

Doc III A

Aerobic transformation in water

Section A7.1.2.2.2

Annex Point IIA7.2.1

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	23/04/2013
Materials and Methods	<p>Section 2.2 and 2.3 (and 5.3.2): The Applicant summary states that '<i>minor deviations in GLP compliance regarding soil composition, counting of radiolabeled ¹⁴C test material at DHI and grain size analyses</i>' for the original study of Jørgensen C. 2010 (ref 1.1). However the GLP compliance statement is clear that the LSC work and the analysis of sediments (composition analysis and grain size determination) was not performed in accordance with GLP. The Quality Assurance statement should highlight the inspection of GLP studies at critical phases of the study. However the Quality Assurance statement provided for Jørgensen C. 2010 (ref 1.1) indicated that the only in-life inspection was at test termination. This is considered inadequate in the opinion of the UK CA. Whilst the analysis of sediments to non-GLP may be accepted as having a relatively minor impact on overall study validation, the analytical LSC work is critical to the validity of the study (since it helps determine mass balance) and should have been performed to GLP in the opinion of the UK CA. Chemical analysis of medetomidine at all time points (and metabolites at a single time point) was performed by a separate lab in accordance with GLP. The supplemental study of Jørgensen C. (2012, ref 1.2) did not include any GLP or Quality Assurance statement. In addition the chemical re-analysis work performed in 2012 and reported in Rinne, V. (2012, ref 1.3) was not considered to have been conducted in accordance with GLP due to the absence of storage stability data for samples stored since the original 2010 study. The absence of GLP and QA for these two references is also considered unacceptable.</p> <p>Section 3.1.4: The quoted specific activity of 889 MBq/mmol is based on the analysis of day 0 water sample concentrations and total ¹⁴C activity performed within the supplemental study of Jørgensen C. 2012 (ref 1.2) (note that this study was not performed to GLP). The quoted specific activity deviates from the value provided by the Applicant (665 MBq/mmol) as confirmed by the certificate of analysis of the test substance. The quoted value also deviates from the value measured in the original study of Jørgensen C. 2011 (ref 1.1) where a value of 998 MBq/mmol were stated (where LSC analysis was non-GLP). No analysis of the test substance stock solution was ever performed. No explanation was given for the discrepancy between the different specific activities and the original certificate of analysis. The use of different values has an influence on the calculation of overall mass balance and calculation of initial dosing levels and the uncertainty around this critical value is considered unacceptable.</p> <p>Section 3.1.5: The molecular weight of medetomidine hydrochloride should be reported as 236.7 g/mol.</p> <p>Section 3.4.7: For the original chemical analysis of sediment samples in Jørgensen C. 2010, extraction was performed using 0.1% HCOOH solutions with tubes shaken for 2 x 10 s followed by centrifugation and solid phase extraction of liquid extracts. Acceptability of the method was confirmed via appropriate quality control samples at between 0.1 ng/ml (the proposed LOQ) up to 8.0 ng/ml. However quality control results were only presented for the 0.9, 3.0 and 8.0ng/ml</p>

Doc III A**Aerobic transformation in water****Section A7.1.2.2.2****Annex Point IIA7.2.1**

spiked samples (above the day 98 sediment concentrations reported to be between 0.271 and 0.294 ng/ml in the original study). The validity of the original extraction procedure for medetomidine cannot be confirmed. The original extraction procedure was therefore investigated in both Jørgensen C. 2012 and Rinne, V. (2012) and subsequently shown to be inadequate. Repeat analysis of day 98 samples was performed using an improved four step extraction procedure of 1 x 1ml of 0.1% HCOOH in water and 3 x 1ml methanol in Rinne (2012). With the revised extraction procedure, medetomidine concentrations in extracts increased to between 0.405 and 0.514ng/ml. However this improved extraction procedure was only repeated for the day 98 samples and therefore the results presented for earlier time point rely on an unacceptable extraction procedure. In the supplemental study of Jørgensen C. 2012 sediment extraction was performed using 0.1% HCOOH with overnight extraction on a rotary shaker with the procedure repeated three times. The residual sediment was further extracted for a total of 21 d with scintillation liquid. Extraction of replicate frozen samples of both test systems for days 42, 56 and 98 was performed. The use of different extraction procedures for different time points and different analyses (i.e. total ¹⁴C and chemical analysis of medetomidine) make comparison of results difficult. The relevance of these extraction procedures in determining the likely biologically available fraction of test material was unclear. No validation of the extraction procedures for the potential metabolites of medetomidine was performed. These aspects are considered unacceptable.

Section 3.4.7: The determination of total ¹⁴C in water and sediment extracts at day 0 and day 98 (study termination) only in the original study is considered unacceptable. Additional analyses of the day 42, 56 and 98 d samples was performed in the supplementary study of Jørgensen C. 2012 based on an improved extraction method. However no determination of sediment unextracted (i.e. bound) residues was performed at any time point. This information is important to ensure that mass balances were acceptable throughout the study and the absence of this information is considered a significant deviation from OECD guidelines. For the determination of total radioactivity in sediment extracts, the extraction procedure involved shaking 2.0 to 2.9 g of sediment with 20ml of scintillation cocktail and then leaving for 14 d. No information to support the validity of this extraction procedure was provided. This procedure also deviated from that used in the chemical analysis of medetomidine used in either the original study or re-analysis work of Rinne, V., (2012) and Jørgensen C. (2012).

Section 3.4.7: The determination of possible transformation products was conducted for the water phase samples from a single time point at day 98 (study termination) in Jørgensen C. 2010. Such limited analysis of metabolites is considered unacceptable and a significant deviation from OECD guidelines.

Results and discussion

Section 4.1: The 'improved' ¹⁴C recovery in the supplemental study was still below the acceptability criteria of 90% recovery in both systems at multiple time points (e.g. down to 83% after 56 d in the coarse sediment). This is unacceptable. Total mass balances may have been improved if sediment bound residues had been analytically determined. However this was never performed.

Section 4.2.4: None of the expected transformation products were detected in the original study (ref 1.1). The Applicant stated this may be due to the formation of metabolites different from the anticipated metabolites. Whilst this may be true the UK CA considers that in such situations the Applicant must take all reasonable steps to quantify and identify the individual metabolites formed even when they do not correspond to the expected metabolites. In addition analysis

Doc III A

Aerobic transformation in water

Section A7.1.2.2.2

Annex Point IIA7.2.1

	<p>was only performed on a single water sample from the Niva Bay system at day 98 in the original study. No analysis of earlier time points was performed, and no analysis of metabolites in sediment extracts was performed. Therefore the formation of metabolites in significant amounts at earlier time points or in the sediment cannot be excluded. The absence of analysis of metabolites across all time points metabolites is considered unacceptable and a significant deviation from OECD guidelines.</p> <p>Section 5.1: The QSAR evaluation of Nfon, E. (2012) is evaluated by the UK CA at AIII 7.1.2.2.3.</p> <p>Section 5.2: Given the uncertainties in the underlying methodology the derivation of K_d and K_{oc} values from this study is highly uncertain. However simply looking at levels of medetomidine in water and sediment would suggest that sorption was actually higher in the fine textured high organic carbon sediment system. This contradicts the Applicants statement.</p> <p>Section 5.2: No specific information was included in the original study report to support the conclusion that medetomidine has a strong affinity to form complexes with metal oxides.</p> <p>Section 5.2 and 5.3: Based on the limited number of data points and the uncertainty over acceptability of mass balance the UK CA did not consider the DT_{50} value proposed to be reliable. However it is clear that medetomidine is relatively persistent in this study, with whole system DT_{50} and DT_{90} values extrapolated beyond the study duration of 98 d.</p> <p>Section 5.2: The UK CA does not consider the statement that '<i>lack of mineralization suggests a degradation route based on biodegradation</i>' to necessarily be a logical conclusion.</p> <p>Table A7_1_2_2_2-2 and -3: The data presented as 'unidentified radioactivity' is based on a calculation method using the parent compound analysis results and an assumption of 100% recovery. This approach is not considered acceptable and any unidentified radioactivity should be analytically determined.</p> <p>Table A7_1_2_2_2-2 and -3: The data presented a 'non-extractable residues' is based on a calculation method using the LSC analysis of water and sediment extracts and assuming 100% recovery. This approach is not considered acceptable and any unextracted sediment radioactivity should be analytically determined.</p>
Conclusion	<p>Section 5.3: Extraction with a more appropriate solvent extraction system was only performed on day 42, 56 and 98 samples (Jørgensen C. 2012). Therefore it is not possible to conclude on the acceptability of mass balance at other, intermediate time points. Acceptability against the guideline mass balance criteria is also influenced by the choice of specific activity, which is currently considered uncertain in the opinion of the UK CA. No analysis to determine the extent of sediment unextracted radioactivity was performed at any time point.</p>
Reliability	<p>Section 5.3.1: Based on the major methodological and reporting deficiencies the UK CA considers the study to be completely unreliable, with a reliability rating of 3.</p>
Acceptability	Not acceptable
Remarks	<p>Based on the major methodological and reporting deficiencies the UK CA considers the study to be completely unreliable, with a reliability rating of 3. Due to the deficiencies the UK CA did not consider that reliable DT_{50} or DT_{90} values could be proposed. However it is clear that medetomidine is relatively persistent</p>

Doc III A**Aerobic transformation in water****Section A7.1.2.2.2****Annex Point IIA7.2.1**

in this study, with whole system DT_{50} and DT_{90} values extrapolated beyond the study duration of 98 d in both test systems. Since this study represents the key study for assessing likely fate and behaviour in aerobic aquatic marine systems, which is the major environmental compartment of concern, a new study will be required before the assessment of this substance can be completed.

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 A7_1_2_2_2-1: Test conditions

Criteria	Details
Composition of medium	According to the OECD guideline, No. 308
Additional substrate	No
Test temperature	15°C ± 2°C.
pH	7.2-7.4 (active) and 7.7-8.8 (inactive)
TOC in sediment	4200-4500 mg/kg d.w (fine sediment) 500 mg/kg d.w (coarse sediment)
TOC water (start value)	8.3 mg/L (fine water-sediment system) 4.5 mg/L (coarse water-sediment system)
Biomass in sediment (HPC)	2.3 x 10 ⁶ (fine active sediment) 6.5 x 10 ⁶ (coarse active sediment)

Table A7_1_2_2_2-2: Biotransformation of medetomidine, expressed as percentage of applied radioactivity (mean ± s.d) in water –sediment system under aerobic conditions. Course sediment (Niva Bay)

Medetomidine		Sampling times (days, hours, or other time period)								
		0	14	28	42	56	98			
Parent compound (based on chemical analysis)	Water	96	61	50	46	38	33.2**			
	Sediment	LLOQ	26	28	28	23	33.5**			
Transformation product 1	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
Transformation product 2	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
Transformation product n	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ			
Unidentified radioactivity, if any (based on parent compound analyses)	Water	4	13	22	26	39	33.3			X
	Sediment	0								
Total CO ₂ (t=transformation study, m= mineralisation study)	Entire system		0.1 (t)	0.2 (t)	0.4 (t)	0.5 (t)	2.9 ± 0.3 (m)			
Volatile organic 1	Entire system									
Total volatile organics										
Non-extractable residues	Sediment				13.6	17	10.6			X
Total % recovery ¹⁴ C (water, sediment and CO ₂)	Water				38*	33.5*	40*			
	Sediment				47.5*	48*	46.5*			
	Entire system				86.4	83	89.4			

LLOQ = Lower limits of quantification

* Data from supplementary DHI study

*** Data from supplementary CRST study

Table A7_1_2_2_2-3: Biotransformation of medetomidine, expressed as percentage of applied radioactivity (mean \pm s.d) in water –sediment system under aerobic conditions. Fine sediment (Niva Harbour)

Medetomidine		Sampling times (days, hours, or other time period)							
		0	14	28	42	56	98		
Parent compound (based on chemical analysis)	Water	79	37	25	20	18	10		
	Sediment	6	62	57	58	84	47		
Transformation product 1	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
Transformation product 2	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
Transformation product n	Water	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
	Sediment	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ		
Unidentified radioactivity, if any (based on parent compound analyses)	Water	15	1	18	22	0	23		X
	Sediment								
Total CO ₂ (t=transformation study, m= mineralisation study)	Entire system		0.1 (t)	0.1 (t)	0.2 (t)	0.2 (t)	1.6 \pm 0.2 (m)		
Volatile organic 1	Entire system								
Total volatile organics									
Non-extractable residues	Sediment				16.3	13.3	18.4		X
Total % recovery ¹⁴ C (water, sediment and CO ₂)	Water				25.5*	20*	16.5*		
	Sediment				58*	66.5*	63.5*		
	Entire system				83.7	86.7	81.6		

LLOQ = Lower limits of quantification

* Data from supplementary DHI study

** Data from supplementary CRST study

Doc III A**Environmental fate – QSAR evaluation****Section A7.1.2.2.3****Annex Point IIA7.2.1**

		1 REFERENCE	Official use only
1.1	Reference	Nfon E. (2012) Environmental fate of the active substance medetomidine and its metabolites in the aquatic environment. Enviresearch Limited, UK. Study number E2012-07 (Unpublished)	
1.2	Data protection	Yes	
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	Not applicable	
2.2	GLP	Not applicable	
2.3	Deviations	Not applicable	
		3 MATERIALS AND METHODS	
3.1	Test material	Medetomidine	
3.1.1	Lot/Batch number	Not applicable	
3.1.2	Specification	Not applicable	
3.1.3	Purity	Not applicable	
3.1.4	Radiolabelling	Not applicable	
3.1.5	Further relevant properties	Molecular weight Saturated vapour pressure Solubility in water Henry's law constant Log Kow Log Koa BAF BCF Koc Biodegradation (BIOWIN3) Data is summarised in Table 1.	
3.2	Reference substance	No	
3.3	Test solution	Not applicable	
3.4	Testing procedure		
3.4.1	Inoculum / test species	Not applicable.	
3.4.2	Test system	The following databases were used:	

Doc III A**Environmental fate – QSAR evaluation****Section A7.1.2.2.3****Annex Point IIA7.2.1**

- KOWWIN: Estimates the log octanol-water partition coefficient ($\log K_{OW}$) of chemicals.
- AOPWIN: Estimates the gas-phase reaction rate for the reaction between the most prevalent atmospheric oxidant, hydroxyl radicals, and a chemical.
- HENRYWIN: Calculates the Henry's Law constant (H) (air/water partition coefficient).
- MPBPWIN: Estimates the melting point, boiling point, and vapor pressure of organic chemicals are estimated using a combination of techniques.
- BIOWIN: Estimates aerobic and anaerobic biodegradability of organic chemicals
- BioHCwin: Estimates biodegradation half-life for compounds containing only carbon and hydrogen (hydrocarbons).
- KOCWIN: This program estimates the organic carbon-normalized sorption coefficient for soil and sediment (K_{OC}).
- WSKOWWIN: Estimates an octanol-water partition coefficient using the KOWWIN program, then estimates a chemical's water solubility from this value.
- WATERNT: Estimates water solubility.
- BCFBAF: This program estimates fish bioconcentration factor and its logarithm using two different methods. The first is the traditional regression based on $\log K_{OW}$ and the second is the Arnot-Gobas method, which calculates BCF from mechanistic first principles.
- HYDROWIN: Estimates aqueous hydrolysis rate constants and half-lives of a variety of chemical structure classes for which hydrolysis may be significant.
- KOAWIN: Estimates the octanol/air partition coefficient (K_{OA}) using the ratio of the octanol/water partition coefficient (K_{OW}) from KOWWIN and the dimensionless Henry's Law constant (K_{AW}) from HENRYWIN.
- AEROWIN Estimates the fraction of airborne substance sorbed

Doc III A**Environmental fate – QSAR evaluation****Section A7.1.2.2.3****Annex Point IIA7.2.1**

to airborne particulates.

- WVOLWIN: Estimates the rate of volatilization of a chemical from rivers and lakes; and calculates the half-life for these two processes from their rates.
- STPWIN: This program predicts the removal of a chemical in a typical activated sludge-based sewage treatment plant. Values are given for total removal and three processes: biodegradation, sorption to sludge, and air stripping that may contribute to removal.
- LEV3EPI: This program contains a level III multimedia fugacity model and predicts partitioning of chemicals among air, soil, sediment, and water under steady state conditions for a default model "environment".
- ECOSAR: The Ecological Structure Activity Relationships (ECOSAR) Class Program estimates the aquatic toxicity of industrial chemicals. The program estimates acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms to fish, aquatic invertebrates, and green algae, and has limited SARs for other salt water and terrestrial species, where data were available.

3.4.3 Method of analysis for transformation /degradation products

The predicted properties are automatically used in the program to model the volatilisation from water, treatability in sewage plants and environmental distribution using a Mackay type level III fugacity model.

The main predictor of aquatic toxicity in the EPI suite is the Ecological Structure Activity Relationships (ECOSAR) program. ECOSAR uses structure activity relationships to predict the aquatic toxicity of untested chemicals based on their structure similarity to chemicals for which aquatic studies are available (USEPA).

4 RESULTS

4.1 Degradation of test substance

Doc III A**Environmental fate – QSAR evaluation****Section A7.1.2.2.3****Annex Point IIA7.2.1**

- | | | |
|-------|-------------------------|---|
| 4.1.1 | Transformation | Abiotic processes such as hydroxylation, N-methylation, dehydrogenation by microorganisms and by conjugation processes by higher level species. |
| 4.1.2 | Transformation products | <p>The reaction products are:</p> <ul style="list-style-type: none">• 3-Hydroxy Dex generated by hydroxylation of the methyl group in the meta position of the benzene ring. This compound further reacts by dehydrogenation to form the carboxy dex (a benzoic acid) or conjugated with glucoronide to form O-Glucoronide.• G-Dex-1 and G-Dex-2 formed by the conjugation of the N-group in the pyridine ring with Glucoronide.• N-Methyl formed by the methylation of the amine group in the pyridine ring. If methylation is followed by hydroxylation, then the 3-Hydroxy N-methyl metabolite is formed.• Carboxy N-methyl formed by dehydrogenation of the 3-Hydroxy N-methyl metabolite and N-Methyl O-Glucoronide formed by conjugation of 3-Hydroxy N-methyl with Glucoronide. |

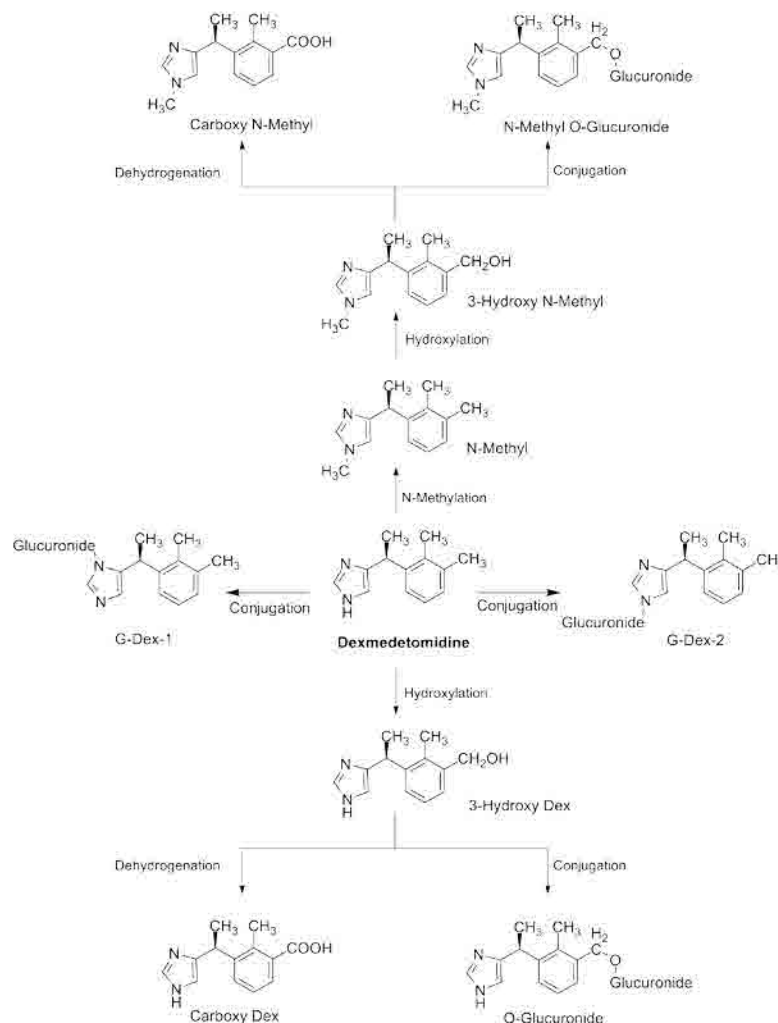
Doc III A

Environmental fate – QSAR evaluation

Section A7.1.2.2.3

Annex Point IIA7.2.1

4.1.3 Graph



4.1.4 Other observations

The physical chemical properties regarding medetomidine were within the same range as known experimental values.

The physical chemical properties of the proposed metabolites are summarised in table 2.

The predicted toxicity for medetomidine was generally higher than experimental values. For example, LC_{50} for fish was predicted to be between 1.55 and 4.40 mg/L. The experimental value is 30 mg/L.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

There is a paucity of physical/chemical property data on medetomidine, therefore the relevant data for this assessment was estimated using the Estimation Program Interface (EPI ver. 4.1) developed by the United States Environmental Protection Agency. The EPI suite is used in the USEPA Pollution Prevention framework for the review of premanufacturing notice chemicals under the Toxic Substance Control Act (TSCA) and for the evaluation of the potential for chemicals to have persistent, bioaccumulative and toxic properties. The programs within the suite are able to predict physical chemical properties from chemical structure, chemical name or CAS number entries. The predicted properties were automatically used in the program to model the volatilisation from water, treatability in sewage plants and environmental distribution using a Mackay type level III fugacity model.

X

5.2 Results and discussion

The main predictor of aquatic toxicity in the EPI suite is the Ecological Structure Activity Relationships (ECOSAR) program. ECOSAR uses structure activity relationships to predict the aquatic toxicity of untested chemicals based on their structure similarity to chemicals for which aquatic studies are available (USEPA). The input required by EPI suite for the prediction of the properties of medetomidine was Simplified Molecular Input Line Entry Specification (SMILES). The SMILES notation CC(c2cncn2)c1c(C)c(C)ccc1 was used for medetomidine and the predicted metabolites were annotated according to the same procedure and evaluated in parallel with medetomidine.

N-methylation of the imidazole ring of medetomidine produce a molecule that is slightly more toxic by a factor of two to aquatic species than the parent compound while oxidation of the benzene ring leads to a dramatic reduction in toxicity and this will quickly detoxify any of the methylated metabolites that may be formed.

It is assumed that medetomidine in the environment will be degraded by a combination of abiotic processes (hydroxylation, N-methylation, dehydrogenation) by microorganisms and conjugation processes by higher trophic level species, generating similar metabolites. While it is unclear which of these reactions will dominate the fate of medetomidine in the environment, it is beyond doubt that the molecule will be degraded in the environment since the medetomidine molecule is composed of a ring structure substituted with hydrocarbon side chains that are generally susceptible to common degradative reactions.

X

The result of the predicted toxicity of medetomidine and its metabolites is presented in Table 3.

5.3 Conclusion

The intrinsic toxicity of the metabolites of medetomidine as predicted in EPIWIN does not in itself indicate a risk to organisms. A key factor determining the risk will be the level in the environmental compartment following normal use of formulations containing medetomidine. Also intrinsic properties such as the rapid biodegradation of these compounds will also decrease the amount available for uptake. The results of level III evaluative assessments presented in the EPI output files indicate the amount of each of the metabolites in the water column after the emission of 1000 kg into an evaluative level III environment ranged from 12 – 15%, and furthermore the bulk of the chemical in water

X

column (> 70%) was removed by reaction. Therefore the weight of evidence seems to indicate the parent compound and its metabolites will not be present in sufficient amounts and for sufficiently long periods following normal use to present a risk to aquatic species.

The predicted properties of the metabolites indicate similar fate properties to the parent compound. The toxicity of the metabolites as predicted in ECOSAR suggests oxidation (hydroxylation and dehydrogenation) of medetomidine generally leads to metabolites with reduced toxicity while metabolites formed by N-methylation are more toxic than medetomidine. These metabolites are quickly detoxified by oxidation of the benzene ring. Since only small amounts of medetomidine is released into the environment during normal use ((the concentrations in the formulated product (0.1% and 1.0% a.s in wet paint)), it is concluded that the risk to aquatic organisms due to environmental metabolism of medetomidine is probably low.

5.3.1 Reliability

The reliability factor is 2. The study is performed according to generally accepted scientific principles.

5.3.2 Deficiencies

None.

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30/04/2013
Materials and Methods	Applicant's version is acceptable, noting the following Section 5.1: The use of a QSAR tool to estimate basic physicochemical properties for use in further QSAR analysis is considered to add a further degree of uncertainty to the endpoints predicted. More reliable estimates may have been possible using measured data for medetomidine. No assessment of the inherent uncertainty in these kinds of QSAR assessments was included and this would have been useful.
Results and discussion	Applicant's version is acceptable, noting the following: Section 5.2: The Applicant assumes that medetomidine will degrade in the environment on the basis of QSAR analysis. However in the opinion of the UK CA such QSAR analysis cannot be used as a substitute for core data on key aspects of the fate and behaviour of a substance in the environment. This QSAR analysis is not considered sufficient to meet the data requirement for information on the aerobic aquatic degradation of medetomidine.
Conclusion	Applicant's version is acceptable, noting the following: Section 5.3: The Applicant draws conclusions on the likely environmental risks posed by the substance utilising a Level III fugacity model. The risks posed by PT21 substances should be assessed in accordance with the OECD ESD and TGD.
Reliability	2
Acceptability	Acceptable (but only as supporting information pending submission of acceptable data on the aerobic aquatic degradation of medetomidine).
Remarks	The UK CA considered the study to be acceptable. However in the opinion of the UK CA such QSAR analysis cannot be used as a substitute for core data on key aspects of the fate and behaviour of a substance in the environment. This QSAR analysis is not considered sufficient to meet the data requirement for information on the aerobic aquatic degradation of medetomidine. Therefore further data will be required on the aerobic aquatic degradation of medetomidine in the marine environment.
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A7_1_2_2_3-1

Physical chemical properties (25°C) of medetomidine estimated in EPIWIN and some known experimental values.

Parameter	Unit	Values predicted by EPI	Known experimental values
Molar mass	g/mol	200.29	
Saturated vapour pressure	Pa	2.01×10^{-04}	
Solubility in water	mg/L	23.56	186 ¹
Henry's law constant	$\text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$	5.40×10^{-02}	
Octanol-water partition coefficient (log K_{ow})	--	3.83	3.1 ²
Octanol-air partition coefficient (log K_{oa})	--	8.49	
Octanol-air partition coefficient (log K_{oa})	--	-4.66	
Bioaccumulation factor (BAF)	L kg^{-1} wet weight	331	
Bioconcentration factor (BCF)	L kg^{-1} wet weight	155.4	
Organic carbon-water partition coefficient (K_{oc})	L kg^{-1}	2767	Mean: 2157 ³ range: (1215 – 4114)
Hydroxyl radical reaction rate constant	$\text{cm}^3 \text{molecule}^{-1} \text{sec}^{-1}$	1.03×10^{-10}	
Ozone reaction	--	No ozone reaction estimation	
Half life in air (12 hr day)	days	0.11	
Half life in water	days	37.5	
Half life in soil	days	75	
Half life in sediment	days	337.5	
Biodegradation (Ultimate survey model – BIOWIN3)		Weeks - months	

¹ Medetomidine – Physical chemical properties. Huntingdon life Sciences report FGT0006. August 2011.

² Medetomidine – Physical chemical properties. Huntingdon life Sciences report FGT0006. August 2011.

³ Adsorption/desorption of [¹⁴C]Medetomidine – RCC Limited Environmental Chemistry and Pharmaanalytics.

Table 1: Physical property data (25°C) of metabolites of medetomidine predicted in EPI.

Parameter	Units	N-methyl	3-Hydroxy Dex	Carboxy Dex	Carboxy N- Methyl	3-Hydroxy-N- Methyl
Molar mass	g/mol	214.31	216.29	230.27	244.3	230.31
Saturated vapour pressure	Pa	1.28×10^{-03}	1.30×10^{-07}	8.30×10^{-07}	5.88×10^{-06}	1.22×10^{-06}
Solubility in water	mg/L	6.794	346.8	119.1	34.03	99.51
Henry's law constant	$\text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$	1.17	2.00×10^{-06}	1.00×10^{-06}	2.13×10^{-05}	2.83×10^{-06}
log K _{ow}	--	4.37	2.36	2.82	3.36	2.91
log K _{oa}	--	7.40	11.50	12.20	11.43	10.67
BAF	$\text{L kg}^{-1} \text{ w.w}$	927	16.9	51.6	164	54.5
BCF	$\text{L kg}^{-1} \text{ w.w}$	356	9.32	3.16	3.16	21.40
K _{oc}	L kg^{-1}	5504	522.9	403.4	647.3	839
biodegradation (ultimate survey model-BIOWIN3)		Weeks-months	Weeks-months	Weeks-months	Weeks-months	Weeks-months

Table 2: Toxicity endpoints of metabolites of medetomidine predicted in EPIWIN.

Compound	Imidazole		Neutral organic SAR		
	Fish LC ₅₀ (mg L ⁻¹)	Daphnia LC ₅₀ (mg L ⁻¹)	Fish LC ₅₀ (mg L ⁻¹)	Daphnia LC ₅₀ (mg L ⁻¹)	algae EC ₅₀ (mg L ⁻¹)
Dexmedetomidine	1.55	0.56	4.40	3.17	2.89
N-Methyl	0.74	0.50	1.62	1.25	1.42
3-Hdroxy Dex	14.52	0.98	82.45	49.70	24.85
Carboxy-Dex	78.86	8.94	36.07	22.98	13.85
Carboxy N-methyl	37.34	7.93	13.17	8.97	6.77
3-HydroxyN-Methyl	6.90	0.868	30.22	19.46	12.18

Doc III A

Aerobic aquatic degradation study

Section A7.1.2.2.1

Annex Point IIA7.2.1

		1	REFERENCE	
1.1	Reference	Lewis C. J. 2014 [¹⁴ C]-Medetomidine: Degradation in marine water-sediment systems under aerobic conditions. Smithers Viscient, UK. Study Number: 3200167 (Unpublished)		
1.2	Data protection	Yes		
1.2.1	Data owner	I-Tech AB		
1.2.2	Criteria for data protection	Data on new a.s. for first Approval.		
		2	GUIDELINES AND QUALITY ASSURANCE	
2.1	Guideline study	Yes. OECD Guideline for Testing of Chemicals, No. 308.		
2.2	GLP	Yes.		
2.3	Deviations	No		
		3	MATERIALS AND METHODS	
3.1	Test material	Medetomidine		
3.1.1	Lot/Batch number	7240MXM028-5		
3.1.2	Specification	As given in section 2		
3.1.3	Purity	██████ % (radiochemical purity)		
3.1.4	Radiolabelling	Yes. Specific activity 1007 MBq/mmol		
3.1.5	Further relevant properties	Molecular weight: 200.3 g/mol (unlabelled) Solubility: 24 g/l pKa: 7.1		
3.1.6	TS inhibitory to microorganisms	Yes. The results of respiration inhibition test are presented in Document IIIA, Section 7_4_1_4.		
3.2	Reference substance	No		
3.3	Test solution	Test solution was prepared by removing the solvent from 390 µL of stock solution (conc. 1.3 mg/mL) under nitrogen. The test substance was dissolve in 4.5 mL water to a concentration of 0.105 mg/mL.		
3.4	Testing procedure			
3.4.1	Inoculum / test species	Microorganisms occurring in the water/sediment system used. No inoculums used.		
3.4.2	Test system	Borosilicate glass cylinders (4.5 cm diameter) containing 3 cm sediment and 9 cm water above the sediment, corresponding to a surface water volume of ca 143 mL was used. The sediment was either a clay loam sediment or loamy sand sediment.		
3.4.3	Test conditions	The flasks were incubated in darkness at 20°C + 2°C.		

Doc III A**Aerobic aquatic degradation study****Section A7.1.2.2.1****Annex Point IIA7.2.1**

3.4.4	Initial TS concentration	10.5 µg [¹⁴ C]-Medetomidine per unit or 0.073 µg/mL medetomidine.	X
3.4.5	Duration of test	100 days.	
3.4.6	Analytical parameter	Microbial biomass Total organic carbon Redox potential pH Oxygen concentration Chemical analyses of TS ¹⁴ C in water and sediment Bound residues Mineralisation of test substance measured as ¹⁴ CO ₂ Transformation of test substance measured as transformation products/metabolites.	
3.4.7	Sampling	Microbial biomass: day 0 and 100 Total organic carbon: day 0 and 100 Redox potential: day 0, 7, 14, 30, 59 and 100 pH: day 0, 7, 14, 30, 59 and 100 Oxygen concentration: day 0, 7, 14, 30, 59 and 100 Chemical analyses of TS: day 0, 7, 14, 30, 59 and 100 ¹⁴ C in water and sediment: 0, 7, 14, 30, 59 and 100 ¹⁴ CO ₂ and ¹⁴ C-organic volatiles: day 0, 7, 14, 30, 59 and 100 Transformation products: 0, 7, 14, 30, 59 and 100	
3.4.8	Method of analysis for transformation /degradation products	Analysis for transformation products were performed by HPLC and TLC.	
3.4.9	Controls	No	
3.4.10	Statistics	Estimation of degradation rates were performed using the CAKE Software (v 1.4).	

4 RESULTS

4.1 Degradation of test substance

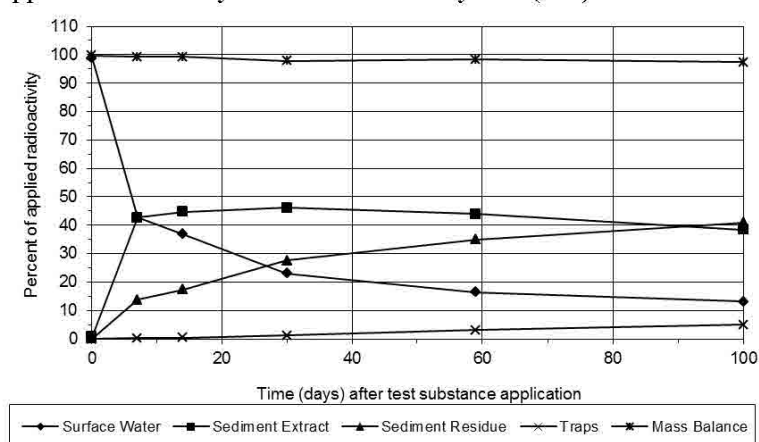
4.1.1 Mineralisation 4.9-5.8 %

4.1.2 Transformation Degradation of medetomidine resulted in formation of minor degradation products (total water and sediment 12-15% at 100 DAT), each present < 5% of applied radioactivity, bound residues and mineralization to carbon dioxide (up to 6% at 100 DAT).

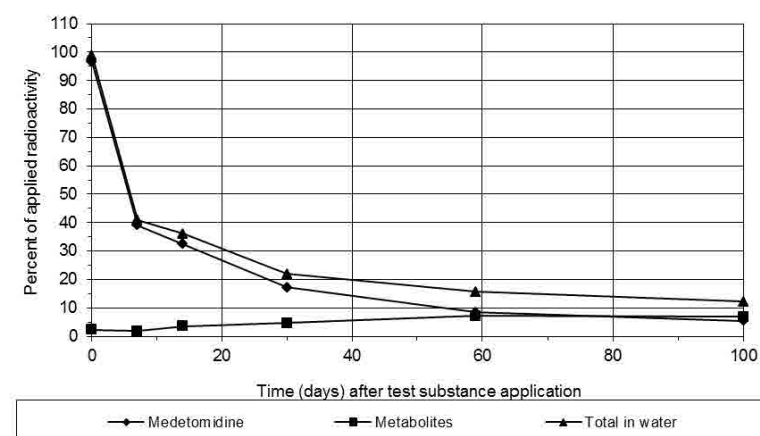
4.1.3 Transformation products Transformation products were not identified based on their concentration < 5% of applied radioactivity.

4.1.4 Graph

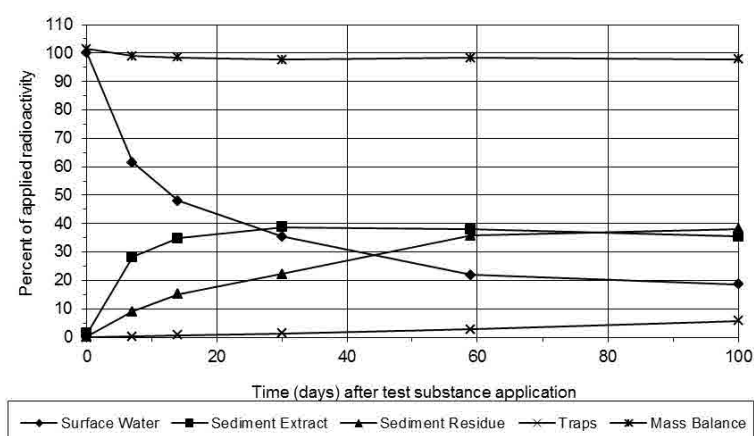
Applied radioactivity in water-sediment system (W1)



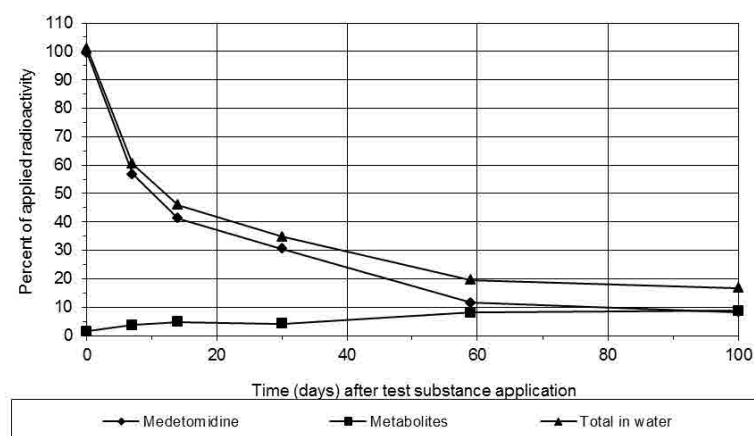
Medetomidine and metabolites in water-sediment system (W1)



Applied radioactivity in water-sediment system (W2)



Medetomidine and metabolites in water-sediment system (W2)



- | | | | |
|-------|--------------------------------------|--|---|
| 4.1.5 | Other observations | Degradation of medetomidine might have occurred during workup. Therefore it was assumed that all radioactivity in the primary sediment extract was medetomidine for calculation of DT ₅₀ -values. | X |
| 4.1.6 | Degradation of TS in abiotic control | No abiotic control used. | |
| 4.1.7 | Intermediates/ degradation products | Degradation products were not identified based on their concentration < 5% of applied radioactivity. | |

5 APPLICANT'S SUMMARY AND CONCLUSION

- | | | | |
|-----|------------------------|---|---|
| 5.1 | Materials and methods | The OECD Guideline 308 was performed to evaluate the degradation of medetomidine in a water/sediment system. | |
| 5.2 | Results and discussion | Medetomidine dissipated from water to sediment (DT ₅₀ 8 to 15 days) throughout the duration of the study resulting in 6 to 8% AR remaining in water as medetomidine at 100 DAT. Some of the medetomidine in sediment (up to ca 40% AR) could be extracted readily easily from sediment but a further proportion (up to ca 13% AR) could only be extracted under harsher conditions and up to ca 5% was recovered from the bound residues. DT ₅₀ values for the decrease in medetomidine levels in the water plus the medetomidine more readily extracted from the sediment from the two water-sediment systems were 54 and 49 days. | X |

Degradation of medetomidine in the water-sediment systems was observed by mineralisation to carbon dioxide (up to 6% AR) and metabolites in surface water (up to 5% AR). Degradation products in sediment were detected (up to 13%) but some might be produced during work-up.

5.3 Conclusion

The overall conclusion was that medetomidine steadily dissipated from water to sediment but more slowly dissipated from sediment. Some of the medetomidine in sediment was tightly bound, some not so tightly bound. Degradation in the total system occurred producing metabolites in water and by mineralisation to carbon dioxide. It can be calculated that approximately 60% of the applied medetomidine remained in the system at 100 DAT.

5.3.1 Reliability

Based on the assessment of materials and methods, the appropriate reliability indicator is 1.

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	12 February 2014
Materials and Methods	<p>The applicant's version is acceptable with the following comments:</p> <p>3.4.2. The test guideline states that one sediment should have a high organic carbon content (defined as 2.5 to 7.5%) and that the difference in organic carbon content between the two sediments should be at least 2%. In this study the organic carbon contents were 1.7% and 0.5%. Although the differences in organic content did not strictly comply with the guideline, the UK CA considered that overall the characteristics of the two sediments were sufficiently different and were acceptable.</p> <p>3.4.4. An aliquot of 100 µL of the application solution (section 3.3) was applied to each test unit. The final concentration in each unit was approximately ten times the tier 1 predicted environmental concentration. This was considered a practical compromise between a relevant environmental concentration and a practical concentration needed for analytical sensitivity.</p>
Results and discussion	<p>The applicant's version is acceptable with the following comments:</p> <p>4.1.2 The largest degradation product in the water phase was reported as 1.7% AR (system W1) and 2.2% (system W2). The largest degradation product in the sediment phase was reported as 4.4% AR (system W1) and 4.5% (system W2). No degradation product was identified and it was not reported whether the largest degradation products in the two phases were the same degradation products. Mineralisation increased slowly through the study and at 100 DAT there was a mean of 4.9% CO₂ in system W1 and 5.8% CO₂ in system W2.</p> <p>4.1.4 The title of the second graph should be 'medetomidine and metabolites in the water phase of water-sediment system (W1). The title of the fourth graph should be 'medetomidine and metabolites in the water phase of water-sediment system (W2).</p>

Results and discussion

4.1.4 The evaluator has produced graphs that show medetomidine in the water phase and sediment phase and the non-extractable residue over the course of the study (Figures 1 and 2 below). These show the dissipation of medetomidine from the water phase, and the rapid rise of medetomidine concentrations in the sediment. They also show the small decline in % AR in the sediment for system W1 by the end of the study and that there is no clear indication of decline in the sediment for W2. Non extractable residues are also presented and there is an increase to approximately 40% AR in both W1 and W2 systems.

4.1.4 The percentage of applied radioactivity recovered as medetomidine used to calculate DT_{50} values is summarised in the following table:

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

4.1.5 The applied radioactivity recovered at each time-point was always greater than 97% and less than 102 % except for a single sample (time-point 59 days, site W2).

Conclusion

The applicant's version is acceptable with the following comments:

5.2 The data for medetomidine was independently fitted according to FOCUS kinetics guidance by the CA evaluator – SFO modelling by EXCEL spreadsheet and FOMC modelling using EXCEL and KINGUI. Examples of the visual fitting are presented after this section (Figures 3 to 9).

Kinetic fitting (surface water data for medetomidine)

Visual fitting using SFO kinetics was poor (Figures 3 and 7) and χ^2 values close to or above 15% (see first table below). The data was fitted with FOMC kinetics and was visually good (Figures 4 and 8) with χ^2 values below 10% in both systems. The applicant rejected FOMC fitting for W1 based on the negative 95% confidence interval value for the parameter beta. However, FOCUS kinetic guidance states that 90% confidence interval values are acceptable for sediment / water studies. The data was modelled using KINGUI and the confidence intervals were acceptable (summarised in the second table below).

Summary of SFO and FOMC modelling:

System	Compartment	Model	DT ₅₀	Visual fitting	χ^2 error (%)
W1	surface water	SFO	8.2	poor	18.7
W2			14.5	poor	14.5
W1	surface water	FOMC	19.1	good	6.2
W2			28.6	good	5.8

FOMC modelling parameters values and confidence intervals (CI):

System	Parameter	Value	σ	Lower CI	Upper CI
W1	M ₀	96.4	3.4796	89.6	103.2
	Alpha	0.8077	0.1721	0.4704	1.145
	beta	3.8957	1.6597	0.6428	7.149
W2	M ₀	99.1	3.0034	93.2473	105.0
	Alpha	0.9626	0.1701	0.6292	1.296
	beta	9.5443	2.8642	3.9305	15.158

Kinetic fitting (sediment data for medetomidine)

A dissipation DT₅₀ value for the sediment from system W1 was calculated according to FOCUS kinetic guidance, taking time zero as the time the peak concentration in the sediment was reached (judged by the evaluator to be 14 days). There were four timepoints and it was the opinion of the evaluator that this was sufficient to adequately model the data and establish a DT₅₀ value (Figure 5). Results are summarised in the table below. There was no clear evidence of a decline (from peak values) in sediment concentrations in system W2 by the end of the study and therefore no DT₅₀ value could be calculated.

System	Compartment	Model	DT ₅₀	Visual fitting	χ^2 error (%)
W1	sediment	SFO	218.0	acceptable	7.9

Kinetic fitting (whole system for medetomidine)

The data for both systems was fitted using SFO kinetics. It was noted that the lack of degradation between the 60 day and final timepoint (at 100 days) in both systems meant that the fitting, instead of being good, was only considered acceptable (Figures 6 and 9). The χ^2 values for both systems was just over 10 %. Fitting by FOMC was not permissible by FOCUS kinetics as applied radioactivity at the end of the study was greater than 10 %, and was not acceptable by DFOP as the value of one rate constant approached zero in each case.

System	Compartment	Model	DT ₅₀	Visual fitting	χ^2 error (%)
W1	system	SFO	52.6	acceptable	10.3
W2			47.9	acceptable	10.0

Reliability

Reliability indicator 1

Acceptability

Acceptable

Remarks

The study has been performed to GLP and follows the OECD guideline 308 with only minor deviations.

The UK CA agreed with the conclusion that no individual metabolite was present at > 5% AR in either phase at any time point and therefore no identification of metabolites was required.

The appropriate DT₅₀ values for modelling if required are as follows:

- Whole system geomean DT₅₀ value: 50.2 days (as no surface water degradation DT₅₀ value could be determined)
- Sediment DT₅₀ value: 1000 days (conservative default value as there was no evidence of decline in system W2)

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**

Figure 1. The %AR present as medetomidine in the water phase, medetomidine in the sediment phase, and the total non-extractable residue (NER) over the course of the study for system W1.

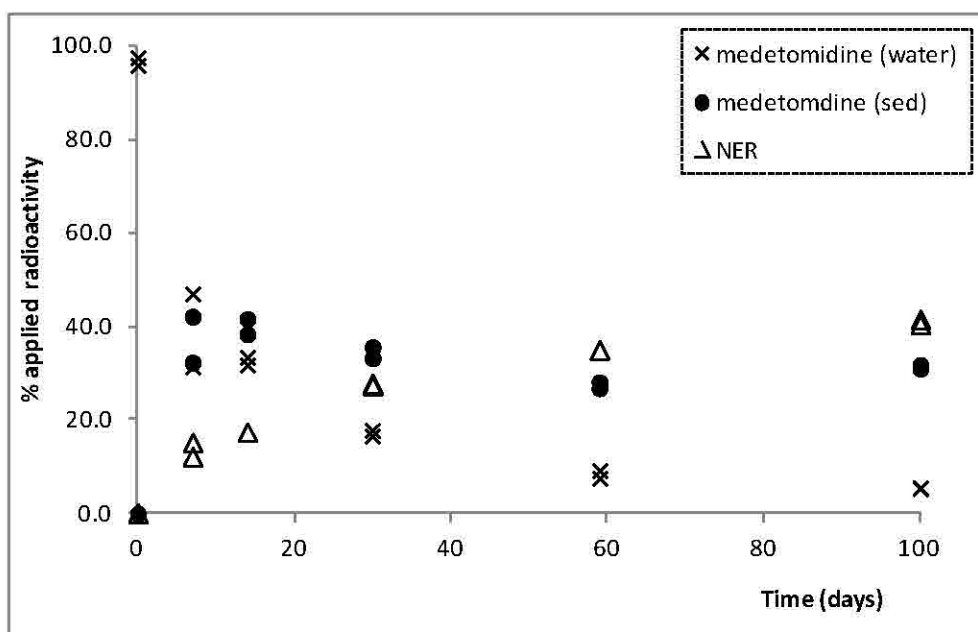


Figure 2. The %AR present as medetomidine in the water phase, medetomidine in the sediment phase, and the total non-extractable residue (NER) over the course of the study for system W2.

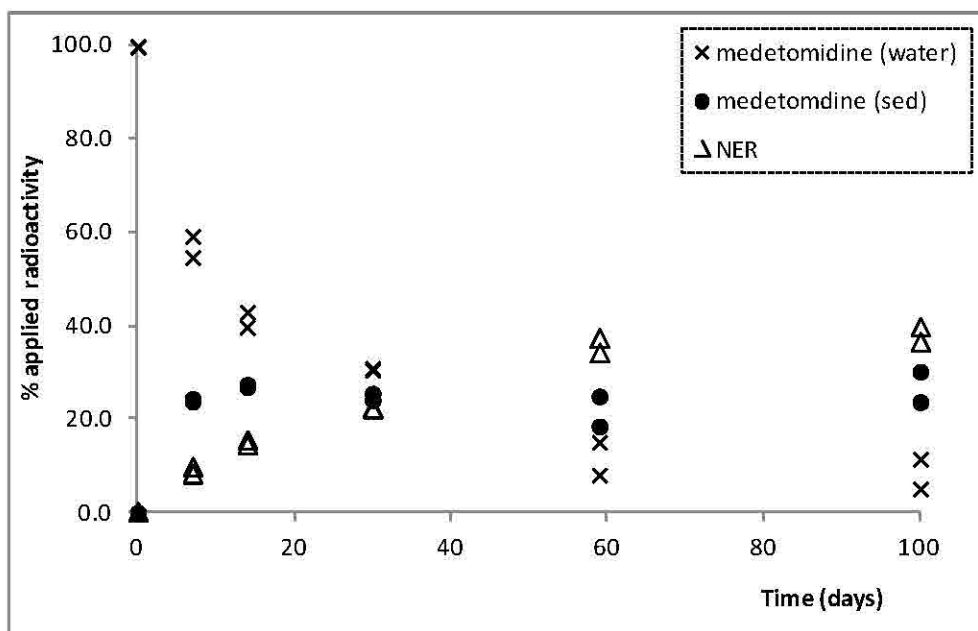
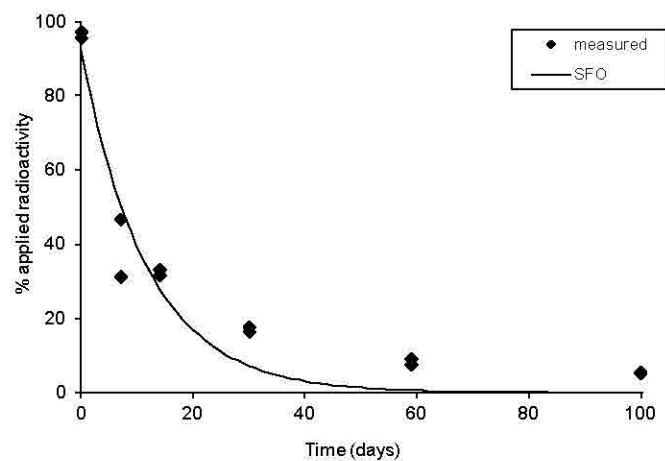


Figure 3. Kinetic evaluation: medetomidine dissipation from the water phase (SFO kinetics by EXCEL spreadsheet) – system W1.

Time (days)	Response (% AR)
0	97.40
0	95.80
7	46.90
7	31.40
14	33.40
14	31.70
30	16.60
30	17.80
59	7.60
59	9.20
100	5.40
100	5.60



Parameter	Value
DT ₅₀ (days)	8.2
DT ₉₀ (days)	27.1
χ^2 (% error)	18.7
Visual fitting	Poor

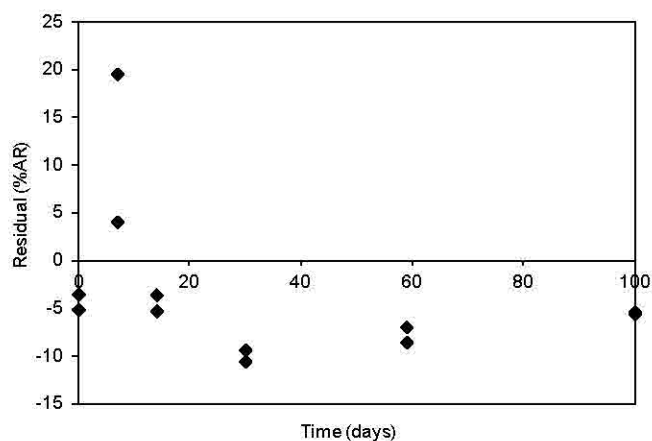
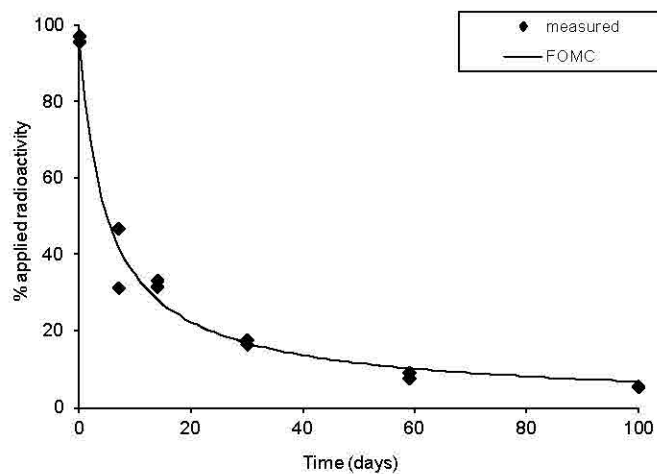


Figure 4. Kinetic evaluation: medetomidine dissipation from the water phase (FOMC kinetics by EXCEL spreadsheet) – system W1.

Time (days)	Response (% AR)
0	97.40
0	95.80
7	46.90
7	31.40
14	33.40
14	31.70
30	16.60
30	17.80
59	7.60
59	9.20
100	5.40
100	5.60



Parameter	Value
DT ₅₀ (days)	19.1 ^a
DT ₉₀ (days)	63.5
χ^2 (% error)	6.16
Visual fitting	Good

^a calculated as $DT_{90}/3.32$

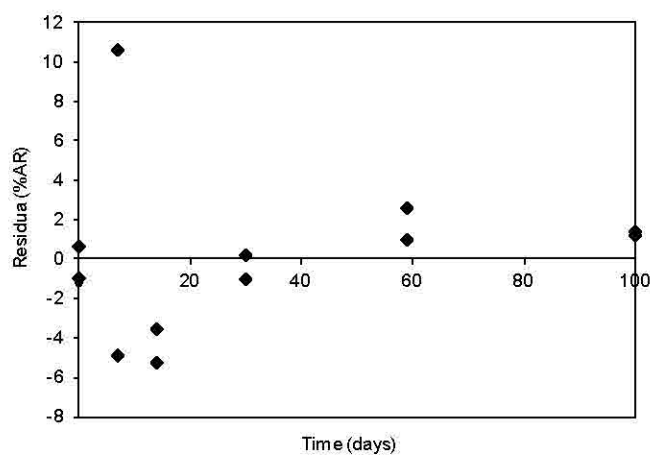
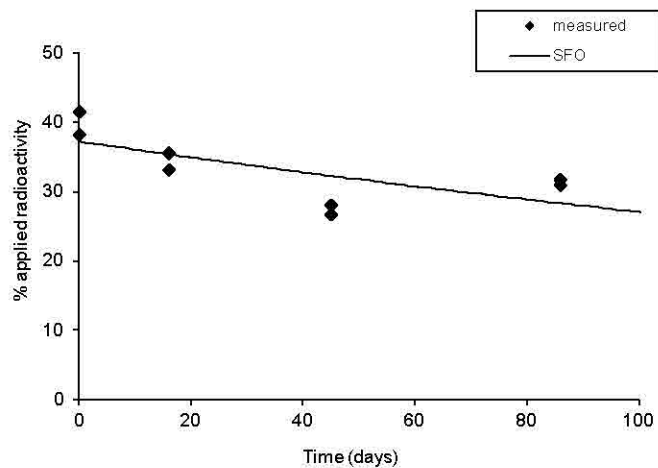


Figure 5. Kinetic evaluation: medetomidine dissipation from the sediment (SFO kinetics by EXCEL spreadsheet) – system W1.

Time (days)	Response (% AR)
0	38.30
0	41.60
16	35.60
16	33.20
45	28.10
45	26.80
86	31.00
86	31.80



Parameter	Value
DT ₅₀ (days)	218.0
DT ₉₀ (days)	724.1
χ^2 (% error)	7.9
Visual fitting	Acceptable

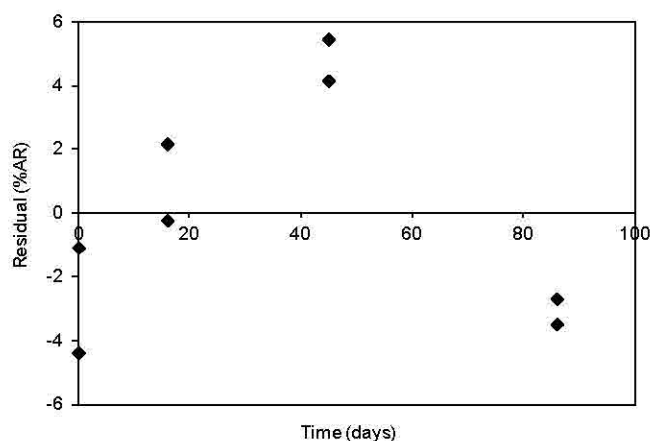
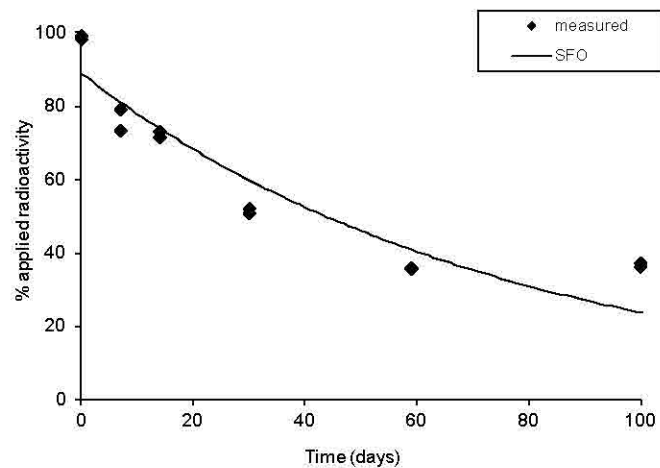


Figure 6. Kinetic evaluation: medetomidine degradation from the whole system (SFO kinetics by EXCEL spreadsheet) – system W1.

Time (days)	Response (% AR)
0	98.40
0	99.30
7	79.20
7	73.50
14	71.70
14	73.30
30	52.20
30	51.00
59	35.70
59	36.00
100	36.40
100	37.40



Parameter	Value
DT ₅₀ (days)	52.6
DT ₉₀ (days)	174.7
χ^2 (% error)	10.3
Visual fitting	Acceptable

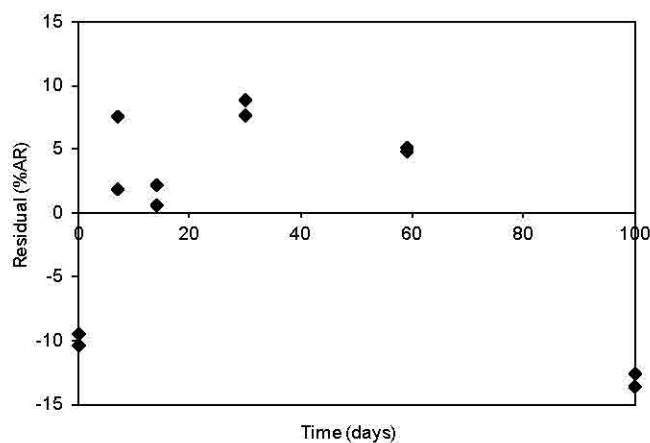
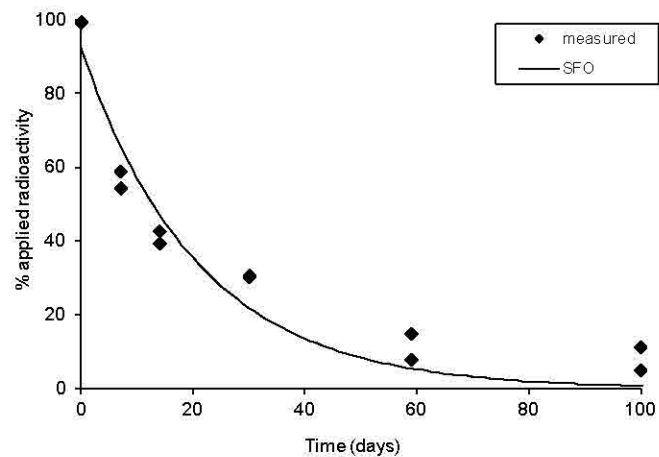


Figure 7. Kinetic evaluation: medetomidine dissipation from the water phase (SFO kinetics by EXCEL spreadsheet) – system W2.

Time (days)	Response (% AR)
0	99.60
0	99.40
7	59.00
7	54.50
14	42.80
14	39.60
30	30.40
30	30.80
59	8.00
59	15.00
100	5.10
100	11.40



Parameter	Value
DT ₅₀ (days)	14.5
DT ₉₀ (days)	48.0
χ^2 (% error)	14.5
Visual fitting	Relatively poor

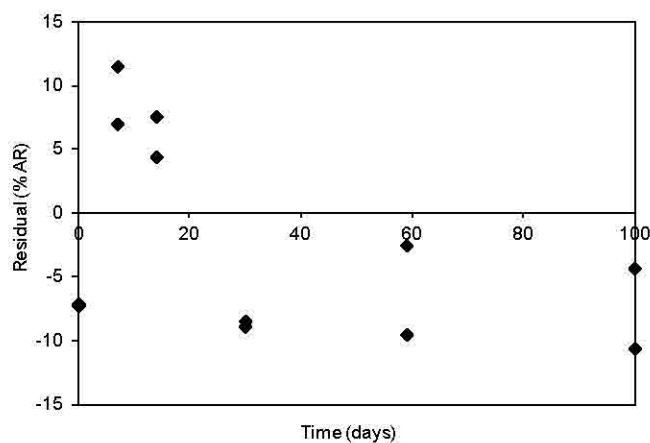
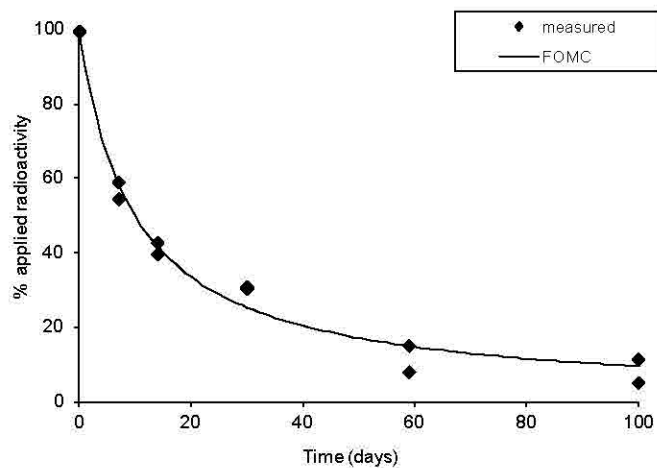


Figure 8. Kinetic evaluation: medetomidine dissipation from the water phase (FOMC kinetics by EXCEL spreadsheet) – system W2.

Time (days)	Response (% AR)
0	99.60
0	99.40
7	59.00
7	54.50
14	42.80
14	39.60
30	30.40
30	30.80
59	8.00
59	15.00
100	5.10
100	11.40



Parameter	Value
DT ₅₀ (days)	28.6 ^a
DT ₉₀ (days)	94.8
χ^2 (% error)	5.78
Visual fitting	Good

^a calculated as $DT_{90}/3.32$

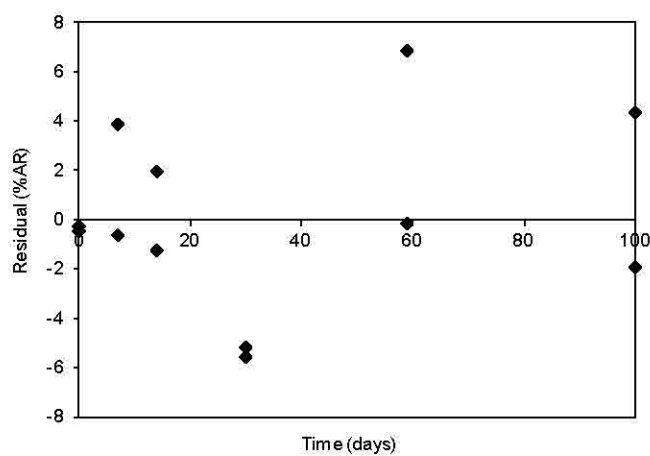
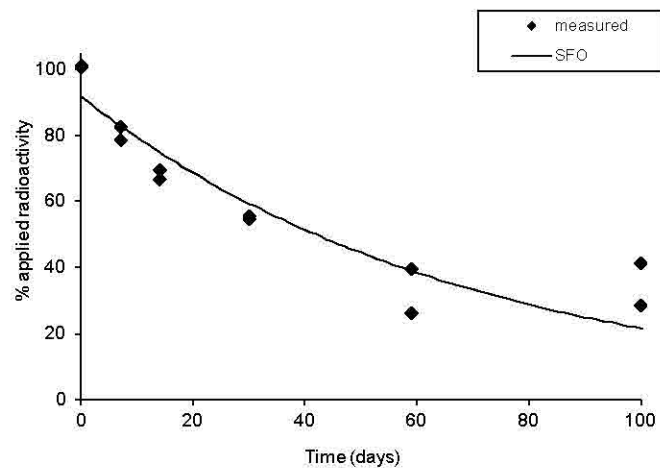


Figure 9. Kinetic evaluation: medetomidine degradation from the whole system (SFO kinetics by EXCEL spreadsheet) – system W2.

Time (days)	Response (% AR)
0	101.30
0	100.80
7	82.70
7	78.80
14	69.60
14	66.90
30	55.80
30	54.80
59	26.40
59	39.80
100	28.70
100	41.50



Parameter	Value
DT ₅₀ (days)	47.9
DT ₉₀ (days)	159.1
χ^2 (% error)	10.0
Visual fitting	Acceptable

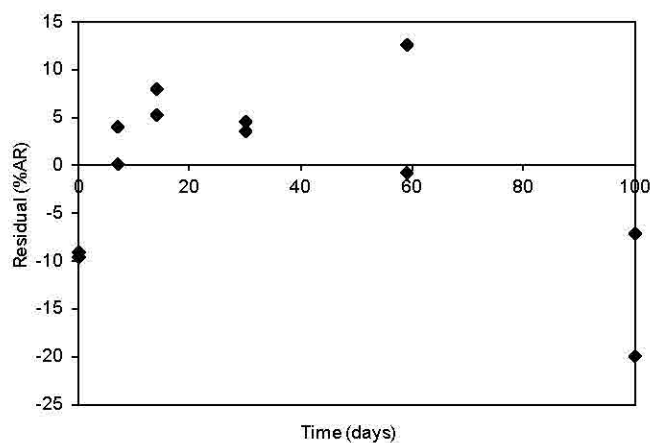


Table A7_1_2_2_1-1: Test conditions

Criteria	Details
Composition of medium	According to the OECD guideline, No. 308.
Additional substrate	No
Test temperature	20°C ± 2°C.
pH	8.2-8.8 (water) and 7.1-8.2 (sediment)
TOC in sediment	1.7 % (fine sediment) 0.5% (coarse sediment)
TOC water	15.3-17.3 ppm
Biomass in sediment (µg carbon/g sediment)	262 (fine sediment) 195 (coarse sediment)

Table A7_1_2_2_2-2: Biotransformation of medetomidine, expressed as percentage of applied radioactivity (mean ± s.d) in water –sediment system under aerobic conditions. Fine sediment (W1)

Table 1
Percent recovery of applied radioactivity from the Site W1 water-sediment system

Unit	Sampling Interval (days)	Surface water (Ext 1)	Primary Sediment Extract (Ext 2)	Secondary Sediment Extract (Ext 3)	Unextracted from Sediment	Total in Sediment	Sodium Hydroxide Traps	Mass Balance
A1	0	97.9	0.6	NA	0.1	0.7	NA	98.6
A2	0	99.9	0.8	NA	0.1	0.9	NA	100.8
Mean		98.9	0.7	NA	0.1	0.8	NA	99.7
A3	7	51.1	33.6	1.6	12.2	47.4	0.3	98.8
A4	7	34.4	47.8	2.1	15.2	65.1	0.3	99.8
Mean		42.8	40.7	1.9	13.7	56.3	0.3	99.3
A5	14	37.4	42.4	2.0	17.4	61.8	0.6	99.8
A6	14	36.1	43.0	2.0	17.4	62.4	0.1	98.6
Mean		36.8	42.7	2.0	17.4	62.1	0.4	99.2
A7	30	23.2	44.7	2.2	27.4	74.3	1.2	98.7
A8	30	22.7	43.3	2.0	27.8	73.1	1.1	96.9
Mean		23.0	44.0	2.1	27.6	73.7	1.2	97.8
A9	59	15.9	41.6	2.8	35.0	79.4	3.2	98.5
A10	59	16.8	40.6	2.7	35.0	78.3	2.9	98.0
Mean		16.4	41.1	2.8	35.0	78.9	3.1	98.3
A11	100	12.7	35.8	1.7	41.5*	79.0	5.1	96.8
A12	100	13.4	36.3	3.0	40.5	79.8	4.6	97.8
Mean		13.1	36.1	2.4	41.0	79.4	4.9	97.3

NA = Not Applicable

The primary sediment extraction solvent was 0.2% formic acid in methanol. The secondary solvent extract was a Soxhlet extraction with the same solvent.

*This sample was used for harsh reflux extraction and a further 12.6% AR was recovered.

Table A7_1_2_2_3: Biotransformation of medetomidine, expressed as percentage of applied radioactivity (mean \pm s.d) in water –sediment system under aerobic conditions. Course sediment W2

Table 2
Percent recovery of applied radioactivity from the Site W2 water-sediment system

Unit	Sampling Interval (days)	Surface water (Ext 1)	Primary Sediment Extract (Ext 2)	Secondary Sediment Extract (Ext 3)	Unextracted from Sediment	Total in Sediment	Sodium Hydroxide Traps	Mass Balance
B1	0	100.4	1.3	NA	0.2	1.5	NA	101.9
B2	0	99.5	1.3	NA	0.2	1.5	NA	101.0
Mean		100.0	1.3	NA	0.2	1.5	NA	101.5
B3	7	63.7	25.6	1.4	8.3	35.3	0.3	99.3
B4	7	59.3	28.1	1.2	9.8	39.1	0.2	98.6
Mean		61.5	26.9	1.3	9.1	37.2	0.3	99.0
B5	14	50.4	30.1	3.0	14.6	47.7	0.6	98.7
B6	14	45.6	34.2	2.1	15.5	51.8	0.7	98.1
Mean		48.0	32.2	2.6	15.1	49.8	0.7	98.4
B7	30	34.7	36.5	2.8	22.4	61.7	1.3	97.7
B8	30	36.2	35.4	2.6	22.2	60.2	1.3	97.7
Mean		35.5	36.0	2.7	22.3	61.0	1.3	97.7
B9	59	18.6	32.3	3.2	37.3	72.8	3.1	94.5
B10	59	25.4	36.6	3.5	34.2	74.3	2.4	102.1
Mean		22.0	34.5	3.4	35.8	73.6	2.8	98.3
B11	100	16.2	31.9	2.4	39.7*	74.0	7.0	97.2
B12	100	20.9	34.7	1.9	36.5	73.1	4.6	98.6
Mean		18.6	33.3	2.2	38.1	73.6	5.8	97.9

NA = Not Applicable

The primary sediment extraction solvent was 0.2% formic acid in methanol. The secondary solvent extract was a Soxhlet extraction with the same solvent.

*This sample was used for harsh reflux extraction and a further 12.8% AR was recovered.

Form for justification of the non-submission of data

III A Section 7.1.2 Annex Point XII.2.1	Rate and route of degradation in aquatic systems including identification of metabolites and degradation products		
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure []	Other justification [x]		
Detailed justification:	Study 7.1.2.2.2 (Water/sediment degradation study (marine water)) are under execution and will be submitted after completion. Therefore no further studies of degradation in aquatic systems have been performed.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	Technically the UK CA agrees with the Applicants justification for non-submission because this data requirement can be met by the submission of an acceptable water/sediment degradation study under annex point IIIA 7.1.2.2.2. However the UK CA considered the existing water sediment study under IIIA 7.1.2.2.2 to be unreliable and further information will be required to fulfil this basic data point. However pending submission and evaluation of an acceptable marine water/sediment study the justification for non-submission of data under this data point can be accepted.		
Conclusion	The Applicants justification will be acceptable pending submission and evaluation of an acceptable marine water/sediment study under IIIA 7.1.2.2.2.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		

III A Section 7.1.2
Annex Point XII.2.1

**Rate and route of degradation in aquatic systems
including identification of metabolites and degradation
products**

Remarks

**Doc III A Section
7.1.3(01)**

Adsorption / Desorption screening test

Annex Point IIA7.7

Official
use only

		1 REFERENCE
1.1	Reference	Dr. Völkel W. 2006. Adsorption/desorption of [¹⁴ C]-Medetomidine RCC Ltd., Environmental Chemistry & Pharamalytics CH-4452 Itingen, Switzerland RCC Study Number: A19451 (Unpublished)
1.2	Data protection	Yes
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	Yes. OECD Guideline for Testing of Chemicals, No. 106.
2.2	GLP	Yes.
2.3	Deviations	Yes. One deviation from the guideline was done; a replacement of soil IV by a river sediment. A sponsor's request.
		3 MATERIALS AND METHODS
3.1	Test material	As given in Doc IIIA, Section 2. New test material: Medetomidine in a ¹⁴ C-labelled form. [¹⁴ C]-Medetomidine.
3.1.1	Lot/Batch number	0001
3.1.2	Specification	As given in Doc IIIA, Section 2 for unlabelled Medetomidine.
3.1.3	Purity	As given in section 2 for unlabelled Medetomidine. [¹⁴ C]-Medetomidine has a purity of [REDACTED] % (RCC Study Number A19451).
3.1.4	Further relevant properties	No
3.1.5	Method of analysis	Percent of the test item adsorbed was measured by liquid scintillation counting (LSC). TLC analysis. The volumes of the supernatants were determined gravimetrically.
3.2	Degradation products	Degradation products tested: No
3.2.1	Method of analysis for degradation products	Not relevant due to no degradation products.
3.3	Reference substance	No reference substance was used.

**Doc III A Section
7.1.3(01)**

Adsorption / Desorption screening test

Annex Point IIA7.7

3.3.1	Method of analysis for reference substance	No reference substance was used.	
3.4	Soil types	Classification, textural class/content and physico-chemical properties of the soils used as adsorbents are shown in Table A7_1_3(01)-1. Soils with different properties were used in order to cover as widely as possible the interactions of a given item with naturally occurring soils.	
3.5	Testing procedure		
3.5.1	Test system	The soils chosen represent a range of soil properties that are believed most important for adsorption, i.e. organic carbon content, pH, clay content and texture. The study was performed in sealed Teflon or glass centrifuge tubes and all experiments including controls were performed in duplicate on a rotary shaker at about 150 strokes per minute, at a constant temperature of $20 \pm 2^\circ\text{C}$ (temperature-controlled room). The tubes were shaken in the dark and the agitation device kept the soil in suspension.	
3.5.2	Test solution and Test conditions	12 μl of [^{14}C]-Medetomidine was added to 100 ml of 0.01 M CaCl_2 . The amount of this solution was determined by LSC to 137 μg [^{14}C]-Medetomidine/ml considering specific radioactivity of 2.15 MBq/mg. The experiment continued for 48 h in darkness at constant temperature $20 \pm 2^\circ\text{C}$. The Teflon tubes were shaken at about 150 stokes per minute to keep the soil in suspension. Adsorption/desorption behaviour of test item, [^{14}C]-Medetomidine, was tested at single concentrations of either 0.011 mg/l or 0.055 mg/l in preliminary and screening tests, respectively. Duplicate samples were taken for LSC measurements.	X
3.6	Test performance		
3.6.1	Preliminary test	According to (a)"OECD 106": Yes. Two soils (I and II), three degree of saturation or soil-to-aqueous phase ratios (1/1, 1/5, 1/25), centrifugation 10 min at about 3300 rpm, agitation time 2, 5, 24 and 48 hours.	X
3.6.2	Screening test: Adsorption	According to (a)"OECD 106": Yes. Five soils (I-V), one degree of saturation or soil/solution ratio (1/25), centrifugation 10 min at about 3300 rpm, agitation intervals 2, 5, 24 and 44 hours.	X X
3.6.3	Screening test: Desorption	According to (a)"OECD 106": Performed. Five soils (I-V), one degree of saturation or soil/solution ratio (1/25), centrifugation 10 min at about 3300 rpm, agitation intervals of 2, 5, 24 and 48 hours.	X
3.6.4	HPLC-method	According to (a)"OECD-HPLC-method" ¹ : No	

¹ OECD (1999) OECD-Guidelines for the Testing of Chemicals. Proposal for a new guideline 121: Estimation of the adsorption coefficient (K_{oc}) on soil and on sewage sludge using High Performance Liquid Chromatography (HPLC), Draft Document (August 1999).

**Doc III A Section
7.1.3(01)**

Adsorption / Desorption screening test

Annex Point IIA7.7

		TLC chromatogram. One dimensional TLC using pre-coated plates of kieselgel with thickness layer of 0.25 mm. The radioactive zones were detected by an automatic TLC analyzer. Area of interest was integrated manually. The radioactivity was measured using liquid scintillation.	X
3.6.5	Other test	The volumes of the supernatants were determined gravimetrically.	
	4 RESULTS		
4.1	Preliminary test	Summary of the preliminary test is presented in Table A7_1_3(01)-2.	
4.2	Screening test: Adsorption	Summary of the screening adsorption test is shown in Table A7_1_3(01)-3.	
4.3	Screening test: Desorption	Summary of the screening desorption test is presented in Table A7_1_3(01)-4.	
4.4	Calculations		
4.4.1	Ka , Kd	Adsorption (Ka) and desorption (Kd) coefficients were calculated according to guideline 106. Soil I 20, 24 Soil II 50, 78 Soil III 45, 65 Soil IV 32, 41 Soil V 45, 74	
4.4.2	Ka _{oc} , Kd _{oc}	Adsorption coefficient and desorption coefficient as a function of the organic carbon content were calculated according to guideline 106.	
4.5	Degradation product(s)	Not applicable. Substance without impurities and stable during the study.	
	5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	Materials and methods	OECD Guideline for Testing of Chemicals, No. 201. Percent of test item adsorbed was measured by liquid scintillation counting (LSC). TLC analysis. The volumes of the supernatants were determined gravimetrically. One deviation from the guideline was done; a replacement of soil IV by a river sediment. A sponsor's request.	
5.2	Results and discussion	Test item is a stable, non-volatile and relatively good soluble in water substance. Radiochemical purity of [¹⁴ C]-Medetomidine was 100%. No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the entire test duration.	
		Soil I Soil II Soil III Soil IV Soil V	
5.2.1	Adsorbed a.s. [%]	41.6 66.1 60.4 52.6 60.2	X
5.2.2	K _a	20 50 45 32 45	
5.2.3	K _d	24 78 65 41 74	
5.2.4	Ka _{oc}	1526 1215 1702 4114 2229	

**Doc III A Section
7.1.3(01)****Adsorption / Desorption screening test****Annex Point IIA7.7**

5.2.5	Ka/Kd	0.3	0.64	0.69	0.78	0.61
5.2.6	Degradation products (% of a.s.)	Not measured.				
5.3	Conclusion	The adsorption / desorption screening test followed OECD guideline 106. One deviation from the guideline was done; a replacement of soil IV by a river sediment at sponsor's request. Soil mobility of the test substance is classified as slightly mobile (K_{oc} 1215-4114ml/g).				
5.3.1	Reliability	Based on the assessment of materials and methods, the appropriate reliability indicator is 1.				
5.3.2	Deficiencies	No.				

**Doc III A Section
7.1.3(01)**

Annex Point IIA7.7

Adsorption / Desorption screening test

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	22/04/2013
Materials and Methods	<p>Applicant version is acceptable, noting the following:-</p> <p>Section 3.5.2: The concentration tested in the Tier 2 screening step stage (0.055 mg/l) was noted to be higher than predicted to occur based on the product specific exposure assessments in Doc IIB.</p> <p>Section 3.6.1: The preliminary test also confirmed that no adsorption according to sample tubes in the absence of test soils.</p> <p>Section 3.6.2: The selected soil: solution ration of 1:25 was chosen to ensure adsorption was within the OECD 106 guideline range of greater than 20% and preferably >50% at equilibrium.</p> <p>Section 3.6.2: The amount of test substance adsorbed to soil was calculated via subtraction based on the aqueous phase concentration (i.e. the indirect method according to OECD 106).</p> <p>Section 3.6.2: The study progressed as far as the Tier 2 Screening test stage according to OECD 106. No Tier 3 determination of Freundlich adsorption isotherms was performed, and therefore no conclusion on the influence of concentration on adsorption could be reached. According to the testing strategy in Annex I of OECD 106 since the $K_d * (m_{sol}/V_0)$ was > 0.3 (indirect method) a full Tier 3 test should have been performed.</p> <p>Section 3.6.3: Desorption had reached approximate equilibrium within 48 h based on the graphical presentation of results in the original study report.</p> <p>Section 3.6.4: Based on the TLC chromatograms after either 44 h of adsorption or 48 h of desorption, the test substance medetomidine was stable with no metabolites formed.</p>
Results and discussion	<p>Applicant version is acceptable, noting the following:-</p> <p>Section 5.2: Sorption was noted to correlate reasonably well with organic carbon content and therefore the derivation of $K_{a,oc}$ values appears valid.</p> <p>Table A7_1_3(01)-4: Since total desorption was less than 75% of the amount adsorbed, the adsorption cannot be considered reversible according to paragraph 79 of OECD 106.</p>
Conclusion	Applicant version is acceptable, noting that the arithmetic mean $K_{a,oc}$ is 2157 ml/g.
Reliability	1
Acceptability	Acceptable
Remarks	The study progressed as far as the Tier 2 Screening test stage according to OECD 106. No Tier 3 determination of Freundlich adsorption isotherms was performed, and therefore no conclusion on the influence of concentration on adsorption could be reached. According to the testing strategy in Annex I of OECD 106 since the $K_d * (m_{sol}/V_0)$ was > 0.3 (indirect method) a full Tier 3 test should have been performed.

Doc III A Section Adsorption / Desorption screening test
7.1.3(01)

Annex Point IIA7.7

	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	

Table A7_1_3(01)-1: Classification and physico-chemical properties of soils used as adsorbents

	Soil I	Soil II	Soil III	Soil IV	Soil V
Soil order	June 2003	October 2001	June 2001	October 2005	October 2001
Classification	Loam	Clay loam	Silty clay loam	Sandy loam	Silt loam
Location	Mechthildshausen	Mussig	Uffholtz	River Sediment	Bretagne 1
Horizon	50°02'N, 8°18'E	48°14'N, 7°31'E	47°49'N, 7°10'E	47°32'N, 7°55'E	47°55'N, 2°49'E
Sand [%]	45.31	21.14	19.02	70.0	17.37
Silt [%]	37.12	42.44	46.94	25.9	65.23
Clay [%]	17.57	36.42	34.04	4.1	17.40
Organic carbon [%]	1.28	4.13	2.67	0.78	2.00
pH (1:1 H ₂ O)	7.37	7.55	5.00	7.29	5.36
Cation exchange capacity (MEQ/100 g)	11.40	39.5	26.85	12.1	12.87
Moisture content in g per 100 g dry soil	2.18	5.73	2.43	1.06	1.15
Special chemical/mineralogical features (C/N ratio)	10.7	7.9	9.2	7.1	9.5
Nitrogen content, %	0.12	0.52	0.29	0.11	0.21
Organic matter (g/100g soil), %	2.21	7.12	4.60	1.34	3.45
Particle size analysis (mm): USDA: < 0.002 (clay) %	17.57	36.42	34.04	4.1	17.40

0.002-0.05 (silt) %	37.12	42.44	46.94	25.9	65.23
> 0.05 (sand) %	45.31	21.14	19.02	70.0	17.37

Table A7_1_3(01)-2: Results of preliminary test:

Test substance	Medetomidine in a form of radioactive labelled molecule: [¹⁴ C]-Medetomidine
Sample purity	100 %
Weighed soil	10 g, 5g and 1 g
Volume of CaCl₂ solution	200 ml
Nominal concentration of a.s. final solution	0.011 mg/l
Analytical concentration final of a.s. solution	22 µg/ml considering its specific radioactivity of 2.15 MBq/mg
Concentration of the test solution (show calculation)	Details are in RCC Study Number: A19451. Specific radioactivity of [¹⁴ C]-Medetomidine is 2.15 MBq/mg.
Details of the analytical method used:	
Method	Liquid scintillation counting (LSC)
Recovery rate	>95 % of their radioactivity content
Detection limit	2 x background radioactivity (10 dpm)

Table A7_1_3(01)-3: Results of screening test - adsorption:

	Soil I	Soil II	Soil III	Soil IV	Soil V
Concentration of test material [mg/l]	0.055	0.055	0.055	0.055	0.055
Mass of the test item in the aqueous solution at adsorption equilibrium, after contact of 44 hours with soil, µg	0.766	0.454	0.485	0.598	0.491
Mass of the test item adsorbed on the soil at adsorption equilibrium, after contact of 44 hours with soil, µg	0.599	0.911	0.881	0.767	0.874
Initial concentration of test solution [mg/l]	0.055	0.055	0.055	0.055	0.055
Decrease in concentration [mg/l]	0.023	0.036	0.033	0.029	0.033
Test material adsorbed [%]	41.6	66.1	60.4	52.6	60.2
Temperature [°C]	20	20	20	20	20

Table A7_1_3(01)-4: Results of screening test - desorption:

	Soil I	Soil II	Soil III	Soil IV	Soil V
Temperature [°C]	20	20	20	20	20
Quantity desorbed [µg]	0.33	0.19	0.25	0.31	0.21
[%] of adsorbed test material, which is desorbed	49.6	23.4	26.3	36.7	23.9

Form for justification of the non-submission of data

III A Section 7.1.4.1		Field study on accumulation in the sediment	
Annex Point XII.2.1			
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified [X]	
Limited exposure []	Other justification []		
Detailed justification:	Adsorption/desorption screening test did not indicate the need for further studies.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	The Applicants non-submission of data for this Annex point is accepted.		
Conclusion	Applicants non-submission of data accepted.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

Form for justification of the non-submission of data

III A Section 7.1.4 Annex Point XII.2.2	Further studies on adsorption and desorption in water/sediment systems	
JUSTIFICATION FOR NON-SUBMISSION OF DATA		Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified [X]
Limited exposure []	Other justification []	
Detailed justification:	Adsorption/desorption screening test did not indicate the need for further studies.	
Undertaking of intended data submission []		
Evaluation by Competent Authorities		
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>		
EVALUATION BY RAPPORTEUR MEMBER STATE		
Date	24/4/2013	
Evaluation of applicant's justification	Applicant's justification accepted.	
Conclusion	Accepted.	
Remarks	None.	
COMMENTS FROM OTHER MEMBER STATE (specify)		
Date	<i>Give date of comments submitted</i>	
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>	
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>	
Remarks		

Form for justification of the non-submission of data

III A Section 7.2.1		Aerobic degradation in soil, initial study	
Annex Point VII.4, XII.1.1			
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure [x]	Other justification []		
Detailed justification:	The active substance will be used in product-type 21: Antifouling products. The products are for use in the marine environment. Dispersion to terrestrial compartments might occur during application, maintenance and removal of the product but will be kept to a minimum. Degradation in soil was therefore not studied.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	The Applicants justification was accepted.. However pending evaluation of the environmental risks to the soil compartment, additional information may be needed to refine the 1 st tier assessment.		
Conclusion	Accepted.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

Form for justification of the non-submission of data

III A Section 7.2.2		Aerobic degradation in soil, further studies	
Annex Point VII.4, XII.1.1			
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure [x]	Other justification []		
Detailed justification:	The active substance will be used in product-type 21: Antifouling products. The products are for use in the marine environment. Dispersion to terrestrial compartments might occur during application, maintenance and removal of the product but will be kept to a minimum. Degradation in soil was therefore not studied.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	The Applicants justification was accepted.. However pending evaluation of the environmental risks to the soil compartment, additional information may be needed to refine the 1 st tier assessment.		
Conclusion	Accepted.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

Form for justification of the non-submission of data

III A Section 7.2.3		Adsorption and mobility in soil, further studies	
Annex Point XII.1.2-1.3			
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure [x]	Other justification []		
Detailed justification:	The active substance will be used in product-type 21: Antifouling products. The products are for use in the marine environment. Dispersion to terrestrial compartments might occur during application, maintenance and removal of the product but will be kept to a minimum. Adsorption and mobility in soil was therefore not studied further.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	This Annex point has been adequately addressed by Annex Point IIIA 7.1.3.		
Conclusion	Addressed.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

Form for justification of the non-submission of data

III A Section 7.3.1 Annex Point VII.5		Phototransformation in air including identification of breakdown products	
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure [x]	Other justification []		
Detailed justification:	The active substance is non-volatile and dispersion to air compartments will be minimal. Studies of phototransformation in air were therefore not performed.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	Accepted		
Conclusion	Accepted.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

Form for justification of the non-submission of data

III A Section 7.3.2 Annex Point XII.3		Fate and behaviour in air, further studies	
JUSTIFICATION FOR NON-SUBMISSION OF DATA			Official use only
Other existing data []	Technically not feasible []	Scientifically unjustified []	
Limited exposure [x]	Other justification []		
Detailed justification:	The active substance is non-volatile and dispersion to air compartments will be minimal. Further studies of fate and behaviour in air were therefore not performed.		
Undertaking of intended data submission []			
Evaluation by Competent Authorities			
<i>Use separate "evaluation boxes" to provide transparency as to the comments and views submitted</i>			
EVALUATION BY RAPPORTEUR MEMBER STATE			
Date	24/04/2013		
Evaluation of applicant's justification	Accepted.		
Conclusion	Accepted.		
Remarks	None.		
COMMENTS FROM OTHER MEMBER STATE (specify)			
Date	<i>Give date of comments submitted</i>		
Evaluation of applicant's justification	<i>Discuss if deviating from view of rapporteur member state</i>		
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>		
Remarks			

**Doc III A section
7.4.1.1 (01)**

Acute toxicity to fish

Annex Point IIA VII.7.1

		1	REFERENCE	
1.1	Reference		Medetomidine: Acute toxicity to zebra fish (<i>Brachydanio Rerio</i>) in a 96-hour static test (Unpublished)	
1.2	Data protection		Yes	
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		Yes. OECD Guideline for Testing of Chemicals, No. 203.	
2.2	GLP		Yes.	
2.3	Deviations		Yes. Reconstituted water with the water hardness of 125 mg/l as CaCO ₃ was used as test water instead of local tap water	
		3	MATERIALS AND METHODS	
3.1	Test material		Medetomidine hydrochloride.	X
3.1.1	Lot/Batch number		24431	
3.1.2	Specification		Medetomidine hydrochloride, used instead of Medetomidine due to higher solubility in water. Final form in water is pH dependent.	
3.1.3	Purity		%	
3.1.4	Composition of Product		Crystalline powder	
3.1.5	Further relevant properties		Molecular weight: 236.7 g/mol Solubility: 24 g/l pKa: 7.1	
3.1.6	Method of analysis		The quantification of the test item was performed by derivatization with pentafluorobenzoylchloride followed by GC analysis with MS-detection	
3.2	Preparation of TS solution for poorly soluble or volatile test substances		Not necessary.	
3.3	Reference substance		No reference substance was used.	
3.3.1	Method of analysis for reference substance		No reference substance was used.	

Doc III A section**Acute toxicity to fish****7.4.1.1 (01)****Annex Point IIA VII.7.1****3.4 Testing procedure**

- | | | | |
|-------|--------------------------------|--|---|
| 3.4.1 | Dilution water | Details on dilution water in tabular form is shown in Table A7_4_1_1(01)-2 | X |
| 3.4.2 | Test organisms | Zebra fish (<i>Brachydanio rerio</i>), see Table A7_4_1_1(01)-3 | |
| 3.4.3 | Test system | Details on test type, renewal of TS solution, laboratory equipment, loading, replicates etc. Is presented in the Table A7_4_1_1(01)-4 | |
| 3.4.4 | Test conditions | pH, test temperature, oxygen concentration etc are shown in Tables A7_4_1_1(01)-5. | |
| 3.4.5 | Duration of the test | 96 hours | |
| 3.4.6 | Test parameter | Mortality is presented in Table A7_4_1_1(01)-6. | |
| 3.4.7 | Sampling | The test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for mortality and visible abnormalities.

For the determination of the actual item concentrations, the samples were taken just before the start test and after 48 and 96 hours. All samples were taken from the approximate centre of the aquaria without mixing of the test media and were deep-frozen (-20°C) immediately after sampling. The concentrations of the test item Medetomidine was analytically measured in all test medium samples taken from all test concentrations at the sampling times at the start of the test and after 96 hours test duration (or taken after 24 hours for the highest test concentration) | |
| 3.4.8 | Monitoring of TS concentration | Yes. 0 and 24 hours (for the highest test concentration), 96 hours test duration | |
| 3.4.9 | Statistics | The LC50 and the 95% confidence interval at the observation dates were calculated by Probit Analysis. | |

4 RESULTS**4.1 Limit Test**

- | | | |
|-------|--|---------------|
| 4.1 | Limit Test | Not performed |
| 4.1.1 | Concentration | Not performed |
| 4.1.2 | Number/
percentage of
animals showing
adverse effects | Not performed |
| 4.1.3 | Nature of adverse
effects | Not performed |

4.2 Results test substance

- | | | |
|-------|--|--|
| 4.2.1 | Initial concentrations of test substance | Medetomidine nominal initial concentration was 0; 0.32; 1.0; 3.2; 10; 32 and 100 mg/l. |
| 4.2.2 | Actual concentrations of | See Table A7_4_1_1(01)-6 and Table A7_4_1_1(01)-9. |

**Doc III A section
7.4.1.1 (01)**

Acute toxicity to fish

Annex Point IIA VII.7.1

	test substance	
4.2.3	Effect data (Mortality)	Mortality data as absolute numbers of immobile fish and as percent of exposed animals is shown in Table A7_4_1_1(01)-6; Report LC ₀ , LC ₅₀ , and LC ₁₀₀ values for 96 h, including 95 % confidence limit, presents in Table A7_4_1_1(01)-7
4.2.4	Concentration / response curve	No concentration/response curve generated. Please refer to Table A7_4_1_1(01)-6 and A7_4_1_1(01)-7 for effect data.
4.2.5	Other effects	Visible abnormalities observed at the test fish is presented in Table A7_4_1_1(01)-6
4.3	Results of controls	
4.3.1	Number/ percentage of animals showing adverse effects	In the control all fish survived until the end of the test. The results is presented in Table A7_4_1_1(01)-6.
4.3.2	Nature of adverse effects	There was no any adverse effect on the test fish observed in the control. See Table A7_4_1_1(01)-6.
4.4	Test with reference substance	Not performed
4.4.1	Concentrations	Not performed
4.4.2	Results	Not performed
5 APPLICANT'S SUMMARY AND CONCLUSION		
5.1	Materials and methods	OECD Guideline for Testing of Chemicals, No. 203. One deviation from the guideline was performed: reconstituted water with the water hardness of 125 mg/l as CaCO ₃ was used as test water instead of local tap water. Technical reason. The quantification of the test item was performed by derivatization with pentafluorobenzoylchloride followed by GC analysis with MS-detection.
5.2	Results and discussion	<p>Test item is a stable, non-volatile and relatively good soluble in water substance. No remarkable observations were made concerning the appearance of the test media. All test media were clear solutions throughout the entire test duration.</p> <p>In the control and the test concentrations up to and including 3.1 mg/l (nominal 3.2 mg/l), all fish survived until the end of the test. However, the body coloration of the test fish was significantly changed compared to the control at all treatments down to the lowest test concentration of 0.39 mg/l (nominal 0.32 mg/l). The test fish in the treatments showed a brighter coloring compared to the control fish.</p> <p>At the test concentrations of 8.4 and 24 mg/l (nominal 10 and 32 mg/l, respectively), all test fish showed strong symptoms of toxicity and the same difference in body coloration. At these test concentrations, one of the test fish died almost after 24 hrs. At the highest test concentration of 83 mg/l (nominal 100 mg/l), all test fish died within 24 hours of exposure.</p>

X

Doc III A section**Acute toxicity to fish****7.4.1.1 (01)****Annex Point IIA VII.7.1**

5.2.1	LC ₀	3.1 mg/l (96-hours)	X
5.2.2	LC ₅₀	30 mg/l (96-hours)	X
5.2.3	LC ₁₀₀	83 mg/l (96-hours)	X
5.3	Conclusion	All validity criteria can be considered as relatively good fulfilled (see Table A7_4_1_1(01)-8).	X
5.3.1	Other Conclusions	The results of the analytical method validation showed that the method was appropriate for the determination of medetomidine in synthetic test water with zebra fish.	
5.3.2	Reliability	Based on the assessment of materials and methods, the reliability indicator can be 1.	
5.3.3	Deficiencies	No.	

**Doc III A section
7.4.1.1 (01)**

Acute toxicity to fish

Annex Point IIA VII.7.1

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15/7/10
Materials and Methods	Applicants version considered acceptable, noting the following: 3.1 The test material is simply stated to be 'medetomidine' rather than medetomidine hydrochloride; the batch number indicated is correct. 3.4.1 In Table A7_4_1_1(01)-2 the pH of the dilution water is not presented but rather details are presented in the vessels (Table A7-4-1-1(01)-5 as is also the case for the oxygen content which was 8.2 mg/l or higher.
Results and discussion	Applicant's version considered acceptable noting the following: 5.2 The NOEC for fish pigmentation was <0.32 mg a.s./l the lowest concentration tested. 5.2.1-5.2.3 End points based on mean measured concentrations. 5.3 It is noted that the validity criteria for mortality only relates to the control fish and not test fish at the higher concentrations in this LC50 determining test. The fish were larger (2.8 cm) than the 2.0 cm recommended in the protocol.
Conclusion	Applicant's version considered acceptable.
Reliability	1
Acceptability	Acceptable .
Remarks	None.
COMMENTS FROM ...	
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	