Section A 6.4.1.1.a **Subchronic toxicity (rodent)**
Annex Point II 6.4 **Oral, rat, 91 days**

		control range for each parameter.
4.5.3	Urinalysis	-
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Analysis of organ weight data revealed that only the liver and colon were affected by caprenin treatment. Significant differences in absolute and relative organ weight were confined to liver, colon, kidneys, heart, spleen. The changes in kidneys, heart and spleen were mild, restricted to organ to body-weight-ratio only, not dose related and therefore considered unrelated to treatment. The lower liver-to-body weight ratios of male rats and absolute liver weights of female rats fed caprenin appear to be related to the reduced amount of fat deposited in the livers of rats on caprenin diets. Liver fat content was significantly lower in high-dose females than in the corn oil controls. The absolute colon weights were significantly greater for mid- and high-dose male rats compared to the corn oil control group. Colon-to body weight ratios were also significantly higher in the mid- and high dose group male rats but only the mid-dose males showed significantly greater colon-to-brain weight ratios. The colon-to body-weight ratio in female rats fed caprenin were higher but not the absolute colon weights compared with corn oil or MCT oil diets. The effects are believed to be related to the poor absorption of behenic acid and its resulting presence in the colon and appear to reflect a hypertrophic adaptation of this organ to a diet that provided greater faecal bulk.
4.6.2	Gross and histopathology	Significant increase in number of 'granulated/pitted/rough kidneys (15/19) were found among high-dose females compared to controls fed corn oil (9/20). Microscopical evaluation of the kidneys could not discern a treatment-related effect. There were no histopathological alterations associated with the administration of caprenin.
4.7	**Other**	Liver fat content was significant lower in high-dose females than in the corn oil controls. Behenic acid was poorly retained by male and female rats.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups with 25 male and 25 female rats received diets with 5.23, 10.23 and 15% (w/w) caprenin, a triglyceride primarily comprising C8:0 (23.2%), C10:0 (26.6%) and C22:0 (45.0%) as fatty acids, respectively for 91 days. Two control groups were fed with corn oil, a triglyceride primarily comprising C16:0 (11.3%), C18:1 (25.1%) and C18:2 (60.7%) as fatty acids, at a dietary concentration of 12.14% (w/w) or MCT oil, a triglyceride primarily comprising C8:0 (70.6%) and C10:0 (25.9%) as fatty acids, at a dietary concentration of 11.21% (w/w). Corn oil was added to all diets at a level no lower than 3% to provide essential fatty acids. Each diet provided the same percentage of calories as fat (26.8%), protein (19.4%) and carbohydrate (52.4%).Weight gain was measured weekly. Blood samples were taken at the end of study and examined for biochemistry and haematological parameters. Gross and histopathology were carried out.
5.2	**Results and discussion**	No evidence of toxicity in weanling rats maintained for 91 consecutive days on diets containing up to 15% (w/w) caprenin, a level that corresponded to 83% (w/w) of the total dietary fat. This conclusion is

Section A 6.4.1.1.a **Subchronic toxicity (rodent)**
Annex Point II 6.4 **Oral, rat, 91 days**

		supported by the absence of treatment-related effects in growth, mortality, haematology and serum chemistry values, or in anatomical or microscopical pathology. The only in vivo observation that was attributed to treatment was the finding that rats maintained on MCT oil diets (which contained the highest proportion of octanoic acid) developed a high incidence of tail desquamation. This finding may be related to direct skin contact with the diet and the mildly irritant properties of MCT oil, which could be due to its high octanoic acid content.	
5.3	**Conclusion**	Caprenin did not show any adverse effects in rats treated under the described conditions. The study also demonstrated that MCT oil, a triglyceride mainly composed of octanoic and decanoic acid, as the primary source of dietary fat produce no adverse systemic effects. Rats fed with 15% (*w/w*) caprenin consumed 13.2 g/kg bw (male) and 14.6 g/kg bw (female) caprenin which results in about 3.5 g/kg bw (male) and 3.9 g/kg bw (female) decanoic acid respectively.	
5.3.1	LO(A)EL	-	
5.3.2	NO(A)EL	NOAEL decanoic acid/male ≥ 3.5 g/kg bw/day NOAEL decanoic acid/female ≥ 3.9 g/kg bw/day	X
5.3.3	Other	-	
5.3.4	Reliability	1	X
5.3.5	Deficiencies	-	

Evaluation by Competent Authorities	
Date	
Materials and Methods	[redacted]
Results and discussion	

Section A 6.4.1.1.a Subchronic toxicity (rodent)

Annex Point II 6.4 **Oral, rat, 91 days**

Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

Table A6_4.1.1 Results at termination of study after 91 days

Parameter	Control Corn oil		Control MCT		low dose		medium dose		high dose	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Mortality	1/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	1/25
body weight gain [g]	548	263	548	274	562	256	548	265	564	256
food consumption[g]	2574	1976	2597	1954	2642	1922	2668	1977	2797	1997
caprenin consumption [g/kg bw/day]	-	-	-	-	4.4	4.9	8.7	9.7	13.2	14.6
Alkaline Phasphatase [IU/litre]	76	50	98	49	81	58	94	63	101	56
ALT [IU/litre]	47	25	56	25	47	28	66	37	70	42
Total protein [g/dL]	6.9	7.6	7.2	7.5	6.7	7.1	6.6	6.8	6.7	6.7
organ weight liver*	2.7	2.76	2.85	2.75	2.45	2.59	2.47	2.67	2.56	2.73
organ weight colon*	0.33	0.44	0.34	0.44	0.33	0.51	0.38	0.51	0.38	0.52

* Mean organ-to-body-weight ratio (organ wt/body wt x 100)

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	
Materials and Methods	[redacted]

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 energy	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

[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. 42, 325, 1951). Ascorbic acid omitted. In addition, 0.015 g Crown Tocomorphum 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

	Official use only
Justification for non-submission of data	
Other existing data [ ] **Technically not feasible** [ ] **Scientifically unjustified** [x]	
Limited exposure [x] **Other justification** [ ]	
Detailed justification: No specific study is available.	
[redacted]	X
Undertaking of intended data submission [ ]	

Evaluation by Competent Authorities	
Date	2009-06-09
Evaluation of applicant's justification	[redacted]
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

Evaluation of applicant's justification

Conclusion

Remarks

[redacted]

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: No specific study has been conducted.

[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
1.1	**Reference**	[redacted]	
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		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*

3.3	**Administration / Exposure; Application of test 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**	
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	

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**	[redacted]
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 A6.6.1 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 octanoic acid Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.667 Ref nr A6.6.1/01
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.13/14, OECD 471
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	Octanoic acid
3.1.1	Lot/Batch number	Product code: 800192
3.1.2	Specification	Not reported
3.1.2.1	Description	Clear, colourless liquid
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>

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: Octanoic acid was dissolved in DMSO (50 mg/mL). 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 Octanoic 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 poured 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	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	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.
4.2	**Cytotoxicity**	The test substance was toxic to all strains used. Most strains showed decreased numbers of revertants from 1500 µg/plate and higher.

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 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 octanoic acid in any strain at any concentration. Cytotoxicity was evident at 1500 µg/plate and higher.
5.3	**Conclusion**	Octanoic acid did not show any evidence of mutagenicity under the described test conditions
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	2009-08-20
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	acceptable
Remarks	

Table A6_6_1-1. Table of bacterial reverse mutation assay; mutagenicity test with octanoic 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	24	16	18	23	39	54	144	127	16	26
62	23	21	23	22	44	53	138	142	21	26
185	23	18	23	21	35	56	125	120	22	24
556	19	15	11	16	25	43	109	115	14	24
1667	14	12	8	9	17	31	75	109	11	16
5000	7	8	3	4	9	13	51	57	9	6
Positive control	420	458	991	247	388	682	553	1721	224	1227

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	26	21	14	17	32	51	142	127	17	25
94	15	12	21	19	37	46	156	134	17	19
188	21	14	18	18	33	46	141	143	19	24
375	26	10	14	15	29	45	126	132	18	22
750	15	15	13	11	25	54	122	124	19	19
1500	10	5	7	11	22	38	84	97	22	15
Positive control	411	589	1416	247	871	994	579	1498	190	1153

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	

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

[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 octanoic acid in cultured Chinese hamster ovary cells Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.660 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**	Octanoic acid
3.1.1	Lot/Batch number	Product code 800192
3.1.2	Specification	Not reported
3.1.2.1	Description	Clear and colourless liquid
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, 100, 300, 500, 750 and 1000 and 1200 µg/mL (4 hours treatment/18 hours fixation) + S9-mix: 0, 100, 300, 500, 750, 1000 and 1200 µg/mL (4 hours treatment/18 hours fixation) Test 2: - S9-mix: 0, 25, 50, 100, 200, 400 and 500 µg/mL (18 hours treatment/18 hours fixation) + S9-mix: 0, 100, 200, 400, 500, 750 and 1000 µg/mL (4 hours treatment/32 hours fixation)	
3.3.2	Way of application	Octanoic acid was dissolved in DMSO (further diluted to result in the selected concentrations); for treatment 1% DMSO solution in cell culture medium. All cultures were incubated at 37°C. Test 1: Cell cultures were exposed to octanoic acid according to the concentration given in 3.3.1. In both the absence and presence of S9-mix the treatment time was 4hours and fixation time was 18 hours after onset of treatment. Test 2: Cell cultures were exposed to octanoic 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 be demonstrable.	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	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: 750 µg/mL +S9 (mitotic index 45% of control) 300 µg/mL +S9 (mitotic index 77% of control) 1000 µg/mL -S9 (mitotic index 48% of control) 500 µg/mL -S9 (mitotic index 83% of control) Test 2: 750 µg/mL +S9 (mitotic index 51% of control) 400 µg/mL +S9 (mitotic index 83% of control) 200 µg/mL -S9 (mitotic index 52% of control) 50 µg/mL -S9 (mitotic index 86% 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 octanoic acid concentration in presence or in absence of S9.
5.3	**Conclusion**	Treatment of Chinese hamster cells with octanoic acid had no effect on chromosome aberrations in presence or in absence of metabolic activation. It is concluded that octanoic acid was not clastogenic under the conditions used in this study.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

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

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

Concentration [µg/mL]	Treatment	Fixation	Mitotic index (1000 cells scored) %	Mitotic index (1000 cells scored) % control	% Metaphases with aberrations ~~Specific~~ structural	% Metaphases with aberrations ~~Unspecific~~ numerical
Test 1						
0	4 h	18 h	8.15	100	0	0
100	-S9		8.2	101	-	-
300			8.15	100	-	-
500			6.75	83	0	0
750			6.25	77	0.5	0
1000			3.9	48	0.5	0
1200			3.05	37	-	-
mitomycin			5.45	67	24.5	0
0	4 h	18 h	7.5	100	0	0
100	+S9		5.95	79	-	-
300			5.75	77	2.5	0.5
500			4.3	57	2.5	0
750			3.35	45	1	0
1000			1.95	26	-	_
1200			0.25	3	-	-
Cyclophosphamide			3.1	41	42	0.5
Test 2						
0	18 h	18 h	8.3	100	0	0
25	-S9		8.0	96	-	-
50			7.15	86	0	0
100			4.95	60	0	0
200			4.3	52	0	0
400			2.85	34	-	-
500			2.0	24	-	-
mytomycin			5.05	61	10.5	0
0	4 h	32 h	8.3	100	0	0
100	+S9		7.85	95	-	-
200			7.95	96	-	-
400			6.85	83	0	0
500			5.95	72	0	0
750			4.2	51	0.5	0
1000			3.2	39	-	-
cyclophosphamide			5.9	71	12.5	0.5

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

1.1	**Reference**	[redacted]
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**	
3.3.1	Concentrations	

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]

3.3.2	Way of application		X
3.3.3	Pre-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

[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

Materials and Methods

[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

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

[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 Octanoic acid; Netherlands Organisation for applied scientific research (TNO), Zeist, The Netherlands TNO-report V99.714 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**	Cells in absence of S9 were only 4 hours exposed to the test substance instead of 24 hours mentioned in protocol because of the observed cytotoxic effects observed after 4 hours. Since the OECD GL prescribes a treatment time of 3-6 hours this deviation is accordance to OECD GL. The deviation is not considered to have influenced the integrity and outcome of the study.

3 MATERIALS AND METHODS

3.1	**Test material**	Octanoic acid
3.1.1	Lot/Batch number	Product code 800192
3.1.2	Specification	Not reported
3.1.2.1	Description	Light yellow 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: 0.20 mM Methylmethanesulphonate (MMS) +S9: 10 µL/mL 3-Methylcholanthrene (MCA)
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	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, 0.2, 0.28, 0.4,0.58, 0.82, 1.2, 1.7, 2.4, 3.4, 4.9, 7.0, 10 mM Test 2: -S9-mix: 0, 0.6, 0.86, 1.2, 1.8, 2.5, 3.6, 4.5, 5.6, 7.0, 7.4, 7.7, 8.1, 8.6, 9.0, 9.5, 10 mM +S9-mix: 0, 2.4, 3.0, 3.7, 4.7, 5.8, 7.3, 8.1, 9.0, 10 mM.	
3.3.2	Way of application	Octanoic acid was dissolved in DMSO at 144.2 mg/mL (1mM). From this stock solution serial dilutions in DMSO were prepared and from each of this dilutions 100 µL were added to a final volume of 10 mL growth medium. $5 \cdot 10^6$ cells were incubated with octanoic acid in growth medium with a final volume of 10 mL for 4 hours with and without S9-mix. <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 $0.1 \cdot 10^6$ cells/mL in growth medium and incubated for about 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 was calculated.	X
3.3.3	Incubation time	Treatment time: 4 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 A6.6.3.1 and Table A6.6.3.2	
4.1.1	without metabolic activation	There was no relevant increase in the mutant frequencies of octanoic acid-treated cultures. In contrast the positive controls clearly fulfilled the criteria for a positive response.	
4.1.2	with metabolic activation	In both assays an increase in mutant frequency (in small colonies approx. 60%) was observed resulting in a reproducible response at the highest dose level of 10 mM octanoic acid. The positive controls clearly fulfilled the criteria for a positive response.	X
4.2	**Cytotoxicity**	At a concentration of 10 mM octanoic acid, in the presence of S9, cytotocicity was observed in both tests resulting in a RTG of 35%.	
		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 octanoic acid in absence of S9 was detected. In presence of S9 the frequency of small colonies was approximately 60% at a concentration of 10 mM octanoic acid. As this concentration has been shown to be cytotoxic in presence of S9 the effect on the frequency of the colonies is rather related to the cytotoxicity than to a mutagenic response of to the test substance.	
5.3	**Conclusion**	Octanoic acid and/or metabolites are not mutagenic in mouse lymphoma L5178Y under these 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	

Table A6.6.3.1 Table for mammalian cell Gene Mutation Assay (mutagenicity experiments)

Octanoic acid (mM)	Relative suspension growth [%]	Relative cloning 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	65	68
0.4	103	96	52	62
0.58	103	93	117	144
0.82	101	103	94	105
1.2	99	86	85	114
1.7	96	86	107	143
2.4	84	88	70	90
3.4	94	87	61	80
4.9	66	99	101	116
7.0	67	103	101	112
10	2			
MMS (0.1 mM)	76	66	470	820
1st experiment, + S9				
0°	100	100	94	130
0.58	101	101	97	132
0.82	106	148	104	96
1.2	104	102	104	139
1.7	101	124	117	128
2.4	100	109	97	122
3.4	82	96	110	157
4.9	77	112	114	138
7.0	72	112	162	196
10	42	78	189	330
Positive control	45	82	615	1027

° mean of negative control

Table A6.6.3.2 Table for mammalian cell Gene Mutation Assay (mutagenicity experiments)

Octanoic acid (mM)	Relative suspension growth [%]	Relative cloning efficacy [%]	Mutant cloning efficiency [mutants/10^6 viable cells]	Mutation frequency ~~[mutants/10^6 viable cells]~~ Mutant cloning efficiency/absolute final cloning efficiency
2nd experiment, -S9				
0°	100	100	76	88
0.6	104	88	107	141
0.86	86	94	73	90
1.2	88	106	101	110
1.8	79	93	79	99
2.5	89	93	85	107
3.6	85	94	67	82
5.6	76	97	76	91
7.4	78	93	70	88
8.1	70	96	97	118
9.0	84	108	117	126
10.0	73	101	123	143
Positive control	64	43	463	1260
2nd experiment, +S9				
0°	100	100	92	116
2.4	100	108	73	87
3.0	95	99	82	106
3.7	87	117	154	169
4.7	84	115	120	134
5.8	86	108	97	116
7.3	84	93	144	198
8.1	68	98	127	167
9.0	57	124	147	153
10.0	40	89	207	298
Positive control	67	59	810	1773

° mean of negative control

Section A6.8.1. Teratogenicity Study

Annex Point IIA6.8.1

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

Section A6.8.1. Teratogenicity Study

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	[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

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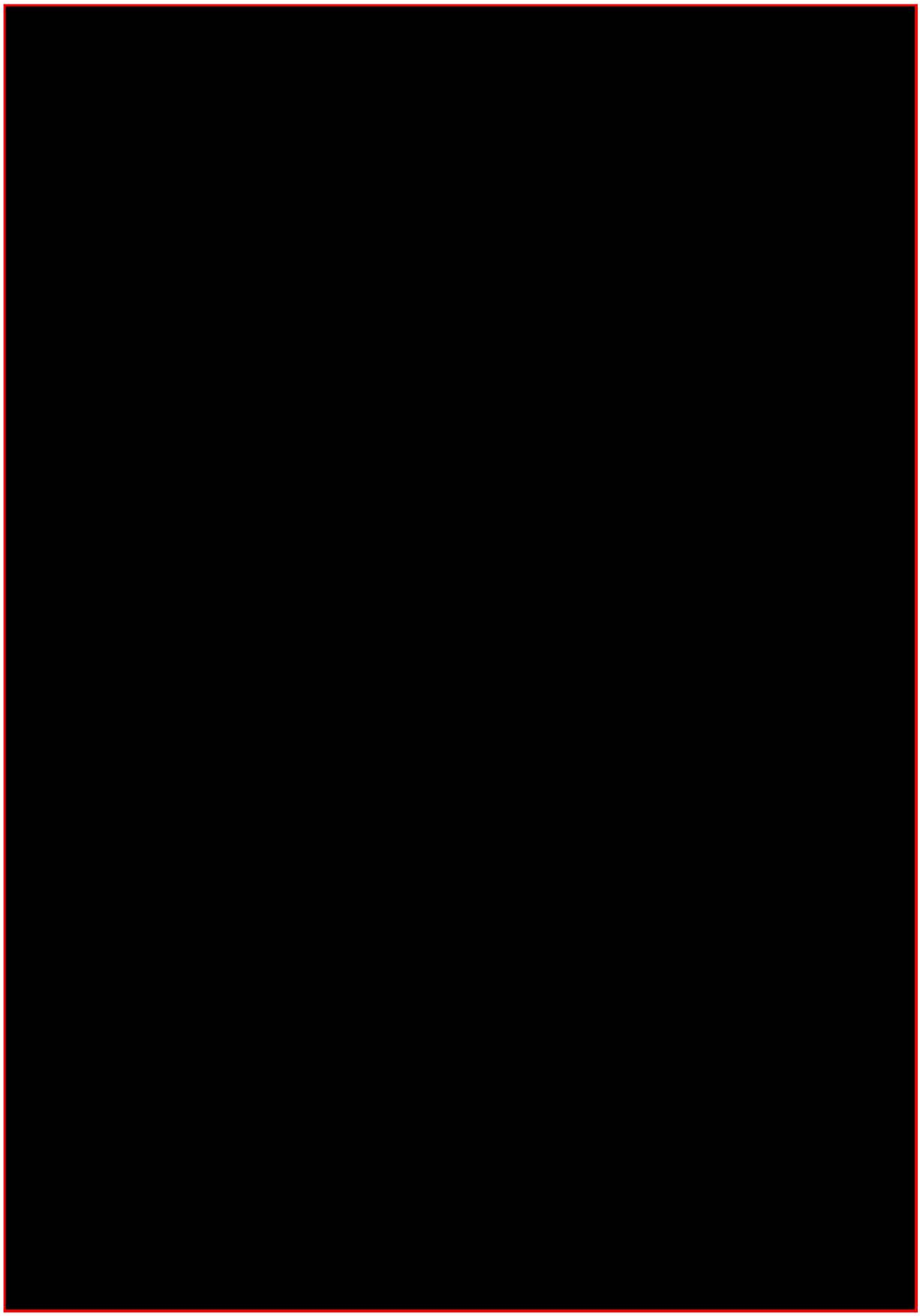
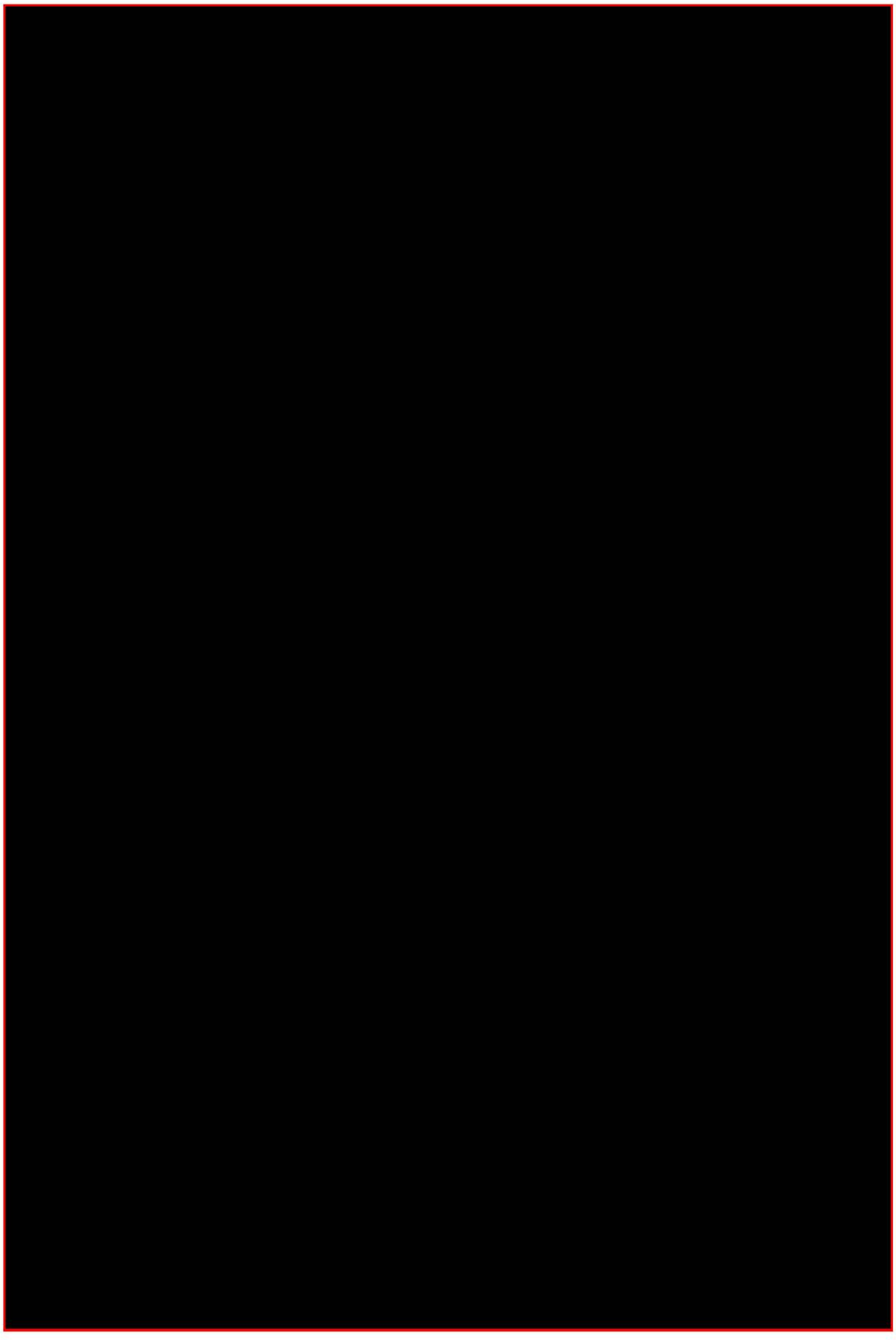
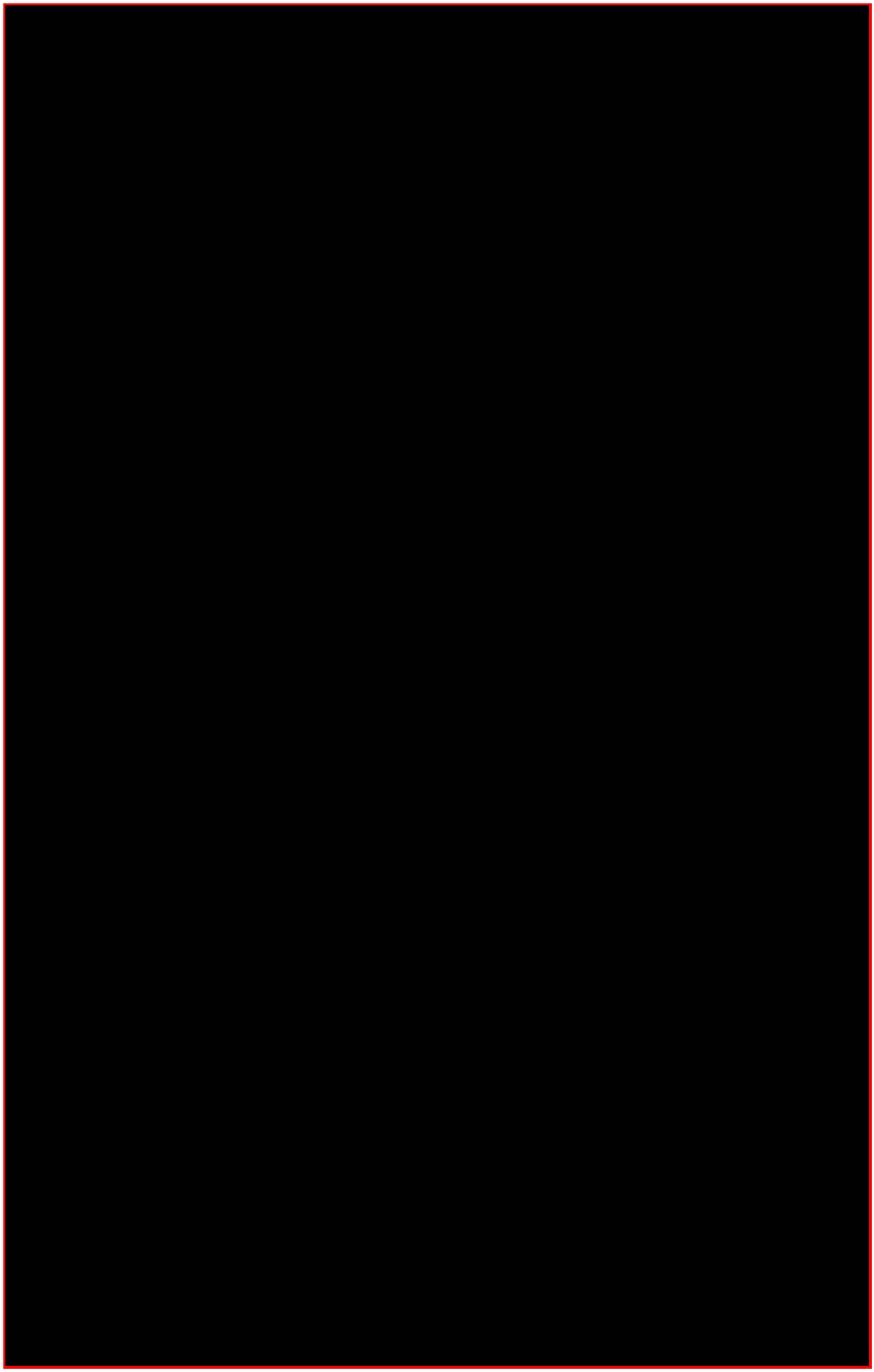
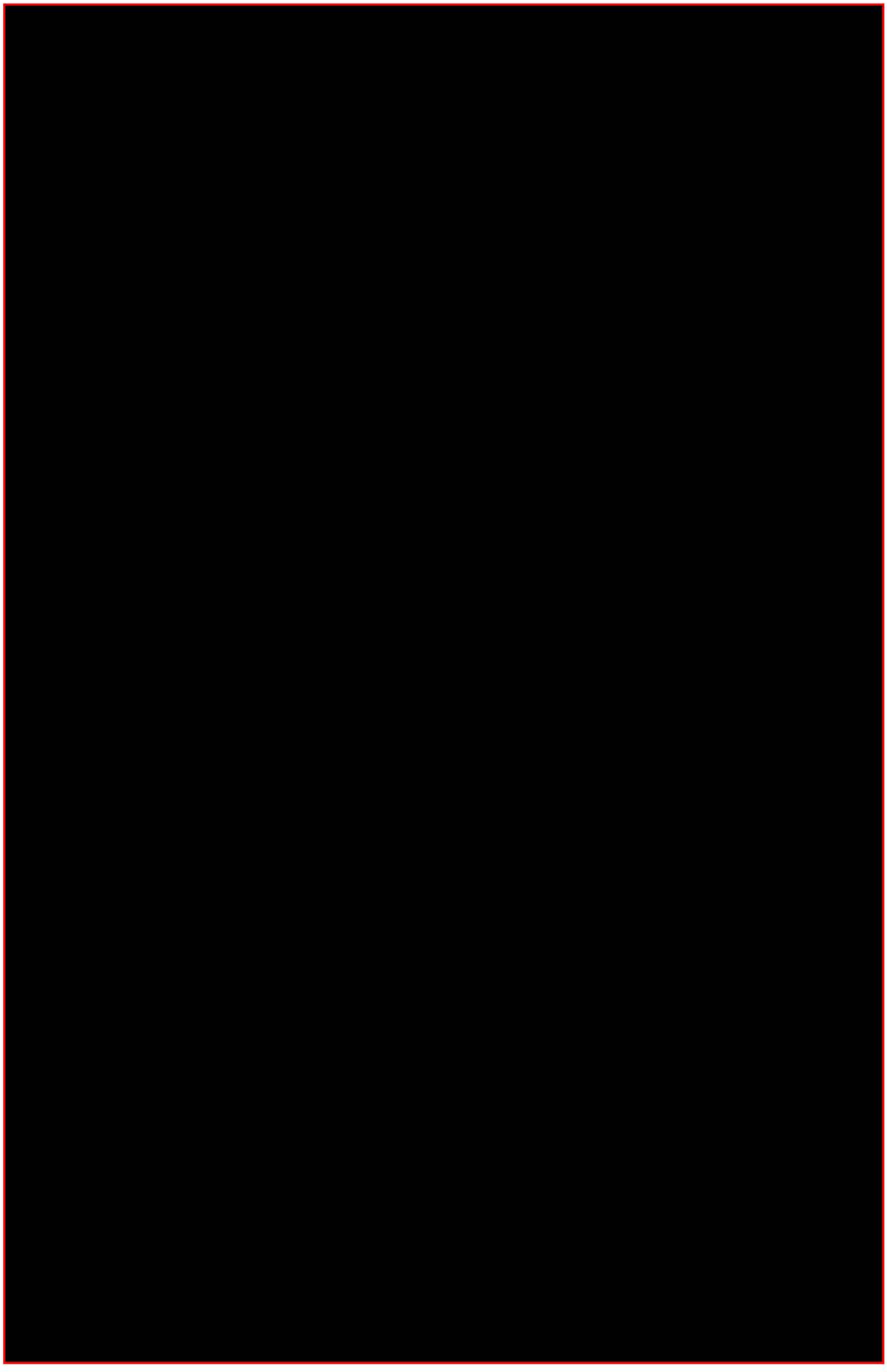
	[redacted]
Conclusion	
Remarks	

[redacted]









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

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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	

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		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	

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	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	