racemate) and dexmedetomidine (the active isomere) and levomedetomidine (inactive isomere) were compared.
5.2	**Results and discussion**	Dexmedetomdidine was equally safe and effective as the corresponding dose of medetomidine as a sedative, analgesic and premedication in beagle dogs. Levomedetomidine caused no observable behavioural effects in conscious dogs. The pharamcokinetics of dexmedetomidine and racemic medetomidine were similar, but the clearance of levomedetomidine was more rapid than that of the other drugs.
5.3	**Conclusion**	Dexmedetomidine was equally safe and effective as the corresponding dose of medetomidine as a sedative, analgesic and premedicant in laboratory beagles. The pharmacokinetics of dexmedetomidine and racemic medetomidine was similar, but clearance of levomedetomidine was more rapid than that of the other drugs. Levomedetomidine did not produce any observational behavioural effects in conscious dogs.
5.3.1	Reliability	2
5.3.2	Deficiencies	No

Doc III A section 6.2.3(01)

Annex Point IIA VI.6.2

Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Give date of action*
Materials and Methods	**Age/weight at study initiation:** The study report states that the animals were aged between 10 months and 3 years throughout the study period. However, it is not clear whether this is the age range at the initiation of the study or the age at the start and end of the study. **Concentration of test substance:** The study report does not state the concentration of the test substance, only the dose that was administered to the animals, expressed as µg/kg bw. **Sampling time:** Blood samples for plasma preparation were taken at 10, 20, 30, 50, 60, 90 and 120 minutes after administration.
Results and discussion	As stated by the Applicant
Conclusion	As stated by the Applicant
Reliability	2
Acceptability	acceptable
Remarks	Although the study did not adhere to GLP, the experiments were performed under the supervision of laboratory head and have subsequently been reviewed and accepted for publication in a peer-review journal.
	COMMENTS FROM ...
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_2_3(01)-1

Not applicable since the study is a comparison of Dexmedetomidine and levomedetomidine – the isomers of medetomidine, in dogs.

	labelled compound	
	absolute amount	**% of dose**
Compound applied		100
Compartments with compound detected		
1. Protective appliances		
2. Liquid used for washing the skin		
3. Skin (with substance not removable)		
4. Blood		
5. Urine		
6. Faeces		
7. Removed organs *specify organs give sum*		
8. Remaining carcass		
9. Exhaled air		
Sum of #4 – 9: blood, excreta, removed organs, remaining carcass (= absorption)		
Sum of all detected labelled compound (#1 – 9) (=recovery)		

Doc III A section 6.2.4/01

Annex Point IIA6.2

Pharmacokinetics of medetomidine as a single dose in rat, dog and cat

			Official use only
		1 REFERENCE	
1.1	**Reference**	Salonen J, 1989, Pharmacokinetics of medetomidine. Acta vet. Scand. Volume **85** pages 49-54 (published)	
1.2	**Data protection**	No	
1.2.1	Data owner	Public domain	
1.2.2	Criteria for data protection	Not claimed	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine HCl , labelled with tritium	
3.1.1	**Lot/Batch number**	No information available	
3.1.2	**Specification**	As given in section 2	
3.1.2.1	**Description**	Pure crystalline form	
3.1.2.2	**Purity**	98%	
3.1.2.3	**Stability**	No information	
3.1.2.4	**Radiolabelling**	Tritium	
3.2	**Test Animals**		
3.2.1	**Species**	Rat, cat, dog	**X**
3.2.2	**Strain**	Sprague Dawley rats, beagle dogs, cats- no information	
3.2.3	**Source**	No information	
3.2.4	**Sex**	Both sexes	
3.2.5	**Age/weight at study initiation**	Sprague Dawley rats mean weight 270 g, beagle dogs mean weight 9.3 kg, and cats mean weight 3.6 kg. No age specified.	
3.2.6	**Number of animals per group**	72 rats, 9 cats and 6 dogs in total. 6 rats in each group. The cats were kept together and the dogs were kept together.	
3.2.7	**Control animals**	No	
3.3	**Administration/ Exposure**	Rats received 80 µg/kg as a single dose subcutaneously and intravenous injection respectively.	**X**

Doc III A section 6.2.4/01

Annex Point IIA6.2

Pharmacokinetics of medetomidine as a single dose in rat, dog and cat

		Dogs received 80 µg/kg as a single dose subcutaneously and intravenous injection respectively. One month later the same dogs received a single intramuscular dose of 80 µg/kg. The cats received a single intramuscular dose of 80 µg/kg.	
3.3.1	**Preparation of test site**	No information stated	
3.3.2	**Concentration of test substance**	80 µg/kg	
3.3.3	**Specific activity of test substance**	Rat 6.96 MBq/kg Dogs 4.7 MBq/kg Cats 1.7 or 4.37 MBq/kg	
3.3.4	**Volume applied**	No information	
3.3.5	**Size of test site**	Not applicable.	
3.3.6	**Exposure period**	Single dose applied, dogs received a follow up dose one month after first exposure at both exposures the follow up time was 72 h Single dose applied to cats and rats and follow up time was 72 h	
3.3.7	**Sampling time**	Rats: at 24, 48, 72 h after exposure, dogs 72 h and cats 72 h	**X**
3.3.8	**Samples**	Blood, serum, urine,	
		4 RESULTS AND DISCUSSION	**X**

Summary of the pharmacokinetic parameters of medetomidine:

	Cmax ng/ml	Tmax (h)	CL ml/min kg	T1/2 (h)
Rat (s.c.)	-	-	-	1.60
Dog (i.v.)	-	-	33.4	0.97
Dog (i.m.)	22	0.5	27.5	1.28
Cat (i.m.)	24.6	0.25	29.5	1.35

4.1	**Toxic effects, clinical signs**	No effects
4.2	**Dermal irritation**	No effects
4.3	**Recovery of labelled compound**	Excretion of radioactivity was from 28.6 to 74.7% of the dose in three days

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Pharmacokinetics of medetomidine as a single dos in rat, dog and cat
5.2	**Results and discussion**	The results showed a rapid distribution, after a s.c. dose, of medetomidine radioactivity into rat tissues including the brains. In plasma/seum a very short half-life was observed (only a few minutes). Differences between dosing routes were small. In each specie most of the radioactivity was excreted in the urine. Fecal excretion was only significant in the rat. No measureable levels of the parent drug was found in excreta. Instead a hydroxylated product(s) and (their) conjugates (except in cat) were present in urine. Other metabolites were not observed.
5.3	**Conclusion**	Elimination occurs mainly by biotransformation in the liver
5.3.1	Reliability	2
5.3.2	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 11/8/2010

Materials and Methods

Administration/exposure: The test substance was administered by subcutaneous injection to rats and intravenous injection to dogs.

Sampling time: Samples were collected from dogs at 24, 48 and 72 hours post administration.

Species: the data obtained from rats was not evaluated as it is a duplicate of the data presented in Salonen (1986) - Document IIIA 6.2.2, and evaluated in the Competent Authority Report.

Results and discussion

Additional data to be included from table 2 of the original study report.

Excretion of radioactivity: mean ±SD as percentage of the original dose (n=6)

Sample	Dog (i.v)	Cat (i.m)
0-24h urine	26.3±9.2	67.0±12.3
24-4h urine	1.5±0.4	6.3±5.6
48-72h urine	0.7±0.3	1.3±1.1
0-24h feces	4.5±1.6	3.3±3.6
24-48h feces	0.9±0.1	1.8±1.2
48-72h feces	0.3±0.1	0.4±0.4
0-72h urine	28.6±9.1	74.7±14.6
0-72h feces	5.7±1.7	5.5±3.2

Conclusion As stated by the Applicant.:

Reliability As stated by the Applicant

Acceptability acceptable / not acceptable

(give reasons if necessary, e.g. if a study is considered acceptable despite a poor reliability indicator. Discuss the relevance of deficiencies and indicate if repeat is necessary.)

Remarks None.

COMMENTS FROM ...

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	

Doc III A section 6.2.5.2

Metabolism in humans

Annex Point IIA VI. 6.2.introduction

Official use only

		1 REFERENCE
1.1	**Reference**	Abbot-85499 Dexmedetomidine A Phase 1, Single-Center, Open-Label Study Evaluating the Metabolism and Excretion of ^{3}H-Dexmedetomidine in Healthy, Adult Vonunteers.
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
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**	No. A Phase 1 Clinical Study.
2.2	**GLP**	According to Good Clininical Practice.
2.3	**Deviations**	
		3 MATERIALS AND METHODS
3.1	**Test material**	^{3}H-Dexmedetomidine HCl
3.1.1	Lot/Batch number	Stock solution A lot no 15-096VH Stock solution B lot no 55585-ST-37
3.1.2	Specification	Stock solution A – non radioactive Dexmedetomidine HCl, 200 µg/mL in 2 mL glass ampoules Stock solution B – ^{3}H-Dexmedetomidine HCL, 6,04 mCi in 9 ml ethanol.
3.1.2.1	**Description**	Stock B Chemical content 8.028 µg/g; Specific activity 3.35 µCi/µg
3.1.2.2	**Purity**	Stock solution A 99,8% optical purity Stock solution B, radiochemical purity 99%, optical purity >99%
3.1.2.3	**Stability**	No information available
3.1.2.4	**Radiolabelling**	Tritiated Dexmedetomidine HCl
3.2	**Test Animals**	
3.2.1	Species	Human
3.2.2	Strain	n.a.
3.2.3	Source	Healthy volunteers
3.2.4	Sex	Five healthy male volunteers
3.2.5	Age/weight at study initiation	Age 27.4 y (18-33) Weight 75.4 kg (71.2-86.0)

Doc III A section 6.2.5.2

Annex Point IIA VI. 6.2.introduction

Metabolism in humans

		Height 178.6 cm (172-190)
3.2.6	Number of animals per group	Five healthy male volunteers
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Preparation of test site	No information available
3.3.2	Concentration of test substance	0.002 mg/kg of labelled dexmedetomidine during a 10 min period.
3.3.3	Specific activity of test substance	Specific activity 3.35 µCi/µg
3.3.4	Volume applied	No information available
3.3.5	Size of test site	No information available
3.3.6	Exposure period	The five male volunteers were injected with labelled dexmedetomidine i.v. during a 10 min period. Blood, urine and feces were collected during a 9 day period. Additional blood samples were obtained on day 23 or 24.
3.3.7	Sampling time	Blood samples will be drawn at 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h from the start of the infusion. Urine collection occurred 2 hour collection intervals relative to the start of the infusion. Fecal collection occurred for the interval 0-24 h relative the start of the infusion. Further, day 2-7 blood samples were taken daily, urine samples collection occured every 12h, fecal collection occurred daily.
3.3.8	Samples	Urine, faeces, blood samples.

Doc III A section 6.2.5.2

Annex Point IIA VI. 6.2.introduction

Metabolism in humans

4 RESULTS AND DISCUSSION

The pattern of total plasma radioactivity was remarkably similar in all subjects. The concentration decreased from a peak mean value of 3.18 ng Eq/g at 10 minutes to 1.51 ng Eq/g at 30 minutes. After 30 minutes, the radioactivity in plasma rose again due to the appearance of the parent drug N-glucuronides (G-dex-1and 2). A smaller increase was noted after 5 hours, probably indicating enterohepatic cycling. Thereafter, the levels of plasma radioactivity declined gradually over a period of 9 days with traces still present up to 24 days. Including the 23- to 24-day time point, the mean half-life for total plasma tritium was estimated to be 10.75 days which comparable to that of tritiated water in humans (9.46 days).

Unchanged dexmedetomidine was not detected in the urine, suggesting extensive metabolism. Dexmedetomidine is metabolised by phase I and phase II pathways and excreted predominately into the urine. The average of 95,17% of the radioactive dose was excreted in the urine, whereas 4.08% was recovered in the feces after 9 days. Approximately 85% of the dose in urine was recovered wihin 24 hours.

Pharmacokinetic parameters are summerized below.

Pharmacokinetics

Dexmedetomidine Pharmacokinetic Parameters		
Parameter	N	Mean value (±SD)
C_{max} (ng Eq/g)	5	3.12 (±0.27)
$t_{½}$ (h)[a]	3	2.85 (± 1.10)
$AUC_{0-\infty}$ (ng x h/g)	3	3.49 (± 0.68)
CL (L/h)	3	42.60 (±7.10)
V_{SS} (L)	3	143.9 (± 15.50)
V_{β} (L)	3	182.1 (±36.0)
a; harmonic mean SD = Standard Deviation; C_{max} = maximum observed plasma concentration; $t_{½}$ = terminal half-life; $AUC_{0-\infty}$ = area under the plasma concentration time curve; CL = clearance; V_{SS} = appearant steady state volume of distriubution; V_{β} = volume distribution associated with β phase;		

N-Glucuronides of dexmedetomidine (G-Dex-1 and G-Dex-2) were the major circulating metabolites, together accounting for 41.37% of AUC_{0-24} for total plasma radioactivity. The H-1 and H-3 metabolites were also major plasma components. Other metabolites include the carboxy (COOH), N-methylated (N-Meth) and the glucoronide conjugate of the hydroxylated dexmedetomidine (G-OH).

Doc III A section 6.2.5.2

Metabolism in humans

Annex Point IIA VI. 6.2.introduction

Plasma Metabolite Distribution

Plasma Component	Intravenous infusion
G-dex 1	35.19
G-dex 2	6.17
H1	20.55
H3	10.45
COOH	minor
N-methylated	minor
G-OH	minor
Unidentified	5.78%
% Dose Excreted	78.97

Urine Metabolism Distribution

Urine Component	Intravenous infusion
G-dex 1 and 2	34%
H1	14.51%
H3	10.4%5
COOH	minor
OH	minor
G-OH	minor
Unidentified	32%

4.3Recovery of labelled

Overall recovery from 98.62% to 101%. Average was 99.25%.

Doc III A section 6.2.1(01)

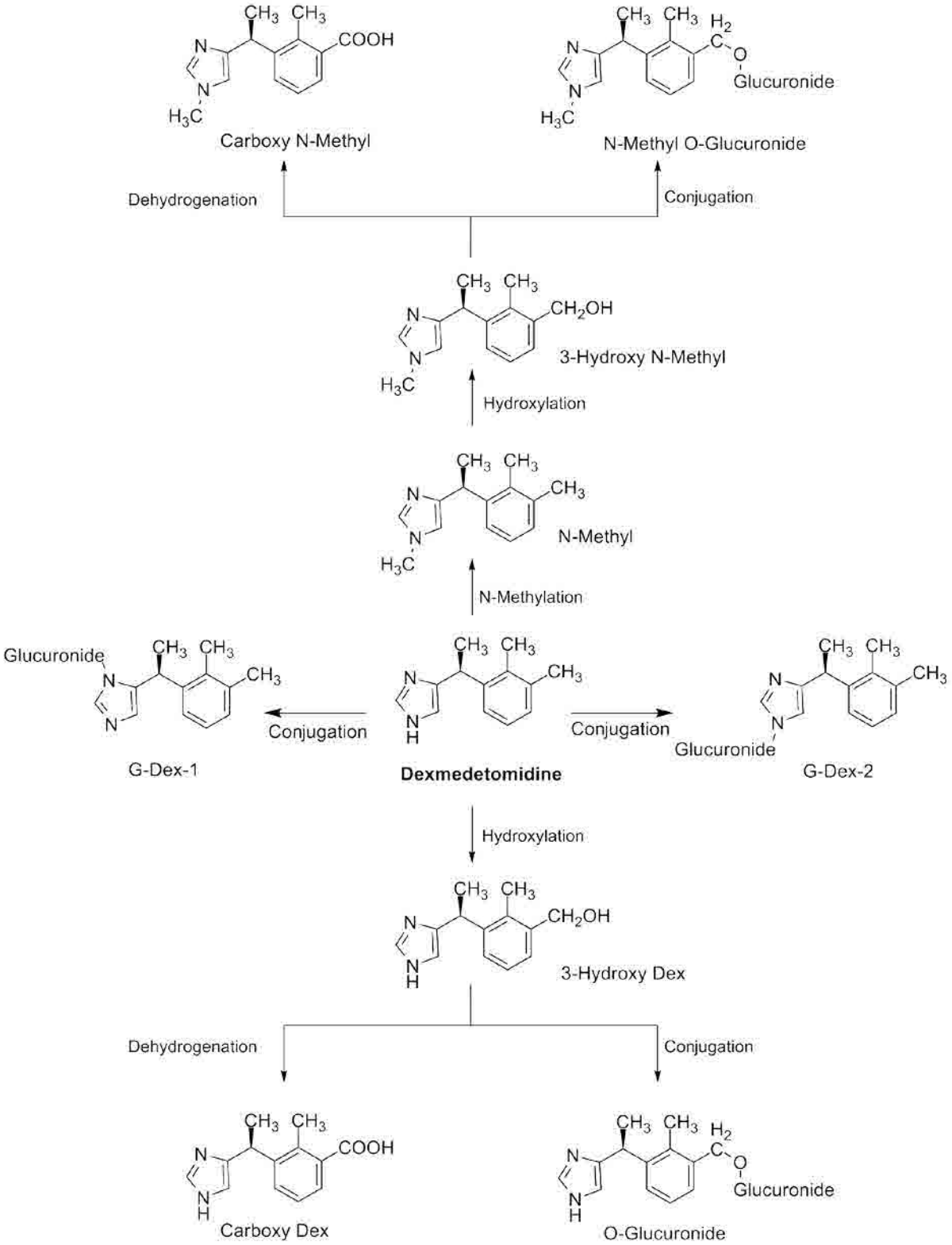
Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in mammals

5 APPLICANT'S SUMMARY AND CONCLUSI

5.1	**Materials and methods**	^{3}H-Dexmedetomidine HCl intravenous administration to humans according to an approved study plan. The study was an open single site phase 1 study.
5.2	**Results and discussion**	Dexmedetomidine is rapidly distributed and rapidly eliminated with a half-life of 2.85 h.. Dexmedetomidine is eliminated by metabolism to inactive metabolites, primarily glucuronides. 80-90% of administrated dose is excreted in the urine and 5-13% in the feces.
5.3	**Conclusion**	Dexmedetomidine undergoes extensive metabolism by phase 1 and phase II pathways.
5.3.1	Reliability	1
5.3.2	Deficiencies	None. A GCP compliant phase 1 study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Jan 2012*
Materials and Methods	*As stated by the applicant*
Results and discussion	*As stated by the applicant*
Conclusion	*As stated by the applicant*
Reliability	*As stated by the applicant*
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
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	


Carboxy N-Methyl
N-Methyl O-Glucuronide
Glucuronide
Dehydrogenation
Conjugation
3-Hydroxy N-Methyl
Hydroxylation
N-Methyl
N-Methylation
Glucuronide
Conjugation
Conjugation
G-Dex-1
Dexmedetomidine
Glucuronide
G-Dex-2
Hydroxylation
3-Hydroxy Dex
Dehydrogenation
Conjugation
Carboxy Dex
O-Glucuronide
Glucuronide

Doc III A section 6.2.5(01)

Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in humans

			Official use only
		1 REFERENCE	
1.1	**Reference**	Mayer M, Machinist J.1997. Summery **Abbott-85499 Drug metabolism Report No 19.** Pharmacology Reviews, FDA, Centre for Drug Evaluation and Research, Division of anaesthetic critical care and addiction drug products. NDA 21-038, volume 1-4. Study No: R&D 97/291 (published)	X
1.2	**Reference**	Karol, M.D. 2000. Pharmacokinetics and interaction pharmadynamics of dexmedetomidine in humans. Baillière's Clinical Anaesthesiology. Vol. 14(2), pp. 261-269.	
1.3	**Data protection**	Yes, data protection is claimed for ref 1.1	
1.3.1	Data owner	[redacted] Reference 1.2. Public domain	
1.3.2	Criteria for data protection	Data on new a.s. for first approval / authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dexmedetomidine HCl	
3.1.1	Lot/Batch number	No information available	
3.1.2	Specification	No information available	
3.1.2.1	**Description**	[^{3}H] dexmedetomidine HCl	
3.1.2.2	**Purity**	No information available	
3.1.2.3	**Stability**	No information available	
3.1.2.4	**Radiolabelling**	No information available	
3.2	**Test Animals**		
3.2.1	Species	Human	
3.2.2	Strain	n.a.	
3.2.3	Source	Healthy volunteers	
3.2.4	Sex	Male	
3.2.5	Age/weight at study	No information available	

Doc III A section 6.2.5(01)

Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in humans

	initiation	
3.2.6	Number of animals per group	5 males
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Preparation of test site	No information available
3.3.2	Concentration of test substance	0.002 mg/kg of labelled dexmedetomidine during a 10 min period.
3.3.3	Specific activity of test substance	No information available
3.3.4	Volume applied	No information available
3.3.5	Size of test site	No information available
3.3.6	Exposure period	The five male volunteers were injected with labelled dexmedetomidine i.v. during a 10 min period. Blood, urine and feces were collected during a 9 day period. Additional blood samples were obtained on day 23 or 24.
3.3.7	Sampling time	No information available.
3.3.8	Samples	Urine, faeces, blood samples.

Doc III A section 6.2.5(01)

Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in humans

4 RESULTS AND DISCUSSION

The pattern of total plasma radioactivity was remarkably similar in all subjects. The concentration decreased from a peak mean value of 3.18 ng Eq/g at 10 minutes to 1.51 ng Eq/g at 30 minutes. After 30 minutes, the radioactivity in plasma rose again due to the appearance of the parent drug N-glucuronides (G-dex-1and 2). A smaller increase was noted after 5 hours, probably indicating enterohepatic cycling.

The average of 95,17% of the radioactive dose was excreted in the urine, whereas 4.08% was recovered in the feces after 9 days. Approximately 85% of the dose in urine was recovered wihin 24 hours.

X

Unchanged dexmedetomidine was not detected in the urine, suggesting extensive metabolism. Dexmedetomidine is metabolised by phase I and phase II pathways and excreted predominately into the urine.

Plasma Metabolite Distribution

Plasma Component	Intravenous infusion
G-dex 1	35.19
G-dex 2	6.17
H1	20.55
H3	10.45
COOH	minor
N-methylated	minor
G-OH	minor
Unidentified	5.78%
% Dose Excreted	78.97

Doc III A section 6.2.5(01)

Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in humans

Urine Metabolism Distribution

Urine Component	Intravenous infusion
G-dex 1 and 2	34%
H1	14.51%
H3	10.4%5
COOH	minor
OH	minor
G-OH	minor
Unidentified	32%

X

4.1 Toxic effects
clinical signs. No effects.

4.2 Dermal irritation No effects

4.3 Recovery of labelled compound Overall recovery from 98.62% to 101%. Average was 99.25%.

Doc III A section 6.2.1(01)

Annex Point IIA VI. 6.2.introduction

Introduction to metabolism in mammals

		5 APPLICANT'S SUMMARY AND CONCLUSI	
5.1	**Materials and methods**	Dexmedetomidine HCl intravenous administration to humans	
5.2	**Results and discussion**	Dexmedetomidine is rapidly distributed and rapidly eliminated with a half-life of 2.2.5 h. Generally, dexmedetomidine does not exhibit pharmacokinetic-based interactions. Dexmedetomidine is eliminated by metabolism to inactive metabolites, primarily glucuronides. 80-90% of administrated dose is excreted in the urine and 5-13% in the feces. There were no apparent ethnicity-related differences in metabolic profile. Dexmedetomidine undergoes extensive metabolism by phase 1 and phase II pathways.	X
5.3	**Conclusion**		
5.3.1	Reliability	3	**X**
5.3.2	Deficiencies	Based on summeries of data.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11/8/2010
Reference	The correct reference is Mayer, MD, Machinist JM. Abbott-85499 Drug Metabolism Report No. 26- Phase I study of the metabolism and excretion of [^{3}H]dexmedetomidine HCL (Abbott-85499.1) in normal male subjects (protocol Dex-96-018). Abbott Laboratories division 46, report No. R&D/97/457, September 1997 (241).
Materials and Methods	As stated by the Applicant.
Results and discussion	Minor typing error. It should read 95.17% and NOT 95,17%. The study report states that the H3 metabolite was NOT detected in urine. Missing information from study report: Comparison of the AUC 0-24 for dexmedetomidine (3.26 h x ng/mL) with that of the total plasma radioactivity over the same time course indicated that inchanged parent drug accounted for an average of 14.7% of the total plasma radioactivity.
Conclusion	As stated by the Applicant
Reliability	2 – A good-quality, robust study in human volunteers.
Acceptability	Acceptable
Remarks	None.
	COMMENTS FROM ...
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	

Doc III A Section 6.3.1 — Repeated dose toxicity - Oral

Annex Point IIA VI.6.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (Unpublished).	
1.2	**Data protection**	Yes, data protection is claimed.	
1.2.1	Data owner	I-Tech AB	
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**	OECD Guideline 407.	**X**
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine hydrochloride.	
3.1.1	Lot/Batch number	1186254	
3.1.2	Specification	Dormitor® for i.v, i.m and s.c administration	
3.1.2.1	**Description**	1 mg/mL prepared in 0.9% NaCl	
3.1.2.2	**Purity**	Not applicable.	
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	Sprague-Dawley	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	8-9 weeks, weight 249-330 g	
3.2.6	Number of animals per group	4 males/dose group.	
3.2.7	Control animals	Yes	

Doc III A Section 6.3.1 — Repeated dose toxicity - Oral

Annex Point IIA VI.6.3

3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	The animals were dosed 7 days/week for 28 days.
3.3.3	Postexposure period	None
3.3.4	**Oral**	
3.3.4.1	**Type**	Gavage
3.3.4.2	**Concentration**	0, 2.5, 3.6 and 4.9 mg/kg bodyweight.
3.3.4.3	**Vehicle**	0.9% NaCl solution.
3.3.4.4	**Concentration in vehicle**	0, 0.5, 0.7 and 1.0 mg/mL.
3.3.4.5	**Total volume applied**	0 for control animals and 4.9 mL/kg for the test animals.
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 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. Loss of rightning reflex and sleeping time were done on day 1 and day 8.
3.4.1.2	**Mortality**	Observations of mortality were made when clinical signs were recorded.
3.4.2	Body weight	The rats were weighed every week.
3.4.3	Food consumption	Food consumption was recorded on day 2 and 23 of the study.
3.4.4	Water consumption	Water consumption was recorded on day 2 and 23 of the study.
3.4.5	Ophthalmoscopic examination	No examination performed.
3.4.6	Haematology	Not performed.
3.4.7	Clinical Chemistry	Not performed.
3.4.8	Urinalysis	Not performed.
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, epididymides and heart

Doc III A Section 6.3.1 **Repeated dose toxicity - Oral**

Annex Point IIA VI.6.3

3.5.2	Gross and histopathology	Gross necropsy was performed and observations in organs and tissues were recorded.
3.5.3	Other examinations	None
3.5.4	Statistics	No statistical test were performed.
3.6	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Control group No clinical signs. 2.5 mg/kg Diarrhea and sedation observed in all animals, tonic spasm in one animal after last dose. 3.6 mg/kg Diarrhea and sedation observed in all animals, aggressive behavior in two animals. 4.9 mg/kg Diarrhea and sedation observed in all animals, weakness, cold and blueness observed in three animals which were euthanized. Aggressive and bizarre behavior in one animal.
4.1.2	Mortality	No deaths occurred during the study but three animals in the 4.9 mg/kg dose group were euthanized on day nine or ten.
4.2	**Body weight gain**	All animals had decreased body weight and weight gain compared to the control group.
4.3	**Food consumption and compound intake**	In the beginning of the study food consumption in all test substance treated groups were decreased compared to the control group. On day 23 there were no differences in food consumption compared to the control group. Water consumption was decreased in the highest dose group only on day 2. On day 23 water consumption was increased in the 2.5 and 3.6 mg/kg groups compared to the control group.
4.4	**Ophtalmoscopic examination**	No examination performed.
4.5	**Blood analysis**	
4.5.1	Haematology	Not performed.
4.5.2	Clinical chemistry	Not performed.
4.5.3	Urinalysis	Not performed.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	In all test substance treated groups the organ weights of heart, liver and kidney were reduced compared to the control group. However, when related to body weight no differences between the groups were noted.

Doc III A Section 6.3.1 **Repeated dose toxicity - Oral**

Annex Point IIA VI.6.3

4.6.2	Gross and histopathology	The three animals which were euthanized on day nine or ten were very dehydrated and had very dark content in the small intestine which indicates bleeding into the gut lumen. There were no macroscopic signs in the animals euthanized on day 28.
4.7	**Other**	None
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Subacute toxicity of medetomedine was studied by repeated oral administration to rats for 28 days according to OECD Guideline 407 as a pilot study for a subchronic oral study. The doses used were 0, 2.5, 3.6 and 4.9 mg/kg bodyweight. The number of animals used in the dose groups and the control groups was 4/group.
5.2	**Results and discussion**	In the performed study sedation was observed, which was pharmacologically expected. Three animals in the 4.9 mg/kg dose group were euthanized due to poor clinical condition. Animals in the 2.5 and 3.6 mg/kg dose groups were in good general clinical condition except for diarrhoea and decreased body weight compared to the control group. No macroscopic signs of toxicity were observed in the animals that were treated for the full 28 days and then necropsied. The animals treated with test substance had decreased heart, liver and kidney weight compared to the control group but this was due to decreased body weight.
5.3	**Conclusion**	4.9 mg/kg bodyweight is a toxic dose of medetomidine for rat and resulted in the need to euthanize 75% of that dose group after 9-10 days. 3.6 mg/kg is therefore suggested as the highest dose level for a subchronic oral study.
5.3.1	LO(A)EL	2.5 mg/kg
5.3.2	NO(A)EL	NOEL not determined.
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Doc III A Section 6.3.1 — Repeated dose toxicity - Oral

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*
Guidelines and Quality Assurance	*This is not a guideline study, it is a range finding study in order to determine the dose level for the 90-day subchronic study.*
Materials and Methods	*As stated by the Applicant*
Results and discussion	*As stated by the Applicant*
Conclusion	*As stated by the Applicant*
Reliability	*1*
Acceptability	acceptable
Remarks	*This study was a range finding study in order to determine the dose level for the 90-day subchronic oral study. As such and due to the low number of animals used and the limited analysis performed no reliable NOAEL or LOAEL can be derived.*
	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_1-1. Results of repeated dose toxicity study

Parameter	Control		low dose 2.5 mg/kg		medium dose 3.6 mg/kg		high dose 4.9 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	4	-	4	-	4	-	4	-		
Mortality	0	-	0	-	0	-	3	-	+	-
clinical signs*	No signs	-	Diarrhoea,	-	Diarrhoea, Aggressive	-	Diarrhoea, Aggressive, Weakness, Cold, Motionless	-	+	-
body weight gain (kg)	0.083	-	0.022	-	0.004	-	-0.033	-	+	-
food consumption / animal (day 23)	23.4	-	23.4	-	23.98	-	13.0	-		-
Heart										
organ weight*	1.372	-	1.248	-	1.275	-	1.133	-	+	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-
Liver										
organ weight*	14.29	-	12.09	-	10.08	-	8.63	-	+	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-
Kidney										
organ weight* L/R	1.199/1.179	-	1.077/1.016	-	1.011/1.001	-	0.986/0.970	-	+	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-
Adrenal gland										
organ weight* L/R	0.034/0.029	-	0.033/0.031	-	0.034/0.036	-	0.057/0.043	-	-	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-
Testicle										
organ weight* L/R	1.733/1.822	-	1.679/1.674	-	1.664/1.644	-	1.834/1.750	-	-	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-
Epididymis										
organ weight* L/R	0.234/0.292	-	0.201/0.204	-	0.199/0.195	-	0.200/0.166	-	-	-
gross pathology*	Not affected	-	Not affected	-	Not affected	-	Not affected	-	-	-

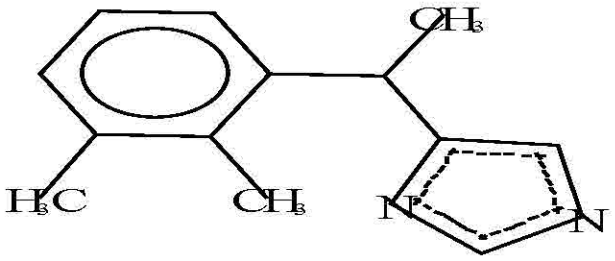
** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

Form for justification of the non-submission of data

III A Section 6_3_2 **Annex Point IIA VI 6.3**	**Repeated Dose Toxicity / Dermal**	
	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 dermal repeated dose toxicity is provided since route-to-route extrapoliations can be drawn from the sections 6.3.4 Study repeated subacute toxicity of medetomidine by subcutaneous administration to rats 28 days, and section 6.3.6 4 Study repeated subacute toxicity of levomedetomidine HCl subcutaneous administration to rats 28 days.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	7th September	
Evaluation of applicant's justification	RMS agrees with the Applicant – Route to route extrapolation from the oral studies or from data produced by parenteral routes of exposure to the dermal route is appropriate for medetomidine. The toxicity of medetomidine is driven by its systemic effects and there are no concerns for potential local dermal effects.	
Conclusion	Justification is acceptable.	
Remarks	None.	

III A Section 6_3_2 Annex Point IIA VI 6.3	Repeated Dose Toxicity / Dermal
	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	

III A Section 6.3.3 Annex Point VI.6.3	Repeated dose toxicity (inhalation)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	No data is provided on repeated dose toxicity (inhalation) as route-to-route extrapoliations can be drawn from section 6.3.7 Subacute toxicity on levomedetomidine by daily intravenous administration to dogs 28 days. This assumption is based on the fact that lungs are highly intervened by blood vessels which encapsels the active substance without passing the gut (the same is true for intravenous administration). A study of acute inhalation toxicicty is also being performed and will be submitted durint the second quarter of 2009. It can also be concluded that medetomidine can be considered as a non-volatile substance due to the following data: The following results are estimates of the vapour pressure of medetomidine (CAS 86347-14-0) from EPI-SUITE (module MPBPWIN version 1.42). There are 3 estimated vapour pressures: the highest is 5.92×10^{-7} mm Hg. This can be converted to Pa using a conversion factor of 133.3. The equivalent value in Pa is 7.89×10^{-5} Pa. The vapour pressure of the hydrochloride salt (CAS 86347-15-1) cannot be calculated with EPI-SUITE. However, the salt form is not expected to have a higher vapour pressure than the neutral form.  medetomidine Experimental Database Structure Match: no data SMILES : c2(c(c(ccc2)C)C)C(C)c1cncn1 CHEM : medetomidine	

III A Section 6.3.3
Annex Point VI.6.3

Repeated dose toxicity (inhalation)

MOL FOR: C13 H16 N2
MOL WT : 200.29
---------- SUMMARY MPBPWIN v1.42 ----------

Boiling Point: 386.32 deg C (Adapted Stein and Brown Method)

Melting Point: 172.21 deg C (Adapted Joback Method)
Melting Point: 111.91 deg C (Gold and Ogle Method)
Mean Melt Pt : 142.06 deg C (Joback; Gold,Ogle Methods)
Selected MP: 132.01 deg C (Weighted Value)

Vapor Pressure Estimations (20 deg C):
(Using BP: 386.32 deg C (estimated))
(Using MP: 172.00 deg C (user entered))
VP: 5.52E-008 mm Hg (Antoine Method)
VP: 2.43E-007 mm Hg (Modified Grain Method)
VP: 5.92E-007 mm Hg (Mackay Method)
Selected VP: 2.43E-007 mm Hg (Modified Grain Method)
Subcooled liquid VP: 9.8E-006 mm Hg (20 deg C, Mod-Grain method)

TYPE	NUM	BOIL DESCRIPTION	COEFF	VALUE
Group	3	-CH3	21.98	65.94
Group	1	>CH-	11.86	11.86
Group	5	CH (aromatic)	28.53	142.65
Group	4	-C (aromatic)	30.76	123.04
Group	2	N (aromatic)	39.88	79.76
Corr	1	Imidazole [NH]	165.00	165.00
*		Equation Constant		198.18

RESULT-uncorr| BOILING POINT in deg Kelvin | 786.43
RESULT- corr | BOILING POINT in deg Kelvin | 659.48
| BOILING POINT in deg C | 386.32

TYPE	NUM	MELT DESCRIPTION	COEFF	VALUE
Group	3	-CH3	-5.10	-15.30
Group	1	>CH-	12.64	12.64
Group	5	CH (aromatic)	8.13	40.65

III A Section 6.3.3 Annex Point VI.6.3	**Repeated dose toxicity (inhalation)**
	Group | 4 | -C (aromatic) | 37.02 | 148.08 Group | 2 | N (aromatic) | 68.40 | 136.80 * | | Equation Constant | | 122.50 ==========+==========+==========+====== === RESULT | MELTING POINT in deg Kelvin | 445.37 | MELTING POINT in deg C | 172.21 ----------
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	7th September 2010
Evaluation of applicant's justification	OK
Conclusion	It is the opinion of the RMS that the justification for the non-submission of a repeated dose inhalation study is acceptable. A repeated dose inhalation study is not justified because medetomidine is not volatile. In addition, from a number of studies investigating the repeated toxicity of medetomidine, there is no evidence to suggest that the lung is a target organ of toxicity. In conclusion, the RMS is of the opinion that route-to-route extrapolation from the oral studies or from data produced by parenteral routes of exposure to the inhalation route is appropriate for medetomidine. The toxicity of medetomidine is driven by its systemic effects and there are no concerns for potential local effects in the respiratory tract.
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*

III A Section 6.3.3 Annex Point VI.6.3	Repeated dose toxicity (inhalation)
Remarks	

Doc III A Section 6.3.4 (01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Subacute toxicity study of medetomidine by repeated subcutaneous administration to rats for 28 days, [redacted] (Unpublished)	X
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 and OECD.	X
2.2	**GLP**	Yes, fulfils known requirements of the US Food and Drug Administration and OECD.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine, also known as FB-785, MPV-785.	
3.1.1	Lot/Batch number	79302	
3.1.2	Specification	As given in section 2	X
3.1.2.1	**Description**	Crystalline powder white to almost white	
3.1.2.2	**Purity**	[redacted]	
3.1.2.3	**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	45 days old, about weight 150 g	
3.2.6	Number of animals per group	10 males and females/dose group, altogether 80 animals were required. 10 additional animals were subjected to the pre-experimental acceptance test (SOP TOX 413) which included external examination and health check.	

Doc III A Section 6.3.4 (01) **Annex Point IIA VI.6.3**	**Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats**	
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Subcutaneous route
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)**
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 IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by 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 injection**	
3.3.7.1	**Vehicle**	Medetomedine was dissolved in Natrosteril, Medipolar .
3.3.7.2	**Concentration in vehicle**	Dose groups:

DOSE GROUP	DOSE LEVEL
1	0 µg/kg
2	100 µg/kg
3	400 µg/kg
4	1600 µg/kg

3.3.7.3	**Total volume applied**	Dosing volume: 1ml/kg
3.3.7.4	**Controls**	Dose group 1. The vehicle physiological saline, natrosteril, medipolar, has been used as control article. Batch 309.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	**Clinical signs**	Clinical signs were recorded 0.5-1 hours and 3-4 hours after dosing.
3.4.1.2	**Mortality**	Once in the morning and once in the after noon 7 days per week. Maximum time between the observations was 18 hours.
3.4.2	Body weight	The rats were weighed every week.
3.4.3	Food consumption	The food consumption was weighed every week.
3.4.4	Water consumption	Monitored by visual inspection on a weekly basis.
3.4.5	Ophthalmoscopic examination	Yes.
3.4.6	Haematology	Blood samples were taken from all animals at the end of the study period by heart puncture during autopsy. The animals were fasted for 18 hours before blood sampling. Water was available to the animals.

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Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

		The following parameters are determined: hematocrit, haemoglobin, red blood cell count, white blood cell count (total and differential), platelet count, reticulocyte count, quick-test, PTT
3.4.7	Clinical Chemistry	Blood chemistry measurements were done to all animals at the end of the study. The animals were fasted for over night before blood sampling. Water was available to the animals. The following parameters are determined: S-Na, S-K, S-Pi, S-Cl, S-Ca, S-prot S-alb, S-krea, S-Uraat, S-Fe, S-trigly, S-Bil, S-Bil-kj, S-glucose, S-kol, S-afos, S-asat, S-alat, S-ld, S-GGT.
3.4.8	Urinalysis	Urine was collected from the animals of the control group and the highest group at the end of the study period. The samples were collected 18 hours into specimen vials using metabolism cages. The following parameters are determined: volume, pH, osmolality, ketones, haemoglobin pigments, proteins, glucose, sediment, erythrocytes, leucocytes, epithelial cells.
3.5	**Sacrifice and pathology**	Autopsies. All animals found dead were put in to the refrigerator to +4 degrees and necropsied. At the end of the study all animals were killed and necropsied in random order. Dosing was continued to the surviving animals until their autopsy. The duration of dosing was, however, reported as 28 days.
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart or other
3.5.2	Gross and histopathology	Yes organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes, peripheral nerve, bone marrow, skin, eyes or other
3.5.3	Other examinations	None
3.5.4	Statistics	DMPI-81 program. Equality if variance was tested by Levene`s test. If analysis of variance were not equal modification of Forsythe was used.
3.6	**Further remarks**	None

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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 (phys. NaCl 1 ml/kg)
All controls behaved normally after dosing and were normal during later observations.
Low dose group (MPV-785 100 µg/kg)
After dosing all animals were sedated. At the last observation about five hours after dosing the animals were normal.
Medium dose group ((MPV-785 400 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 ((MPV-785 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 (phys. NaCl 1 ml/kg)
No clinical signs.
Low dose group (MPV-785 100 µg/kg)
One male had reddish fur posteriously on back.
Medium dose group ((MPV-785 400 µg/kg)
7 males and 6 females had opacity in eye/eyes.
High dose group ((MPV-785 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 (high significant in the low and high dose groups and significant in the medium dose group). Packed cell volume was decreased significantly in the medium dose group and highly significantly in 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. There was a significant decrease in the males and highly significant in the 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**	In the performed study increased sadation as a function of dose was

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Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

	discussion	observed which was farmacologicaly expected. The animals tolerated 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	

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Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by 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	25th August 2010
Reference	The study author is incorrect. It should be [redacted]
Guidelines and Quality Assurance	The study does not conform to a specific OECD guideline, however, it very closely follows the guidelines outlined in OECD 407: Repeated dose 28-day oral toxicity study in rodents. In addition, the study was performed before GLP and therefore in not GLP compliant.
Materials and Methods	The specification is not given in section 2. This section does not exist.
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	None
	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. Results of clinical chemistry haematology and urinalysis

(Use this or similar table, if relevant effects occur and if time sequence is important.Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

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
Not applicable

Parameter	Control		low dose		medium dose		high dose		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
number of animals examined										
Mortality										
clinical signs*										
body weight										
food consumption										
clinical chemistry*										
haematology*										
urinalysis*										
Organ x organ weight*										
gross pathology*										
microscopic pathology*										
Organ y										

* *specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

Doc III A Section 6.3.4 (01)

Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Subacute toxicity study of medetomidine by repeated subcutaneous administration to rats 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 and OECD.	X
2.2	**GLP**	Yes, fulfils known requirements of the US Food and Drug Administration and OECD.	X
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Medetomidine, also known as FB-785, MPV-785.	
3.1.1	Lot/Batch number	79302	
3.1.2	Specification	Medetomdine hydrochloride	
3.1.2.1	**Description**	Crystalline powder white to almost white	
3.1.2.2	**Purity**	[redacted]	
3.1.2.3	**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	45 days old, about weight 150 g	
3.2.6	Number of animals per group	10 males and females/dose group, altogether 80 animals were required. 10 additional animals were subjected to the pre-experimental acceptance test (SOP TOX 413) which included external examination and health check.	

Doc III A Section 6.3.4 (01) **Annex Point IIA VI.6.3**	**Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats**	
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Subcutaneous route
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)**
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 IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by 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 injection**	
3.3.7.1	**Vehicle**	Medetomedine was dissolved in Natrosteril, Medipolar .
3.3.7.2	**Concentration in vehicle**	Dose groups:

DOSE GROUP	DOSE LEVEL
1	0 µg/kg
2	100 µg/kg
3	400 µg/kg
4	1600 µg/kg

3.3.7.3	**Total volume applied**	Dosing volume: 1ml/kg
3.3.7.4	**Controls**	Dose group 1. The vehicle physiological saline, natrosteril, medipolar, has been used as control article.
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	**Clinical signs**	Clinical signs were recorded 0.5-1 hours and 3-4 hours after dosing.
3.4.1.2	**Mortality**	Once in the morning and once in the after noon 7 days per week. Maximum time between the observations was 18 hours.
3.4.2	Body weight	The rats were weighed every week.
3.4.3	Food consumption	The food consumption was weighed every week.
3.4.4	Water consumption	Monitored by visual inspection on a weekly basis.
3.4.5	Ophthalmoscopic examination	Yes.
3.4.6	Haematology	Blood samples were taken from all animals at the end of the study period by heart puncture during autopsy. The animals were fasted for 18 hours before blood sampling. Water was available to the animals.

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Annex Point IIA VI.6.3

Repeated dose toxicity - Subacute toxicity by subcutaneous administration to rats

		The following parameters are determined: hematocrit, haemoglobin, red blood cell count, white blood cell count (total and differential), platelet count, reticulocyte count, quick-test, PTT
3.4.7	Clinical Chemistry	Blood chemistry measurements were done to all animals at the end of the study. The animals were fasted for over night before blood sampling. Water was available to the animals. The following parameters are determined: S-Na, S-K, S-Pi, S-Cl, S-Ca, S-prot S-alb, S-krea, S-Uraat, S-Fe, S-trigly, S-Bil, S-Bil-kj, S-glucose, S-kol, S-afos, S-asat, S-alat, S-ld, S-GGT.
3.4.8	Urinalysis	Urine was collected from the animals of the control group and the highest group at the end of the study period. The samples were collected 18 hours into specimen vials using metabolism cages. The following parameters are determined: volume, pH, osmolality, ketones, haemoglobin pigments, proteins, glucose, sediment, erythrocytes, leucocytes, epithelial cells.
3.5	**Sacrifice and pathology**	Autopsies. All animals found dead were put in to the refrigerator to +4 degrees and necropsied. At the end of the study all animals were killed and necropsied in random order. Dosing was continued to the surviving animals until their autopsy. The duration of dosing was, however, reported as 28 days.
3.5.1	Organ Weights	Yes organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart or other
3.5.2	Gross and histopathology	Yes organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, gall bladder (mouse), lymph nodes, peripheral nerve, bone marrow, skin, eyes or other
3.5.3	Other examinations	None
3.5.4	Statistics	DMPI-81 program. Equality if variance was tested by Levene`s test. If analysis of variance were not equal modification of Forsythe was used.
3.6	**Further remarks**	None