Section A 6.4.1.1.b — Subchronic toxicity (rodent)

Annex Point II 6.4 — **Oral, rat, 47 week study**

Official use only

		1 REFERENCE
1.1	**Reference**	Harkins, R.W. & Sarett, H.P. (1968); nutritional evaluation of medium-chain triglyceride in the rat; The Journal of the American oil chemists' society, 1968, Vol. 45; page 26-30; no A6.4.1.1.b/01 and A6.8.2/01.
1.2	**Data protection**	No
1.2.1	Data owner	published
1.2.2		none
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		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**	Medium-chain triglycerides (MCT) containing 51% octanoic acid (C8:0) **35% decanoic acid (C10:0)** 2% (C12:0) 0.9% (16:0)
3.1.1	Lot/Batch number	Not reported
3.1.2	Specification	A detailed analysis of all use materials is reported.
3.1.2.1	Description	Source and nature of the material are described in sufficient detail.
3.1.2.2	Purity	The percentage decanoic acid is analytically determined and can be considered as 100%
3.1.2.3	Stability	Prepared from food grade material.
3.2	**Test Animals**	
3.2.1	Species	rat
3.2.2	Strain	Wistar
3.2.3	Source	Not reported
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	Not reported
3.2.6	Number of animals per group	15 male/15 female per group
3.2.7	Control animals	yes
3.3	**Administration/ Exposure**	Oral

Section A 6.4.1.1.b — Subchronic toxicity (rodent)

Annex Point II 6.4 — **Oral, rat, 47 week study**

3.3.1	Duration of treatment	47 weeks	
3.3.2	Frequency of exposure	7 days per week, ad libitum	
3.3.3	Postexposure period	none	
3.3.4	**Oral**		
3.3.4.1	Type	in food	
3.3.4.2	Concentration	40% of the calories in food from or MCT (active ingredient) plus 2.5% safflower oil to supplement with essential fatty acids 38%of the calories in the food from carbohydrate 22% of the calories in food from protein mineral and vitamin mixture calculated decanoic acid concentration : 5.1 g/kg bw/day	X
3.3.4.3	Vehicle	-	
3.3.4.4	Concentration in vehicle	-	
3.3.4.5	Total volume applied	-	
3.3.4.6	Controls	40% of the calories in food provided by dietary fat consisting of: - oleo oil (plus 2.5% safflower oil per diet to supplement with essential fatty acids) or - butter fat (plus 2.5% safflower oil) or - coconut oil (plus 2.5% safflower oil) or - corn oil or - safflower oil 38%of the calories in the food from carbohydrate 22% of the calories in food from protein mineral and vitamin mixture. The predominant fatty acids in control dietary fats were: Oleo oil – 22.1% C16:0; 18.4% C18:0; 48.2% C18:1; 12.5% C18:2. Butter fat – 22.8% C16:0; 10.5% C18:0; 23.3% C18:1; 18.8% C18:2. Coconut oil – 36.8% C12:0; 17.2% C14:0; 10.0% C16:0; 11.0% C18:2. Corn oil – 13.4% C16:0; 26.2% C18:1; 57.8% C18:2. Safflower oil – 10.0% C18:1; 80.8% C18:2.	X
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	No effects reported	
3.4.1.2	Mortality	Not markedly different in the groups receiving the various fats during the study. On average 2.5 rats died per group of 15 males and 1.7 rats per group of 15 females. In the group receiving MCT (decanoic acid) 3 males and 2 females died during died.	
3.4.2	Body weight	Recorded after 4, 8, 47 weeks of treatment	
3.4.3	Food consumption	Food intake was recorded.	X
3.4.4	Water consumption	Not reported	

Section A 6.4.1.1.b **Subchronic toxicity (rodent)**
Annex Point II 6.4 **Oral, rat, 47 week study**

3.4.5	Ophthalmoscopic examination	Not reported	
3.4.6	Haematology	Not reported	
3.4.7	Clinical Chemisty	yes total cholesterol in blood; phospholipids levels in the liver.	
3.4.8	Urinalysis	Not reported	
3.4.9	Feces	yes all animals collected daily and pooled in weekly samples, samples from week 3, 10, 21, 35 and 47 examined Parameters: analysed for fat, total nitrogen as parameter for protein, calcium	
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	yes organs: liver, kidneys, adrenals, testes, epididymal fat pads, spleen, heart, femur	
3.5.2	Gross and histopathology	yes all dose groups/ high dose group and controls, other dose groups only if effects organs: liver, kidneys, adrenals, testes, epididymal fat pads, spleen, heart, femur Histology: liver, intestines	X
3.5.3	Other examinations	Liver and carcass were analysed for fat and protein and phospholipidlevel in liver fat-content of fat pad was analysed and fatty acids measured by gas chromatography after methylation	
3.5.4	Statistics	Not reported	
3.6	**Further remarks**	none	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Not reported	
4.1.2	Mortality	An average of 2.5 rats died per group of 15 male rats and 1.7 per group of 15 female animals during 47 weeks (mortality was not markedly different in the groups receiving the various fats during the study) In the group receiving MCT 3 male and 2 females died during study	
4.2	**Body weight gain**	Weight gains in animals fed with MCT were only slightly less than with other fats. (less than 10% difference)	
4.3	**Food consumption and compound intake**	Not reported	
4.4	**Ophtalmoscopic examination**	not reported	
4.5	**Blood analysis**		
4.5.1	Haematology	not reported	
4.5.2	Clinical chemistry	Animals consuming MCT had the lowest level of carcass fat. Levels of protein and ash in the carcass were similar with all dietary fats. Fatty acid composition of depot fat was influenced by dietary fat. The high level of C_{12} in coconut oil and $_{C18:2}$ in corn oil and safflower oil	

Section A 6.4.1.1.b	Subchronic toxicity (rodent)
Annex Point II 6.4	Oral, rat, 47 week study

		were reflected in the high level of these fatty acids in the epididymal fat. Lower levels of C_8 and C_{10}, 0.4 and 4.9% respectively were found in the fad pads of the rats fed MCT although these fatty acids comprised about 85% of the dietary fat but 21.9% plamitic acid and 30.8% of oleic acid were found. Total plasma cholesterol level in male rats, fed with MCT diet were lower than in other animals during the study. This was not found in female rats. At the end of study level of cholesterol (animals fasted 18 hours) was lowest in animals fed with corn oil and safflower diet. The highest plasma cholesterol levels were found in animals receiving the coconut oil diet. Total liver lipids and cholesterol levels were lower in male and female on MCT diet than in those receiving the other dietary fats. Phospholipide levels were not affected. The difference between total lipids and the sum of phospholipids and cholesterol presumably represents the triglyceride fraction, which was also lower in the MCT groups than in those on the other diets.	
4.5.3	Urinalysis	not reported	
4.5.4	Faeces	Faecal excretion and dietary intake were used for calculation net absorption of fat, protein and calcium. The net absorption of MCT was higher than that of the other dietary fats; there was little difference in protein or calcium absorption.	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Determined organ weights were similar in all groups. The weight of the epididymal fat pads was 2.2% of the body weight with MCT diet and 2.5 to 3.1% of the body weight in the groups receiving the other dietary fats.	X
4.6.2	Gross and histopathology	Histological examination of liver and intestine show no marked differences among the groups receiving different diets	
4.7	**Other**	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	non-guideline study, A casein diet containing 19.6% MCT and 2.5% safflower oil, the latter to supply essential fatty acids, was compared with similar diets containing conventional dietary fats when given to groups of 15 male and 15 female rats over a period of 47 weeks. Weight gain, fatty acid composition of depot fat and liver, cholesterol and phospholipids in blood and organ weights were determined.	
5.2	**Results and discussion**	Fatty acid composition of depot fat was influenced by dietary fat. The high level of C12 in coconut oil and C18:2 in corn oil and safflower oil were reflected in the high level of these fatty acids in the epididymal fat. Lower levels of C8 and C10, 0.4 and 4.9% respectively were found in the fad pads of the rats fed MCT although these fatty acids comprised about 85% of the dietary fat. High levels of plamitic acid (21.9%) and of oleic acid (30.8%) were in the fat pads of the rats fed MCT although only traces of these fatty acids were in the diet. These data suggest that C8 and C10 are rapidly metabolised to smaller units and little of these are directly incorporate into tissue fat; C16 and C18:2 are the main fatty acids synthesized.	

Section A 6.4.1.1.b **Subchronic toxicity (rodent)**

Annex Point II 6.4 **Oral, rat, 47 week study**

		Total plasma cholesterol level in male rats, fed with MCT diet were lower than in other animals during the study. At the end of study level of cholesterol (animals fasted 18 hours) was lowest in animals fed with corn oil and safflower diet. The differences in findings between earlier and terminal values may have been attributable in part to differences in the conditions under which the blood samples were taken or may reflect changes in age of the animals. They are not considered to be adverse effects of MCT. No clinical signs have been reported therefore it is most likely that no adverse effects could be observed. This is supported by the lack of effects neither in organ weight nor in the histology of liver and intestine among the groups receiving different diets. Weight gains in animals fed with MCT were less than 10% lower than in animals with other fats. And is therefore not called adverse.	
5.3	**Conclusion**	Decanoic acid (35 % in MCT) did not show any adverse effects in rats treated under the described conditions	X
5.3.1	LO(A)EL	-	
5.3.2	NO(A)EL	NOAEL decanoic acid ≥ 5.1 g/kg bw/day	X
5.3.3	Other	Nutritional evaluation study to investigate the effects of MCT (medium-chain triglyceride) when feed to rats including effects reproduction and lactation. No effects related to reproduction were found.	
5.3.4	Reliability	2 This study was performed not according to a guideline study for regulatory purposes. Nevertheless the goal of the study to evaluate the nutritional properties of medium-chain triglycerides (MCT) including any effects on the normal growth or development of offspring make this study suitable to judge the possible effects of decanoic acid during a multigeneration exposure.	
5.3.5	Deficiencies	-	

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	

Section A 6.4.1.1.b Subchronic toxicity (rodent)

Annex Point II 6.4 **Oral, rat, 47 week study**

Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6_4.1.1.-1. Plasma cholesterol levels in rats fed various dietary fats

	weeks				
	7	14	21	35	47
males					
MCT	84	85	92	99	100
Oleo oil	105	110	116	117	86
Butter fat	110	108	123	126	92
Coconut oil	112	115	128	135	113
Corn oil	110	104	118	115	81
Safflower oil	100	97	109	105	82
Females					
MCT	109	107	119	126	124
Oleo oil	106	104	107	116	102
Butter fat	110	108	125	122	126
Coconut oil	124	125	142	148	125
Corn oil	96	96	103	112	93
Safflower oil	88	83	101	107	90

Composition of diet and dietary fat:

Table 1 of publication:

TABLE I

Composition of Diets

	Diet 1–6 40% Fat en ovrlop	Diet 7 Low fat
	%	%
Fat[a]	21.0	2.5
Casein (ANRC 91.4% protein)	26.2	26.2
Amidex[b]	44.5	63.9
Nonnutritive fiber	4.0	4.0
Mineral mixture[c]	4.0	4.0
Vitamin mixture[d]	0.35	0.35

[a] Diets 1–4 contained mainly MCT, oleo oil, butterfat, and coconut oil respectively, with 2.5% safflower oil added to insure adequate essential fatty acids. (The level of the fat in the MCT diet was increased slightly since MCT provides only 8.4 cal/g.) Diets 5 and 6 contained corn oil and safflower oil respectively.

[b] Partially hydrolyzed corn starch, Corn Products Company, New York.

[c] J. H. Jones and C. Foster (J. Nutr. 24, 245, 1942) with 10 ppm F added as NaF.

[d] H. P. Sarett and L. P. Shipper (J. Nutr. 72, 326, 1954). Ascorbic acid omitted. In addition, 0.015 g Cosm. Fecomorphum and 0.015 g dl-α-tocopheryl acetate were added per 100-g diet.

Table 4 of publication:

	Fatty acids, %											
	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	Other
Dietary Fat												
MCT[a]	51.0	35.0	2.0		0.9			1.4	9.0			0.7
Oleo oil[a]				2.9	22.1	4.8	13.4	43.2	12.5			1.1
Butter fat[a]	1.9	3.3	2.9	8.1	22.8	3.8	10.5	29.3	13.3			10.1
Coconut oil[a]	8.1	7.2	36.8	17.2	10.0		2.4	7.2	11.0			0.1
Corn oil					13.4		1.4	26.2	57.8			1.2
Safflower oil					6.7		1.9	10.0	80.8	0.2		0.4

Section A 6.4.1.2. Subchronic toxicity (non-rodent)

Annex Point IIA6.4. oral

Justification for non-submission of data			Official use only
Other existing data [ ]	Technically not feasible [ ]	Scientifically unjustified [x]	
Limited exposure [x]	Other justification [ ]		
Detailed justification:	[redacted]		X

Undertaking of intended data submission [ ]

Evaluation by Competent Authorities

Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.4.2.1. Subchronic toxicity (rodent)

Annex Point IIA6.4. Dermal

Official use only

Justification for non-submission of data

Other existing data [ ] Technically not feasible [ ] Scientifically unjustified [x]

Limited exposure [ ] Other justification [ ]

Detailed justification: [redacted]

Undertaking of intended data submission [ ]

Evaluation by Competent Authorities

Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6. 4.3.1. Subchronic toxicity (rodent)

Annex Point IIA6.4. Inhalation

Justification for non-submission of data			Official use only
Other existing data [ ]	Technically not feasible [ ]	Scientifically unjustified [x]	
Limited exposure […]	Other justification [ ]		
Detailed justification:	[redacted]		
Undertaking of intended data submission [ ]			

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.5 Chronic toxicity, rodent

Annex Point II 6.5

Justification for non-submission of data			Official use only
Other existing data [ ]	Technically not feasible [ ]	Scientifically unjustified [x]	
Limited exposure […]	Other justification [ ]		
Detailed justification:	[redacted]		X X X

Section A 6.5 Chronic toxicity, rodent

Annex Point II 6.5

[redacted] X

X

Undertaking of intended data submission [ ]

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A 6.5 Chronic toxicity, rodent

Annex Point II 6.5

Official use only

Justification for non-submission of data

Other existing data [X] Technically not feasible [ ] Scientifically unjustified [X]

Limited exposure […] Other justification [ ]

Detailed justification:

[redacted]

Undertaking of intended data submission [ ]

Evaluation by Competent Authorities	
	[redacted]
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 **Gene mutation in bacteria**
Ames test (+/- S9) using *S. typhimurium* and *E.coli*

			Official use only
		1 REFERENCE	
1.1	**Reference**	Van Ommen, B. (1999) Bacterial reverse mutation test with decanoic acid Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.668 Ref nr A6.6.1/01	
1.2	**Data protection**	Yes	
1.2.1	Data owner	S.A. Sopura	
1.2.2			
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	2000/32/EC B.13/14, OECD 471	
2.2	**GLP**	Yes	
2.3	**Deviations**	As positive control for WP2 uvrA without S9 N-ethyl-N-nitrosourea (100 µg/plate) was used in contrast to the GL recommendations. This is considered to be of no relevance for the integrity and validity of the test	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Decanoic acid	
3.1.1	Lot/Batch number	Product code: 802169	
3.1.2	Specification	Not reported	
3.1.2.1	Description	Coulourless cristals	
3.1.2.2	Purity	Not reported	
3.1.2.3	Stability	Not reported	
3.2	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100 E. coli: WP2 uvrA	
3.2.2	Deficiencies / Proficiencies	Histidine deficient S. typhimurium Tryptophan deficient E.coli	
3.2.3	Metabolic activation system	S9 mix prepared from livers of male Wistar rats induced with Arochlor 1254	
3.2.4	Positive control	<u>In absence of S9:</u> Sodium acide at 1.0 µg/plate for TA 1535 and TA100 9-Aminoacridine at 80 µg/plate for TA 1537 2-Nitrofluorene at 2 µg/plate for TA 98 N-ethyl-N-nitrosourea at 100 µg/plate for WP2 *uvrA* <u>In presence of S9:</u>	X

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 **Gene mutation in bacteria**
Ames test (+/- S9) using *S. typhimurium* and *E.coli*

		2-Aminoantracene at 2 µg/plate for TA 1535, TA 98, TA 100, 80 µg/plate for WP2 *uvrA* Benzo(a)pyrene at 4 µg/plate for TA 1537
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Mutagenicity tests: Test 1:0, 62, 185, 556, 1667, 5000 µg/plate (+/- S9) Test 2: 0, 94, 188, 375, 750, 1500 µg/plate (+/- S9)
3.3.2	Way of application	Stock solutions: Decanoic acid was dissolved in DMSO. The bacterial suspensions (0.1 mL) were mixed with soft agar (2.0 mL, supplemented with l-histidine and trypophane respectively), 0.1 mL of Decanoic acid stock solutions or vehicle control, 0.5 mL S9 mix (for experiments in presence of metabolic activation) or 0.5 ml sodium phosphate 100 mM (for experiments in absence of metabolic activation) before being pured onto minimal agar plates. The plates were then incubated for 3 days at 37°C. All determinations were made in triplicates.
3.3.3	Pre-incubation time	None
3.3.4	Other modifications	-
3.4	**Examinations**	Mutagenicity: frequency of revertant colonies Criteria for a positive response: The test was considered to be negative if the colony count in relation to the negative (vehicle) control was not doubled at any concentration. The test was considered to be mutagenic if a concentration-related increase or if a positive response reproducible in two independent assays is observed. Cytotoxicity was defined as a reduction in the number of revertant colonies and/or clearing of the background lawn of bacterial growth.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	In the concentrations of 1666 and 5000 µg/plate, the test substance precipitated. Therefore the concentrations have not been included in the evaluation of the results. None of the observed results fulfilled the criteria of a positive response (see Table A6.6.1/01-1). Positive control compounds gave a clear positive result.
4.1.2	with metabolic activation	In the concentrations of 1666 and 5000 µg/plate, the test substance precipitated. Therefore the concentrations have not been included in the evaluation of the results. None of the observed results fulfilled the criteria of a positive response (see Table A6.6.1/01-1). Positive control compounds gave a clear positive result.

Section A6.6.1		Genotoxicity in vitro
Annex Point IIA6.6.1		**Gene mutation in bacteria** Ames test (+/- S9) using *S. typhimurium* and *E.coli*
4.2	**Cytotoxicity**	Slightly reduced growth (reduced number of revertant colonies) at 1666 and 5000 µg/plate for strain TA 100 in absence and presence of S9.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Evaluation of the in vitro gene mutation potential in S. typhimurium strains and E. coli; no relevant deviation from guidelines (2000/32/EC B.13/14, OECD 471)
5.2	**Results and discussion**	There were no relevant effects on the number of revertant colonies of decanoic acid in any strain at any concentration. Only slightly reduced growth at 1666 and 5000 µg/plate for TA 100 in absence and presence of S9 were reported.
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6_6_1-1. Table of bacterial reverse mutation assay; mutagenicity test with decanoic acid

Test 1

concen-tration [µg/plate]	Number of mutant cells/strain (mean)									
	TA 1535		TA 1537		TA 98		TA 100		E.coli	
	— S9	+ S9	— S9	+ S9	— S9	+ S9	— S9	+ S9	— S9	+ S9
0	19	16	12	24	41	61	157	156	26	23
62	24	14	21	24	45	57	144	156	29	24
185	17	18	15	16	35	52	140	154	24	21
556	11	15	5	13	30	47	105	139	22	20
Positive control	461	485	1261	307	787	884	651	1849	442	1175

Test 2

concen-tration [µg/plate]	Number of mutant cells/strain (mean)									
	TA 1535		TA 1537		TA 98		TA 100		E.coli	
	— S9	+ S9	— S9	+ S9	— S9	+ S9	— S9	+ S9	— S9	+ S9
0	21	17	11	14	39	60	149	148	30	29
94	21	18	13	10	50	66	154	147	26	32
188	20	19	5	10	34	47	128	132	26	30
375	13	12	9	9	27	43	143	144	21	28
750	13	7	4	7	22	24	91	123	20	21
1500	12	19	2	2	17	29	71	95	11	14
Positive control	410	459	475	214	515	281	509	1369	242	1058

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 **Gene mutation in bacteria**
Ames test (+/- S9) using *S. typhimurium* and *E.coli*

[redacted]

Official use only

1.1 Reference

1.2 Data protection

1.2.1 Data owner

1.2.2

1.2.3 Criteria for data protection

2.1 Guideline study

2.2 GLP

2.3 Deviations

3.1 Test material

3.1.1 Lot/Batch number

3.1.2 Specification

3.1.2.1 Description

3.1.2.2 Purity

3.1.2.3 Stability

3.2 Study Type

3.2.1 Organism/cell type

3.2.2 Deficiencies / Proficiencies

3.2.3 Metabolic activation system

3.2.4 Positive control

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 **Gene mutation in bacteria**
Ames test (+/- S9) using *S. typhimurium* and *E.coli*

[redacted]

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

Section A6.6.1 **Genotoxicity in vitro**

Annex Point IIA6.6.1 **Gene mutation in bacteria**
Ames test (+/- S9) using *S. typhimurium* and *E.coli*

5.1	**Materials and methods**	[redacted]
5.2	**Results and discussion**	
5.3	**Conclusion**	
5.3.1	Reliability	
5.3.2	Deficiencies	

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[redacted]

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

Official use only

		1 REFERENCE
1.1	**Reference**	De Vogel, N. (1999); Chromosomal aberration test with decanoic acid in cultured Chinese hamster ovary cells Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.661 Ref nr A6.6.2/01
1.2	**Data protection**	Yes
1.2.1	Data owner	S.A. Sopura, Courcelles, Belgium
1.2.2		
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	2000/32/EC B.10, OECD 473
2.2	**GLP**	Yes
2.3	**Deviations**	none
		3 MATERIALS AND METHODS
3.1	**Test material**	Decanoic acid
3.1.1	Lot/Batch number	Product code 802169
3.1.2	Specification	Not reported
3.1.2.1	Description	colourless cristals
3.1.2.2	Purity	Not reported
3.1.2.3	Stability	Not reported
3.2	**Study Type**	In Vitro mammalian chromosome aberration test
3.2.1	Organism/cell type	Chinese hamster Ovary cells(CHO K-1 line)
3.2.2	Deficiencies / Proficiencies	-
3.2.3	Metabolic activation system	S9 mix prepared from livers of male Wistar rats induced with Arochlor 1254 prior to sacrifice;
3.2.4	Positive control	Test 1: -S9: 0.1 µg/mL mitomycin C +S9: 3.75 µg/mL cyclophosphamide Test 2: -S9: 0.025 µg/mL mitomycin C +S9: 2 µg/mL cyclophosphamide
3.3	**Administration / Exposure; Application of test**	

Section A6.6.2 **Genotoxicity in vitro**

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

	substance		
3.3.1	Concentrations	Chromosome aberration test: Test 1: - S9-mix: 0, 50, 100, 200, 300, 400 and 500 µg/mL (4 hours treatment/18 hours fixation) + S9-mix: 0, 50, 100, 200, 300, 400 and 500 µg/mL (4 hours treatment/18 hours fixation) Test 2: - S9-mix: 0, 5, 10, 25, 50, 75, and 100 µg/mL (18 hours treatment/18 hours fixation) + S9-mix: 0, 50, 100, 200, 300, 350 and 400 µg/mL (4 hours treatment/32 hours fixation)	
3.3.2	Way of application	Decanoic acid was dissolved in DMSO (500 mg/mL); for treatment: 1% DMSO solution in cell culture medium. All cultures were incubated at 37°C. Test 1: Cell cultures were exposed to decanoic acid according to the concentration given in 3.3.1. In both the absence and presence of S9-mix the treatment time was 4 hours and fixation time was 18 hours after onset of treatment. Test 2: Cell cultures were exposed to decanoic acid according to the concentration given in 3.3.1. . In the absence of S9-mix the cells were harvested after a treatment period of 18 hours. In the presence of S9-mix the cells were treated for 4 hours and harvested 32 hours after onset of the treatment. Two hours before harvest mitosis was arrested by addition of colcemid (final concentration 0.1 mM medium). After hypotonic treatment, fixation and staining 200 metaphases (from two cultures per concentration) were counted for aberrations. In addition at least 1000 cells were evaluated to determine the mitotic index. The highest concentration for metaphase evaluation should suppress the mitotic activity by about 50-70% compared to controls.	
3.3.3	Pre-incubation time	1 day	
3.3.4	Other modifications	-	
3.4	**Examinations**	Mitotic index: number of metaphases in a total of at least 1000 cells Aberrations test: 200 well-spread metaphases per concentration of the test substance and of the negative and positive control were analysed. Metaphases with specific aberrations (breaks, exchanges, deletions, fragments, minutes), unspecific aberrations (gaps, premature chromosome condensation, chromosome decay) and numerical aberrations (metaphases with >21 chromsomes) Criteria of a positive result: - if the number of specific chromosomal aberrations is markedly increased in comparison with controls - or if an increased number of exchange figures appears together with a high number of specific chromosomal aberrations like breaks and fragments. In addition a dose-related response in the number of aberrations should	X

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

		be demonstrable.
3.4.1	Number of cells evaluated	Where possible for negative controls and test substance concentrations: totally 200 metaphases per concentration (50 metaphases per slide)

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	See Table 6.6.2.1 There was no biological relevant and statistically significant increase in metaphases with specific chromosomal aberrations at any concentration. The positive control fulfilled the criteria of a positive response (markedly increased metaphases with specific aberrations).
4.1.2	with metabolic activation	See Table 6.6.2.1 There was no biological relevant and statistically significant increase in metaphases with specific chromosomal aberrations at any concentration. The positive control fulfilled the criteria of a positive response (markedly increased metaphases with specific aberrations).
4.2	**Cytotoxicity**	The highest and lowest concentrations respectively selected of the original study were scored by the mitotic index Test 1: 200 µg/mL +S9 (mitotic index 48% of control) 50 µg/mL +S9 (mitotic index 80% of control) 300 µg/mL -S9 (mitotic index 48% of control) 100 µg/mL -S9 (mitotic index 98% of control) Test 2: 350 µg/mL +S9 (mitotic index 50% of control) 200 µg/mL +S9 (mitotic index 80% of control) 50 µg/mL -S9 (mitotic index 47% of control) 10 µg/mL -S9 (mitotic index 82% of control)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Evaluation of the in vitro cytogenetic potential in mammalian cells (Chinese hamster ovary cells); no relevant deviation from guidelines (2000/32/EC B10, OECD 473)
5.2	**Results and discussion**	There were no relevant increases in the number of metaphases with specific aberrations at any decanoic acid concentration in presence or in absence of S9.
5.3	**Conclusion**	Treatment of Chinese hamster cells with decanoic acid had no effect on chromosome aberrations in presence or in absence of metabolic activation. It is concluded that decanoic acid was not clastogenic under the conditions used in this study
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

Table 6.6.2.1. Table for Cytogenetic In-Vitro-Test: Chromosomal aberration study

Concentration [µg/mL]	Treatment	Fixation	Mitotic index (1000 cells scored)		% Metaphases with aberrations	
			%	% control	~~specific~~ structural	~~unspecific~~ numerical
Test 1						
0	4 h	18 h	8.5	100	0.25	0
50	-S9		7.95	94	-	-
100			8.35	98	0	0
200			6.7	79	0.5	0
300			4.1	48	0.25	0
400			2.5	29	-	-
500			0.75	9	-	-
mitomycin			5.7	67	39.5	0
0	4 h	18 h	7.95	100	1	0.5
50	+S9		6.35	80	2.5	0
100			3.9	49	2	0.5
200			3.85	48	0	0
300			2.85	36	-	-
400			1.45	18	-	-
Cyclophosphamide			3.75	47	53.5	0
Test 2						
0	18 h	18 h	7.4	100	0	0
5	-S9		6.9	92	-	-
10			6.15	82	0	0
25			5.2	69	0	0
50			3.55	47	0	0
75			3.15	42	-	-
100			1.65	22	-	-
mytomycin			4.5	60	13.5	0
0	4 h	32 h	8.25	100	0	0.5
50	+S9		7.5	91	-	-
100			6.5	79	-	-
200			6.6	80	0	0
300			4.9	59	0	0
350			4.15	50	0.5	0.5
400			2.25	27	-	-
cyclophosphamide			6.4	78	20.5	0

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

		[redacted]	Official use only
1.1	**Reference**		
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2			
1.2.3	Criteria for data protection		
2.1	**Guideline study**		
2.2	**GLP**		
2.3	**Deviations**		
3.1	**Test material**		
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.2.1	Description		
3.1.2.2	Purity		
3.1.2.3	Stability		
3.2	**Study Type**		
3.2.1	Organism/cell type		
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system		
3.2.4	Positive control		
3.3	**Administration / Exposure; Application of test**		

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

	substance	[redacted]
3.3.1	Concentrations	
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	X

Section A6.6.2 Genotoxicity in vitro

Annex Point II6.6.2 **Cytogenicity in mammalian cells**
Chromosome aberration study in Chinese hamster ovary cells

3.4.1	Number of cells evaluated	[redacted]
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	
5.1	**Materials and methods**	
5.2	**Results and discussion**	
5.3	**Conclusion**	
5.3.1	Reliability	
5.3.2	Deficiencies	

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

[redacted]

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

Official use only

		1 REFERENCE
1.1	**Reference**	Steenwinkel, M.J. S.T. (1999); Gene mutation test at the TK-locus of L5178Y cells with Decanoic acid; Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.715 Ref nr A6.6.3/01
1.2	**Data protection**	Yes
1.2.1	Data owner	S.A. Sopura, Courcelles, Belgium
1.2.2		
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	2000/32/EC B.17 OECD 476
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	Decanoic acid
3.1.1	Lot/Batch number	Product code 802169
3.1.2	Specification	Not reported
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	Not reported
3.1.2.3	Stability	Not reported
3.2	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	Mouse lymphoma L5178Y cells
3.2.2	Deficiencies / Proficiencies	Thymidine kinase deficiency
3.2.3	Metabolic activation system	S9 mix prepared from livers of male Wistar rats induced with Arochlor 1254 prior to sacrifice
3.2.4	Positive control	-S9: Methylmethanesulphonate (MMS) (Test 1: 0.10 µL/mL, test 2: 0.20 µL/mL) +S9: 10 µL/mL 3-Methylcholanthrene (MCA) (test1 and test 2)
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Mutagenicity test (1st and 2nd experiment): Test 1: -S9-mix: 0, 0.2. 0.28, 0.4, 0.58, 0.82, 1.2, 1.7, 2.4, 3.4, 4.9, 7.0, 10 mM +S9-mix: 0, 0.24, 0.34,0.48, 0.69, 0.96, 1.4, 1.7, 2.4, 3.4, 4.9, 7.0,

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

		10 mM Test 2: -S9-mix: 0, 0.42, 0.6, 0.86, 1.2, 1.5, 1.9, 2.1, 2.4, 2.5, 2.6, 2.8, 2.9, 3.1, 3.2, 3.4 mM +S9-mix: 0, 0.44, 0.63, 0.9, 1.1, 1.4, 1.8, 2.0, 2.2, 2.4 mM.	
3.3.2	Way of application	Decanoic acid was dissolved in DMSO. From this stock solution serial dilutions in DMSO were prepared and from each of this dilutions 100 (120) µL were added to a final volume of 10 mL growth medium. $5 \cdot 10^6$ cells were incubated with decanoic acid in 10 mL growth medium for 4.5 hours in the 1st test and 4 hours in the 2nd test without S9-mix. In the assay with S9-mix $5 \cdot 10^6$ cells were incubated with decanoic acid in 10 mL growth medium for 4 hours. <u>Cytotoxicity test</u> The cytotoxicity of the test substance was determined by counting the cells after exposure and by measuring the relative suspension growth (RSG) and the relative total growth (RTG). <u>Gene mutation assay</u> After washing the cells were resuspended at a density of $0.2 \cdot 10^6$ cells/mL in growth medium and incubated for about 44-48 hours – expression period. For determining the frequency of TFT-resistant mutants 200 µL of each dilution at 10.000 cells/mL were transferred to each well of 96-well plates and 10-14 days incubated to determine the cloning efficiency. The TK mutant frequency per 10^6 clonable cells were calculated.	X
3.3.3	Pre-incubation time	Treatment time: 4 and 4.5 hours in presence and absence of S9, respectively Expression period: 2 days	
3.3.4	Other modifications	-	
3.4	**Examinations**	See also 3.3.2 The cytotoxicity was determined by counting the cells after exposure and by measuring the relative suspension growth (RSG) and the relative total growth (RTG). Reduction of cell count after treatment or of the RSG and the RTG is a measure for cytotocicity of the test substance. Mutagenicity: The average mutant frequency of the negative controls should fall within the range of 40-300 TFT-resistant mutants per 10^6 clonabel cells The average cloning efficiency of the negative controls should not be less than 60% or more than 140%. The mutants frequency of the positive controls should be higher than 400 TFT-resistant mutants per 10^6 clonable cells, and should at least be 2-fold higher than the corresponding negative control. unless the material to be tested shows no cytotoxicity at the highest possible concentration, the highest test substance concentration should result in a clear cytotoxic response. A response is considered to be positive if the induced mutant frequency is more than 100 mutants per 10^6 clonable cells.	X
3.4.1	Number of cells evaluated	The TK mutant frequency per 10^6 clonable cells were calculated.	

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	See Table 6.1.3
4.1.1	without metabolic activation	There was no relevant increase in the mutant frequencies of decanoic acid-treated cultures. In contrast the positive controls clearly fulfilled the criteria for a positive response.
4.1.2	with metabolic activation	In one of the assays a positive response was observed at a concentration of 2.2 mM decanoic acid. Since this is a single response without any dose related effect, this finding is considered fortuitous and not as indication of mutagenic activity of the test substance.
4.2	**Cytotoxicity**	In absence as well as in presence of S9-mix decanoic acid was toxic to the cells resulting in a dose related decrease of RSG and RGT. In absence of S9-mix degrease of RTG was observed above a concentration of 1.5 mM decanoic acid and at 3.3 mM for RTG (33%). In presence of S9-mix the RTG was decreased above a concentration of 0.9 mM decanoic acid. At the highest dose of 2.4 mm the RTG was 12% in the 1st assay and 27% in the 2nd assay.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In vitro evaluation of gene mutation in mammalian cells; no relevant deviation from test guidelines (2000/32/EC B.17, OECD 476)
5.2	**Results and discussion**	No mutagenic response after treatment of mouse lymphoma L5178Y cells with decanoic acid was detected in absence of S9. In presence of S9 a single response occurred at 2.2 mM decanoic acid. His result is not considered to be relevant since the effect was not dose related effect and appeared in only one of the two tests. In absence as well as in presence of S9-mix decanoic acid was toxic to the cells resulting in a dose related decrease of RSG and RGT.
5.3	**Conclusion**	Decanoic acid and/or metabolites are not mutagenic in mouse lymphoma L5178Y under this test conditions.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3

Gene mutation in mammalian cells
In vitro gene mutation in Mouse lymphoma L5178Y cells

Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

Table A6.6.3 Table for mammalian cell Gene Mutation Assay (mutagenicity experiments)

Decanoic acid (mM)	Relative suspension growth [%]	Relative colning efficacy [%]	Mutant cloning efficiency [mutants/10^6 viable cells]	Mutation frequency ~~[mutants/10^6 viable cells]~~ Mutant cloning efficiency/absolute final cloning efficiency
1st experiment, - S9				
0 °	100	100	83	101
0.2	95	107	121	137
0.28	98	88	44	60
0.4	93	100	79	96
0.58	77	107	70	79
0.82	121	134	88	80
1.2	104	103	91	107
1.7	63	132	70	64
2.4	33	124	110	108
Positive control	101	71	457	786
1st experiment, + S9				
0°	100	100	76	82
0.2	107	75	58	84
0.28	111	108	91	91
0.4	106	104	73	76
0.58	100	90	101	120
0.82	103	90	97	116
1.2	82	79	94	128
1.7	67	89	85	103
2.4	17	70	101	155
Positive control	72	68	450	710
2nd experiment, -S9				
0°	100	100	67	80
0.6	95	97	64	79
0.86	97	103	76	88
1.2	96	107	110	123
1.5	90	111	79	85
1.9	76	97	82	101
2.4	75	91	82	108
2.6	46	91	58	76
2.9	43	103	67	77
3.1	35	101	79	93
3.3	33	111	140	151
Positive control	62	40	413	1229
2nd experiment, +S9				
0°	100	100	97.5	104
0.44	95	106	82	82
0.63	93	68	101	156
0.9	91	125	120	101
1.1	86	95	58	65
1.4	73	80	140	185
1.8	53	84	110	138
1.9	68	64	117	192
2.2	40	71	144	214
2.4	29	91	149	174
Positive control	59	61	664	1107

° mean of negative control

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

		[redacted]	Official use only
1.1	**Reference**		
1.2	**Data protection**		
1.2.1	Data owner		
1.2.2			
1.2.3	Criteria for data protection		
2.1	**Guideline study**		
2.2	**GLP**		
2.3	**Deviations**		
3.1	**Test material**		
3.1.1	Lot/Batch number		
3.1.2	Specification		
3.1.2.1	Description		
3.1.2.2	Purity		
3.1.2.3	Stability		
3.2	**Study Type**		
3.2.1	Organism/cell type		
3.2.2	Deficiencies / Proficiencies		
3.2.3	Metabolic activation system		
3.2.4	Positive control		
3.3	**Administration / Exposure; Application of test substance**		

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

3.3.1	Concentrations	[redacted]
3.3.2	Way of application	X
3.3.3	Incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	X

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

4.1	**Genotoxicity**	[redacted]	
4.1.1	without metabolic activation		
4.1.2	with metabolic activation		X
4.2	**Cytotoxicity**		
5.1	**Materials and methods**		
5.2	**Results and discussion**		
5.3	**Conclusion**		
5.3.1	Reliability		
5.3.2	Deficiencies		

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	

Section A6.6.3 Genotoxicity in vitro

Annex Point II6.6.3 **Gene mutation in mammalian cells**
In vitro gene mutation in Mouse lymphoma L5178Y cells

Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	

[redacted]

[redacted]

Section A6.8.1. Teratogenicity Study

Annex Point IIA6.8.1

Official use only

Other existing data [X]

Limited exposure […]

Detailed justification:

[redacted]

X

X

X

Section A6.8.1. Teratogenicity Study

Annex Point IIA6.8.1

[redacted]		X
		X
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	

Section A6.8.1. Teratogenicity Study

Annex Point IIA6.8.1

	[redacted]
Conclusion	
Remarks	

QSAR Prediction Reporting Format (QPRF)
(version 1.1, May 2008)

Please fill in the fields of the QPRF with information about the prediction and the substance for which the prediction is made. The information that you provide will be used to facilitate considerations on the adequacy of the prediction (model result) in relation to a defined regulatory purpose.

The adequacy of a prediction depends on the following conditions: a) **the (Q)SAR model is scientifically valid**: the scientific validity is established according to the OECD principles for (Q)SAR validation; b) **the (Q)SAR model is applicable to the query chemical**: a (Q)SAR is applicable if the query chemical falls within the defined applicability domain of the model; c) **the (Q)SAR result is reliable**: a valid (Q)SAR that is applied to a chemical falling within its applicability domain provides a reliable result; d) **the (Q)SAR model is relevant for the regulatory purpose**: the predicted endpoint can be used directly or following an extrapolation, possibly in combination with other information, for a particular regulatory purpose.

A (Q)SAR prediction (model result) may be considered adequate if it is reliable and relevant, and depending on the totality of information available in a weight-of-evidence assessment (see Section 4 of the QPRF).

1. Substance

This section is aimed at defining the substance for which the (Q)SAR prediction is made.

1.1 CAS number: 334-48-5.

1.2 EC number: 206-376-4

1.3 Chemical name: n-decanoic acid *(IUPAC; xxx (CAS)*

1.4 Structural formula: $C_{10}H_{20}O_2$

1.5 Structure codes: *Report available structural information for the substance, including the structure code used to run the model. If you used a SMILES or InChI code, report the code in the corresponding field below. If you have used any another format (e.g. mol file), please include the corresponding structural representation as supporting information.*

- **a. SMILES**: CCCCCCCCCC(=O)O (used for the model prediction).
- **b. InChI:** 1/C10H20O2/c1-2-3-4-5-6-7-8-9-10(11)12/h2-9H2,1H3,(H,11,12) *(not used for the model prediction).*
- **c. Other structural representation**: no
- **d. Stereochemical features:** Substance is not a stereo-isomer.

2. General information

General information about the compilation of the current QPRF is provided in this section.

2.1 Date of QPRF: *2009-08-10*

2.2 QPRF author and contact details:

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Biozide
Biocides
T: +43-(0)1-313 04/3407
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martin.paparella@umweltbundesamt.at

3. Prediction

The information provided in this section will help to facilitate considerations on the scientific validity of the model (as defined in the OECD Principles for the validation of (Q)SAR models) and the reliability of the prediction. Detailed information on the model are stored in the corresponding QMRF which is devised to reflect as much as possible the OECD principles. Remember that the QMRF and the QPRF are complementary, and a QPRF should always be associated with a defined QMRF.

3.1 Endpoint (OECD Principle 1)

a. Endpoint:

Developmental Toxicant: Chemical compounds were categorized into toxicant or non toxicant according to FDA risk factors.

FDA classes	Definition	CAESAR Binary class
Category A	Negative human studies	Non developmental toxicant
Category B	Negative animal studies & No human studies executed OR Positive animal studies & Negative human studies	
Category C	Positive animal studies & No human studies executed OR No studies at all	Developmental toxicant
Category D	Positive human studies	
Category X	Animal OR human studies show abnormalities AND/OR Evidence of foetal risk based on human experience	

b. Dependent variable: *Not applicable, complex endpoint depending on experts interpretation of several variables like prä-, peri- and postnatal lethality, weight gain, functional deficits, rate of malformations, variations, retardations, maternal toxicity, epidemiological evidence.*

3.2 Algorithm (OECD Principle 2)

a. Model or submodel name: *Identify the model used to make the prediction and possibly report its name as stored in the corresponding QMRF; in the QMRF the model name is reported in the field QSAR identifier. Examples: "BIOWIN for Biodegradation"; "TOPKAT Developmental Toxicity Potential". If applicable identify the specific submodel or algorithm applicable to the specific chemical Examples: "BIOWIN 1"; TOPKAT Skin Irritation Acyclics (Acids, Amines, Esters) MOD v SEV Model"; "ECOSAR esters model".*

b. Model version: *Identify, where relevant, the version number and/or date of the model and submodel.*

c. Reference to QMRF: *Provide relevant information about the QMRF that stores information about the model used to make the prediction. Possible useful pieces of information are: availability, source, reference number (if any) of the QMRF. Examples: "The corresponding QMRF named 'BIOWIN for Biodegradation' has been downloaded from the JRC QSAR Model Database"; "The corresponding QMRF named 'TOPKAT Skin Irritation Acyclics (Acids, Amines, Esters) MOD v SEV Model' has been newly compiled".*

d. Predicted value (model result): *DEVELOPMENTAL NON TOXICANT*

e. Predicted value (comments): *qualitative result, two categories: dev.tox or not dev.tox. for definition see 3.1.*

f. Input for prediction: *SMILES, see 1.5.*

g. Descriptor values: *Where appropriate, report the values (experimental or calculated data) for numerical descriptors and indicate which values were used for making the prediction.*

3.3 Applicability domain (OECD principle 3)

a. Domains: *Discuss whether the query chemical falls in the applicability domain of the model as defined in the corresponding QMRF (section 5 of QMRF, Defining the applicability domain – OECD Principle 3). If additional software/methods were used to assess the applicability domain then they should also be documented in this section. Include a discussion about:*

- **i.** *descriptor domain*
- **ii.** *structural fragment domain (e.g., discuss whether the chemical contains fragments that are not represented in the model training set)*
- **iii.** *mechanism domain (discuss whether the chemical is known or considered to act according to the mechanism of action associated with the used model)*
- **iv.** *metabolic domain, if relevant*

b. Structural analogues: *List the structural analogues that are present in the training or test sets, or accessible from other sources (in this case you should*

explain how the structural analogue was retrieved[1]) and why they are considered analogues). For each analogue, report the CAS number, the structural formula, the SMILES code, and the source (e.g., training set, test set or other source). For an expert system (like Derek for Windows or TOPKAT), the example compounds or structurally related analogues with their experimental data should be provided here.

c. Considerations on structural analogues: *Discuss how predicted and experimental data for analogues support the prediction of the chemical under consideration.*

d.

3.4 The uncertainty of the prediction (OECD principle 4)

If possible, comment on the uncertainty of the prediction for this chemical, taking into account relevant information (e.g. variability of the experimental results).

3.5 The chemical and biological mechanisms according to the model underpinning the predicted result (OECD principle 5).

Discuss the mechanistic interpretation of the model prediction for this specific chemical. For an expert system based on structural alerts (e.g. Derek for Windows, Oncologic™) the rationale for the structural alert fired should be provided.

4. Adequacy (Optional)

The information provided in this section might be useful, depending on the reporting needs and formats of the regulatory framework of interest.

This information aims to facilitate considerations about the adequacy of the (Q)SAR prediction (result) estimate. A (Q)SAR prediction may or may not be considered adequate ("fit-for-purpose"), depending on whether the prediction is sufficiently reliable and relevant in relation to the particular regulatory purpose. The adequacy of the prediction also depends on the availability of other information, and is determined in a weight-of-evidence assessment.

4.1 Regulatory purpose: *Biocidal Products Directive - evaluation of active substance for Annex inclusion; QSAR as additional support to old publications*

4.2 Approach for regulatory interpretation of the model result:

Model result integrated into total weight of evidence evaluation; No developmental toxicity concern supported by following arguments:

- The detailed knowledge of the metabolic pathways that are similar for all fatty acids: complete catabolism for energy supply or conversion to fat suitable for storage (see chapter 3.1.1).
- The lack of toxicologically relevant effects also at the very high doses in the available oral repeated dose studies
- The results from the acute mammalian toxicology studies, indicating only concern for skin and eye irritation
- The absence of effects in the three standard in vitro genotoxicity tests (see chapter 3.6. below)

[1] Various software tools (e.g. the OECD QSAR Toolbox) could be used to support the search of analogues.

- The nature of Decanoic acid, that is a linear saturated fatty acid and the ubiquity of Decanoic acid and other similar fatty acids in nature: Decanoic acid is naturally present in many types of food in its free form or as triglyceride (see Gubler 2006, Ref A 6/05). Short term uptake as natural food source from cheese or coconut oil may be estimated to be above 10 mg/ person day (=estimation from average Swiss cheese consumption; 178 mg/person day = estimation from average coconut oil consumption; up to 2000 mg/person day = estimation from 100 g sheep cheese; see Document III-A 6.5). The lowest estimate is in the range of the proposed AEL.
- Scott et al. 1994 (A6.8.1/01 in reference list) reports that Octanoic acid was applied as single dose of 3228 mg/kg bw on day 12 of gestation, rats were killed and analysed on day 20 of gestation. No teratogenic effects were reported. The difference between octanoic acid and teratogenic valporic acid (= 2-propyl pentanoic acid) is explained to be related to the plasma level and half live that are magnitudes lower for octantanoic acid.
- Mei-Jen Liu and Gary M. Pollack 1993 (A6.8.1/02 in reference list) reports the toxicokinetics and metabolism of valporic acid, cyclohexanecarboxylic acid, 1-methyl-1-cyclohexanecarboxylic acid and octanoic acid in Sprague-Dawley rats (4 animals per dose, 3 doses, intravenous application, analysis in serum and urine). It was shown that octanoic acid differs significantly from the other substances: Plasma half lives are very short (<5 minutes), no enterohepatic circulation and no recovery in urine, neither as parental substance nor as glucoronide-metabolites. This finding is explained by the fact that it is a naturally occurring substrate with a linear structure that allows easy mitochondrial ß-oxidation.
- **The CAESAR developmental toxicity QSAR also supports the absence of concern for developmental toxicity**.

4.3 **Outcome:** Supportive information for assumption of no concern with regard to developmental toxicity.

4.4 **Conclusion:** *Provide an assessment of whether the final result is considered adequate for a regulatory conclusion, or whether additional information is required (and, if so, what this additional information should be).*

Section A6.8.2 Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2 **Oral, rat**

Official use only

	1 REFERENCE	
1.1	**Reference**	Harkins, R.W. & Sarett, H.P. (1968); nutritional evaluation of medium-chain triglyceride in the rat; The Journal of the American oil chemists' society, 1968, Vol. 45; page 26-30; No A6.4.1.1.b/01 and A6.8/01.
1.2	**Data protection**	No
1.2.1	Data owner	published
1.2.2	Companies with letter of access	none
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No
2.2	**GLP**	No
2.3	**Deviations**	-
	3 MATERIALS AND METHODS	
Test material		Medium-chain triglycerides (MCT) containing 51% octanoic acid (C8:0) **35% decanoic acid (C10:0)** 2% (C12:0) 0.9% (16:0)
3.1.1	Lot/Batch number	Not reported
3.1.2	Specification	A detailed analysis of all use materials is reported.
3.1.2.1	Description	Source and nature of the material are described in sufficient detail.
3.1.2.2	Purity	
3.1.2.3	Stability	Not reported
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	McCollum-Wisconsin
3.2.3	Source	Not reported
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	P: young adults (not further specified)
3.2.6	Number of animals per group	Not reported
3.2.7	Mating	P: 3 weeks after treatment started F1: 15 weeks of age
3.2.8	Duration of mating	Not reported
3.2.9	Deviations from standard protocol	-
3.2.10	Control animals	Yes

Section A6.8.2		Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2		Oral, rat	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Animal assignment to dosage groups	Not reported	
3.3.2	Duration of exposure before mating	P:3 weeks	
3.3.3	Duration of exposure in general P, F1, F2 males, females	P: exposure during pregnancy and lactation F1: after weaning rats were raised on same diets as fed to their mother At 12 weeks of age each F1 group was divided into 3 subgroups. One subgroup was continued on the same diet whereas the two other subgroups were switched to the diets containing one of the other two fats. After 3weeks the F1 females were mated. F2: after weaning rats were raised on same diets as fed to their mother Oral	
3.3.4	Type	in food	
3.3.5	Concentration	40% of the calories in food from or MCT (active ingredient) plus 2.5% safflower oil to supplement with essential fatty acids 38%of the calories in the food from carbohydrate 22% of the calories in food from protein mineral and vitamin mixture	X
3.3.6	Vehicle	-	
3.3.7	Concentration in vehicle	-	
3.3.8	Total volume applied	-	
3.3.9	Controls	Control-group 1: containing 40% of the calories in food from oleo oil otherwise as treatment group Control-group 2: low-fat diet containing 2.5% safflower oil otherwise as treatment group	X
3.4	**Examinations**		
3.4.1	Clinical signs	No effects reported	X
3.4.2	Body weight	Recorded after 4, 8, 47 weeks of treatment	X
3.4.3	Food/water consumption	7 days per week, ad libitum Food intake was recorded.	
3.4.4	Oestrus cycle	Not reported	
3.4.5	Sperm parameters	Not reported	
3.4.6	Offspring	number of pups, live births, birth weight and weight gain	
3.4.7	Organ weights P and F1	Not reported	
3.4.8	Histopathology P and F1	Not reported	
3.4.9	Histopathology F1 not selected for mating, F2		
3.5	**Further remarks**	Volume of milk secretion in P	

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Oral, rat**

		analysis of fatty acids in milk of P	
		4 RESULTS AND DISCUSSION	
4.1	**Effects**		
4.1.1	Parent males	No effects	
4.1.2	Parent females	Milk secretion showed no difference in mothers because of the diets. Although 85% of the dietary fatty acids were C_8 and C_{10} in the MCT group, these constituted only 24% of the milk fat fatty acids. In contrast the fatty acids in the milk secreted by the oleo acid group were similar to those contained in the dietary fat. Level of fat in milk of animals received MCT was slightly lower with more medium chain fatty acids (C_8 and C_{10}) than in rats receiving oleo oil.	
4.1.3	F1 males	Findings in average birth weight and number of pubs per litter were similar in all 3 diets Wight gain during weaning was lower on the low fat diet than on the MCT or oleo oil diet. Mortality during lactation period was 6% (MCT), 7% (oleo oil) and 2% (low fat diet) respectively	
4.1.4	F1 females	Findings in average birth weight and number of pubs per litter were similar in all 3 diets Mortality during lactation period was 6% (MCT), 7% (oleo oil) and 2% (low fat diet) respectively Milk secretion in F1 mothers was low when fed on MCT diet for 2 generations.	
4.1.5	F2 males	Number of pubs per litter and birth weights were similar for all subgroups Highest weight gain at weaning (21 days) were found in the groups on the oleo oil diet except for the slightly low value in that group which had previously received the low-fat diet. Intermediate weaning weights were found in the groups receiving the MCT diet, and lowest weaning weights were found in groups receiving the low-fat diet. Mortality in groups receiving MCT was 22% for subgroup previously on MCT, 20% for subgroup previously on low-fat diet and 6% for subgroup previously on oleo oil diet. Mortality was 7% or less on other 6 subgroups. No difference in subsequent growth of all animals shown.	X
4.1.6	F2 females	Number of pubs per litter and birth weights were similar for all subgroups Highest weight gain at weaning (21 days) were found in the groups on the oleo oil diet except for the slightly low value in that group which had previously received the low-fat diet. Intermediate weaning weights were found in the groups receiving the MCT diet, and lowest weaning weights were found in groups receiving the low-fat diet. Mortality in groups receiving MCT was 22% for subgroup previously on MCT, 20% for subgroup previously on low-fat diet and 6% for subgroup previously on oleo oil diet. Mortality was 7% or less on other 6 subgroups. No difference in subsequent growth of all animals shown.	X
4.2	**Other**	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	non-guideline study, groups of male and female rats were fed with MCT or other fat diets started 3 weeks before mating. F1 was fed with diet of mothers after weaning. At 12 weeks of age each F1 group was divided into 3 subgroups. One subgroup was continued on the same diet whereas the	

Section A6.8.2	Multigeneration Reproduction Toxicity Study	
Annex Point IIA6.8.2	Oral, rat	
	two other subgroups were switched to the diets containing one of the other two fats. After 3weeks the F1 females were mated. Number of pups, live births, birth weight, mortality during lactation and weight gain was recorded. Also volume of milk secretion (P and F1 mothers) and analysis of fatty acids in milk of P mothers were examined.	
5.2 Results and discussion	. Feeding of MCT in 1st generation does not implicate any adverse effects either in fertility of the parents or in health of the pubs. Feeding MCT in high concentrations over 2 generations resulted in low milk secretion in F1 mothers which suggested that this factor may have affected weight gain and mortality of the pubs. Still lowest weaning weights in pubs were found in groups receiving the low-fat diet which indicates that reasonable fat content in the diet is required for a healthy pub development. This indicates that mortality and low weight gain in pubs of MCT-fed mothers (F1) is not the result of an adverse effect to MCT but rather results in the lack of high chain fatty acids (partly essential fatty acids) which are difficult or not possible to be synthesised by the body.	
5.3 Conclusion	Decanoic acid (35 % in MCT) did not show any adverse effects either in fertility of the parents or in health of the pubs under the described conditions. The described effects in F1 mothers and their pubs are rather caused by the lack of high chain fatty acids which partly have to be supplied with the food especially in lactation animals to enrich the milk sufficiently since the body is not able to synthesise them in decent amount. Therefore the effects in the pubs are caused by deficiency disease rather than by excessive MCT supply followed by adverse effects.	
5.3.1 LO(A)EL		
5.3.1.1 Parent males	n.a.	
5.3.1.2 Parent females	n.a.	
5.3.1.3 F1 males	n.a.	
5.3.1.4 F1 females, F2 male, female	n.a.	
5.3.2 NO(A)EL		
5.3.2.1 Parent males, females F1 males, females F2 males, females	NOAEL decanoic acid ≥ 5.1 g/kg bw/day	X
5.3.3 Reliability	2 This study was performed not according to a guideline study for regulatory purposes. Nevertheless the goal of the study to evaluate the nutritional properties of medium-chain triglycerides (MCT) including any effects on the normal growth or development of offspring make this study suitable to judge the possible effects of decanoic acid during a multigeneration exposure.	
	Decanoic acid occurs is nature and is part of the human diet, it occurs as free acid in 147 individual food items (Gubler 2006; IIC/02) and as triglyceride, which is completely absorbed after ingestion and metabolised (see DOC IIA)to free decanoic acid in the liver. In practice, human intake from both sources has to be considered as systemic. In the next paragraphs the consumption is discussed in some detail.	X
	Human dietary intake of decanoic acid is much higher from fat	

Section A6.8.2 **Multigeneration Reproduction Toxicity Study**
Annex Point IIA6.8.2 **Oral, rat**

	consumption. Spychinger (2003; IIB/02) reports that the average consumption of coconut oil in Germany is 1 kg per person. Based on analytical data from the German organisation DGF (Deutsche Gesellschaft für Fettwissenschaft; IIB/03) coconut fat contains between 5.0 – 8.0 % decanoic acid; taking an average of 6.5 % this translates to an average daily consumption of 178 mg/day per person.
5.3.4 Deficiencies	-

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.2	Multigeneration Reproduction Toxicity Study
Annex Point IIA6.8.2	Oral, rat
	either of the two substances. The RMS fused the two study summaries to increase the readability of the CAR.

Table A6_8_2-1. Fatty acid composition obtained from lactating rats receiving MCT or oleo oil-containing diet

	Milk fat [%]	Fatty acids **[%]** in milk fat / fatty acid no. of carbon atoms											
		8	10	12	14	16	18	16:1	18:1	18:2	18:3	20:4	other
MCT	8.2	6.5	16.8	10.3	11.5	29.9	4.7	0.9	11.7	6.5	0.3	0.3	0.4
Oleo oil	9.8	2.2	5.8	4.4	6.6	20.8	9.4	2.4	86.7	8.0	0.9	0.4	2.8

Composition of diet and dietary fat:

Table I of publication:

Composition of Diets

	Diet 1–6 40% Fat calories	Diet 7 Low fat
	%	%
Fat[a]	21.0	2.5
Casein (ANRC 91.4% protein)	26.2	26.2
Amidex[b]	44.5	63.0
Nonnutritive fiber	4.0	4.0
Mineral mixture[c]	4.0	4.0
Vitamin mixture[d]	0.35	0.35

Table IV of publication:

	Fatty acids, %											
	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	Other
Dietary Fat												
MCT[a]	51.0	35.0	2.0		0.9			1.4	9.0			0.7
Oleo oil[a]				2.9	22.1	4.8	13.4	43.2	12.5			1.1
Butter fat[a]	1.9	3.3	2.9	8.1	22.8	3.8	10.5	23.3	13.3			10.1
Coconut oil[a]	8.1	7.2	36.8	17.2	10.0		2.4	7.2	11.0			0.1
Corn oil					13.4		1.4	26.2	57.8			1.2
Safflower oil					6.7		1.9	10.0	80.8	0.2		0.4

Table VIII of publication

TABLE VIII

Birth Weight and Body Weights of Rats Born and Nursed by Mothers Receiving MCT, Oleo Oil, and Low-Fat Diets

Dietary fat[a]		Pups per litter	Day: Birth	Day: 6	Day: 12	Day: 18	Day: 21	Day: 49	Day: 63	Day: 105
			Male and Female				Male			
			Weight, g per rat				Weight, g per rat			
F1 Generation										
MCT		9.0	6.4	13	21	34	45	181		309
Oleo oil		9.1	6.1	14	24	34	47	186		326
Low fat		9.6	6.4	13	22	29	39	165		286
F2 Generation										
MCT	MCT	9.2	6.5	12	23	35	45		261	
Oleo oil	MCT	7.0	6.7	12	23	36	45		242	
Low fat	MCT	9.4	6.6	12	23	36	43		243	
MCT	Oleo oil	9.4	6.8	13	25	39	49		249	
Oleo oil	Oleo oil	9.2	6.3	12	24	39	47		244	
Low fat	Oleo oil	10.5	6.0	11	23	35	43		248	
MCT	Low fat	10.8	6.2	11	21	31	36		244	
Oleo oil	Low fat	8.8	6.4	12	25	36	39		245	
Low fat	Low fat	9.3	6.5	12	23	32	38		243	

[a] All diets contained 2.5% safflower oil.

Table IX of publication

TABLE IX

Milk Secreted by F_0 and F_1 Generation Lactating Rats Receiving MCT, Oleo Oil, and Low-Fat Diets[a]

Dietary fat[b]		Day 3	6	9	12	15	18	21	Total
		g milk							
F_0 Generation									
MCT		3.9	4.7	5.0	5.5	7.1	7.3	7.3	40.2
Oleo oil		4.3	6.5	4.5	5.3	6.5	6.0	6.0	41.2
Low fat		4.5	5.6	6.3	6.2	5.4	6.7	8.4	43.1
F_1 Generation									
MCT	MCT	1.7	0.8	4.0	2.3	4.0	5.2	6.3	24.3
Oleo oil	MCT	1.3	2.0	2.8	4.3	6.2	9.0	8.8	34.4
Low fat	MCT	2.4	3.9	5.0	5.0	6.7	8.7	5.5	37.2
MCT	Oleo oil	2.2	3.6	6.2	7.0	6.6	10.2	9.2	45.0
Oleo oil	Oleo oil	1.5	2.2	4.8	5.8	6.3	10.8	9.8	41.2
Low fat	Oleo oil	2.2	4.8	7.5	8.7	8.3	8.3	10.0	49.8
MCT	Low fat	1.8	6.5	5.3	4.0	7.5	11.2	10.0	46.3
Oleo oil	Low fat	2.0	4.2	5.2	4.4	7.6	9.6	8.0	41.0
Low fat	Low fat	1.6	3.9	4.4	5.3	6.6	7.7	9.1	38.6

[a] Milk secretion was estimated as the increase in weight of each litter during a one-hour lactation period; the mother was removed from the litter for six hours beforehand.
[b] All diets contained 2.5% safflower oil.

Section A7.1.1.1.2 Annex Point IIA7.6.2.2	**Phototransformation in water including identity of transformation products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	Technically not feasible [ ] Scientifically unjustified [X]	
Limited exposure [ ]	Other justification [X]	
Detailed justification:	[redacted]	X
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	[redacted]	

Section A7.1.1.1.1 Annex Point IIA7.6.2.1	**Hydrolysis as a function of pH and identification of breakdown products**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	[redacted]	X X
Undertaking of intended data submission [ ]		

Evaluation by Competent Authorities	
Date	[redacted]
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Biodegradability (ready)

Official use only

1 REFERENCE

1.1	**Reference**	Seyfried Birgit (2006); DECANOIC ACID: READY BIODEGRADABILITY IN A MANOMETRIC RESPIROMETRY TEST; RCC LTD, Itingen, Switzerland; RCC Study Number: A86567; Ref nr A7.1.1.2.1/02
1.2	**Data protection**	Yes
1.2.1	Data owner	SOPURA N.V.
1.2.2		
1.2.3	Criteria for data protection	Data on existing a.s. submitted for the first time for entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	92/69/EEC C.4-D, OECD 301 F.
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

Section A7.1.1.2.1 Biodegradability (ready)
Annex Point IIA7.6.1.1

3.1	**Test material**	Decanoic acid
3.1.1	Lot/Batch number	03108595700
3.1.2	Specification	Not reported
3.1.3	Purity	99%
3.1.4	Further relevant properties	Not reported
3.1.5	Composition of Product	Not applicable
3.1.6	TS inhibitory to microorganisms	Yes Test material can inhibit microbial growth. However, since the outcome is readily biodegradable no inhibition was seen at the tested concentration.
3.1.7	Specific chemical analysis	Provided by supplier
3.2	**Reference substance**	Sodium benzoate
3.2.1	Initial concentration of reference substance	100mg/L
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	Inoculum: Aerobic activated sludge from a wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland) treating predominantly domestic wastewater.
3.3.2	Test system	The study was performed with aerobic activated sludge from a wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland) treating predominantly domestic wastewater. The sludge was washed twice with tap water by centrifugation and the supernatant liquid phase was decanted. A homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet to dry weight was calculated. Based on this ratio, calculated amounts of wet sludge were suspended in test water (see Section 2.4.1) to obtain a concentration equivalent to 4 g (±10%) dry material per litre. During holding, the sludge was aerated at room temperature until use. Prior to use, the sludge was first thoroughly mixed and then diluted with test water to a concentration of 1 g per liter (dry weight basis). Based on the determined dry weight of this diluted activated sludge defined amounts were added to test water to obtain a final concentration of 30 mg dry material per litre. **Test Water** The test water was prepared according to the testing guidelines. Analytical grade salts were dissolved in purified water to obtain the following stock solutions: a) KH_2PO_4 8.50 g/L K_2HPO_4 21.75 g/L Na_2HPO_4 x $2H_2O$ 33.40 g/L NH_4Cl 0.50 g/L

x

Section A7.1.1.2.1 Biodegradability (ready)
Annex Point IIA7.6.1.1

		The pH of this solution was 7.4. b) $MgSO_4$ x $7H_2O$ 22.50 g/L c) $CaCl_2$ x $2H_2O$ 36.40 g/L d) $FeCl_3$ x $6H_2O$ 0.25 g/L, stabilized with one drop of concentrated HCl per litre To obtain the final test water, 10 mL of stock solution a) and 1 mL each of stock solutions b) – d) were combined and made up to 1000 mL with purified water. The pH was measured to be 7.5.	
3.3.3	Test conditions	**Apparatus**: The test flasks (500-mL Erlenmeyer flasks, labelled with all necessary information to ensure unmistakable identification) were incubated under continuous stirring in a SAPROMAT D12 (Voith GmbH, Heidenheim, Germany). Oxygen consumption was recorded manually by taking a daily reading at least on each working day. **Principle**: Electro-chemical analysis process: The biodegradation process consumes the dissolved oxygen in the liquid and generates CO2. The CO2 is adsorbed by soda lime and the total pressure decreases in the airtight test flasks. The pressure drop is detected and converted into an electrical signal by means of an electrode type manometer. The consumed oxygen is replaced by electrolytically generated oxygen from a copper sulfate solution. **Test duration**: 28 days **Light conditions**: Darkness **Test temperature**: 22 °C, maintained with a built-in thermostat and checked once per week. **pH**: Prior to test start, the pH was measured in each test flask before the addition of the activated sludge inoculum (Table 3). At the end of incubation, the pH was measured again in each test flask.	x
3.3.4	Method of preparation of test solution	The test item was weighed by means of an analytical balance and transferred to the designated test flasks with test water. No emulsifiers or solvents were used. The reference item sodium benzoate was tested simultaneously under the same conditions as the test item, and functioned as a procedure control. A stock solution containing 2.5 g sodium benzoate per liter test water was prepared by completely dissolving 250 mg sodium benzoate in 100 mL of test water. From this stock solution, 10 mL aliquots were added to the corresponding test flasks containing test water. Finally, with the exception of the abiotic control flask, activated sludge was added to each test flask (see Section 2.3). The final test volume was 250 mL per test flask.	

Section A7.1.1.2.1
Annex Point IIA7.6.1.1

Biodegradability (ready)

3.3.5	Initial TS concentration	100 mg/L
3.3.6	Duration of test	28 days
3.3.7	Analytical parameter	Measurement of pressure drop
3.3.8	Sampling	Measurement directly in flask
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	Not required, test substance contains no nitrogen
3.3.11	Controls	Abiotic degradation: No degradation Toxicity control: Not toxic
3.3.12	Statistics	Not reported

4 RESULTS

Section A7.1.1.2.1 Biodegradability (ready)
Annex Point IIA7.6.1.1

4.1 Degradation of test substance

4.1.1 Graph

Figure 1: Oxygen consumption in the test flasks during the incubation period

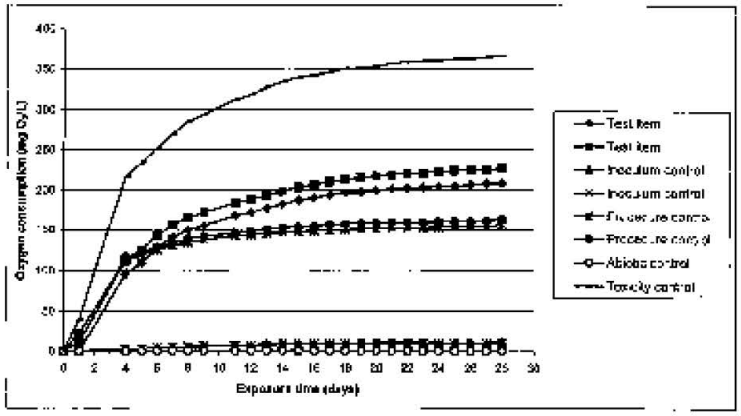
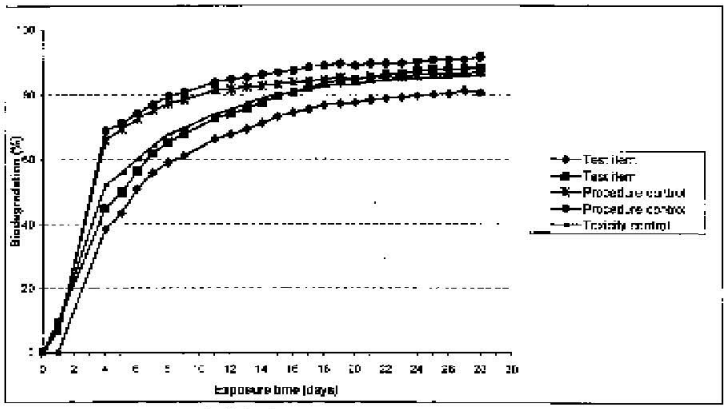


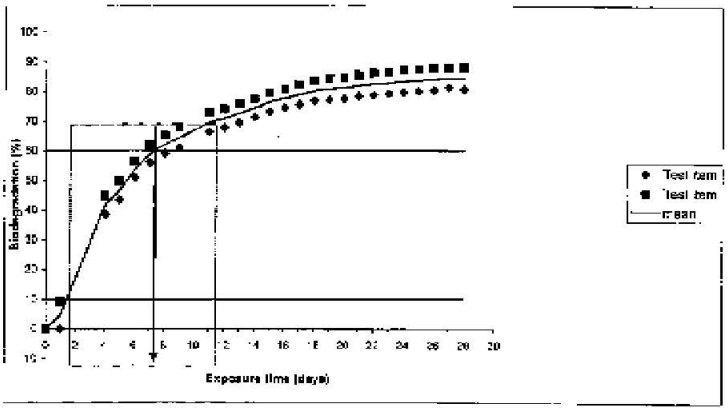
Figure 2: Biodegradation in the test flasks during the incubation period

A: Overview over the whole test series



(based on values presented in Table 2)

B: 10-day window for the biodegradation of the test item



4.1.2 Degradation

The biochemical oxygen demand (BOD) of deanoic acid in the test media significantly increased from Exposure Day 1 until test

Section A7.1.1.2.1 **Biodegradability (ready)**
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		termination after 28 days. After five days of exposure, the mean biodegradation of Decanoic acid amounted to 62%. The pass level for ready biodegradability, i.e. biodegradation of at least 60% of the ThOD in a 10-day window within the 28-day period of the test, was reached. At the end of the 28-day exposure period, the mean biodegradation of Decanoic acid amounted to 92%. Consequently, Decanoic acid was found to be readily biodegradable under the test conditions within 28 days.	
4.1.3	Other observations	The percent biodegradation in the toxicity control, containing both the test item and the reference item, was calculated based on the sum of the ThOD of the test item and the reference item. In the toxicity control, the biochemical oxygen demand over the 28-day exposure period was similar, but significantly higher than in the two procedure controls, containing only the reference item. Within 14 days of exposure, biodegradation amounted to 88%. Thus, according to the test guidelines, the test item had no inhibitory effect on activated sludge microorganisms at the tested concentration of 100 mg/L, because biodegradation in the toxicity control was >25% within 14 days.	
4.1.4	Degradation of TS in abiotic control	No degradation	
4.1.5	Degradation of reference substance	The percent biodegradation of the reference item sodium benzoate was calculated based on the theoretical oxygen demand of 1.67 mg O_2/mg. In the procedure controls, the reference item was degraded by an average of 84% by Exposure Day 14, thus confirming suitability of the activated sludge. At the end of the test (Day 28), the reference item degraded by an average of 89%.	x
4.1.6	Intermediates/ degradation products	Not reported	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was performed in compliance with the testing guidelines 92/69/EEC C.4-D, OECD 301 F. There is no deviation from the guidelines, the results are as easy to interpret, the biodegradation of the reference substance and the results of the toxic control do not show any limitation of the test.	
5.2	**Results and discussion**	Decanoic acid was found to be readily biodegradable under the test conditions within 28 days.	
5.3	**Conclusion**	Decanoic acid fulfils all criteria required by the testing guidelines for readily biodegradable and is therefore classified readily biodegradable.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

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Biodegradability (ready)

Evaluation by Competent Authorities	
Date	[redacted]
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
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 A7_1_1_2-1: Guidline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Aerobic activated sludge
Species	-
Strain	-
Source	Aerobic activated sludge from a wastewater treatment plant treating predominantly domestic wastewater.
Sampling site	ARA Ergolz II, Füllinsdorf, Switzerland
Laboratory culture	No
Method of cultivation	N.a.
Preparation of inoculum for exposure	The sludge was washed twice with tap water by centrifugation and the supernatant liquid phase was decanted. A homogenized aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet to dry weight was calculated.
Pretreatment	Based on the ratio of wet to dry weight, calculated amounts of wet sludge were suspended in test water to obtain a concentration equivalent to 4 g (±10%) dry material per litre. During holding, the sludge was aerated at room temperature until use.
Initial cell concentration	Prior to use, the sludge was first thoroughly mixed and then diluted with test water to a concentration of 1 g per litre (dry weight basis). Based on the determined dry weight of this diluted activated sludge defined amounts were added to test water to obtain a final concentration of 30 mg dry material per litre.

Table A7_1_1_2-3: Test system

Criteria	**Details**
Culturing apparatus	The test flasks (500-mL Erlenmeyer flasks, labeled with all necessary information to ensure unmistakable identification) were incubated under continuous stirring in a SAPROMAT D12 (Voith GmbH, Heidenheim, Germany).
Number of culture flasks/concentration	2/concentration
Aeration device	The consumed oxygen is replaced by electrolytically generated oxygen from a copper sulfate solution.
Measuring equipment	Electro-chemical analysis process: The biodegradation process consumes the dissolved oxygen in the liquid and generates CO_2. The CO_2 is adsorbed by soda lime and the total pressure decreases in the airtight test flasks. The pressure drop is detected and converted into an electrical signal by means of an electrode type manometer.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	The test water was prepared according to the testing guidelines. Analytical grade salts were dissolved in purified water to obtain the following stock solutions: a) KH_2PO_4 8.50 g/L K_2HPO_4 21.75 g/L Na_2HPO_4 x $2H_2O$ 33.40 g/L NH_4Cl 0.50 g/L The pH of this solution was 7.4. b) $MgSO_4$ x $7H_2O$ 22.50 g/L c) $CaCl_2$ x $2H_2O$ 36.40 g/L d) $FeCl_3$ x $6H_2O$ 0.25 g/L, stabilized with one drop of concentrated HCl per litre
Additional substrate	No
Test temperature	22 °C, maintained with a built-in thermostat
pH	The pH measured in all flasks at the start of the test was 7.5. At the end of exposure (Day 28), pH values of 7.6 – 8.6 were measured. The results are presented in the table below. Table 3. pH values at the beginning and at the end of the test.

Replicate No	Identification	pH	
		Start	End
[illegible]	Decanoic acid	7.5	7.7
[illegible]	Octanoic acid	7.5	7.6
[illegible]	[illegible] control	[illegible]	[illegible]
2	[illegible] control	7.5	7.5
1	Procedure control	7.5	8.2
[illegible]	Procedure control	7.5	8.5
1	Aniline	7.5	7.4
1	Toxicity control	7.5	8.2

Aeration of dilution water	No
Suspended solids concentration	4 g (± 10%) dry mater per litre.
Other relevant citeria	Stirring of test solution

Table A7_1_1_2-5: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	X	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test	X	
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%	X	
Percentage of removal of reference substance reaches pass level by day 14	92%	

5.3.2.1 Criteria for poorly soluble test substances	5.3.2.2	5.3.2.3
5.3.2.4	**5.3.2.5**	**5.3.2.6**
5.3.2.7	**5.3.2.8**	**5.3.2.9**

Table A7_1_1_2-6: Pass levels and validity criteria for inherent biodegradability tests

	fulfilled	**not fulfilled**
Pass levels		
20% removal (DOC or COD);	n.a.	
Pass values reached within 10-d window (within 28-d test period)	n.a.	
Removal of reference substance (DOC or COD) > 70 % within 14 d	n.a.	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70 % within 14 days (OECD 302 B)		
Percentage of DOC-removal of reference compound ≥ 40 % within 7 days and ≥ 65 % within 14 days Average residual amount of test compound in blank tests ≥ 40 % (OECD 302 C)		
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)		

Criteria for poorly soluble test substances	5.3.2.10	5.3.2.11
	5.3.2.12	**5.3.2.13**
	5.3.2.14	**5.3.2.15**

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Section A7.1.1.2.2 Annex Point IIA7.6.1.2	Biodegradability (inherent)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible** [ ] **Scientifically unjustified** [X]	
Limited exposure [ ]	**Other justification** [X]	
Detailed justification:	This study is not necessary because n-decanoic acid is readily biodegradable.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	March 2010	
Evaluation of applicant's justification	Agree with applicant's version.	
Conclusion	Agree with applicant's version.	
Remarks	-	