Section A7.1.1.1.1 Annex Point IIA7 — Hydrolysis as a function of pH and identification of breakdown products

			Official use only
		1 REFERENCE	
1.1	**Reference**	A 7.1.1.1.1	
		[redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	McLaughlin Gormley King Company (MGK)	
1.2.2.	Companies with letter of access	Pet and Garden Manufacturing Ltd.	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA guideline N 161-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	^{14}C-Methyl nonyl ketone	
3.1.1	Lot/Batch number		
3.1.2	Specification	Liquid	
3.1.3	Purity	98.1% (the radiochemical purity)	
3.1.4	Further relevant properties		
3.2	**Reference substance**	No	
3.2.1	Initial concentration of reference substance		
3.3	**Test solution**		
3.4	**Testing procedure**	Non-entry field	
3.4.1	Test system	Hydrolysis was performed for 30 days in a thermostated incubator.	
3.4.2	Temperature	25°C	
3.4.3	pH	pH 5, pH 7, pH 9	
3.4.4	Duration of the test	30 days	
3.4.5	Number of replicates		
3.4.6	Sampling		

3.4.7	Analytical methods	TLC, HPLC, GC/MS, LSC
3.5	**Preliminary test**	

4 RESULTS

4.1	**Concentration and hydrolysis values**	***Hydrolysis of Methyl Nonyl Ketone (MNK) was conducted in a test at a nominal test concentration of 1 mg/L in four aqueous buffer solutions: pH 5, 100 mM acetate buffer; pH 7, 100mM TRIS; pH 7, 10 mM (HEPES); and pH 9, 100Mm borate buffer. To determine the percent of methyl nonyl ketone in each test sample, HPLC was used. LSC was used to determine the total activity of the test compound at each sample point.*** ***The data generated during this study prove that ^{14}C-Methyl nonyl ketone does not hydrolyze in the pH range of 5-9. Also, ^{14}C-Methyl nonyl ketone is not affected by buffer type or concentration at pH 7.*** ***TLC was used to confirm the percent of MNK in the terminal samples (Day 30). Additional analysis by MS was performed on the Day 30 pH 7 (TRIS) sample and confirmed that MNK remained stable.*** ***The overall ^{14}C-mass balance indicated no significant los of ^{14}C-activity from any of the test systems. The mean mass balance was 95.1%, 95.4%, 95.3% and 96.1% of IMD for the pH 5, pH 7 (TRIS), pH 7 (HEPES) and pH 9 tests systems, respectively.***
4.2	**Hydrolysis rate constant (k_h)**	Because of lack of degradation, the rate constants were determined to be statically no significantly different from 0, Yielding infinite half-lives.
4.3	**Dissipation time**	-
4.4	**Concentration – time data**	-

4.5 Specification of the transformation products -

	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Hydrolysis of ^{14}C-Methyl nonyl ketone was tested in aqueous buffer solutions at three different pH-values (pH 5, pH 7, and pH 9) and at a temperature of 25°C. The concentration of MNK in the buffer solutions was 1.0 mg/l. The solutions were incubated in the dark under sterile conditions. Sampling times were 0, 1, 3,7,14, 21 and 30 days after treatment (DAT)	
5.2 Results and discussion	It can be concluded that Methyl nonyl ketone is hydrolytically stable in the whole investigated pH-range (pH 5 to pH 9). No half-lives were calculated. No hydrolysis studies were carried out with metabolites, because no relevant metabolites appeared. BREAKDOWN PRODUCTS: -	
5.2.1 k_H	-	
5.2.2 DT_{50}	-	
5.2.3 r^2	-	
5.3 Conclusion	It can be concluded that Methyl nonyl ketone is hydrolytically stable in the whole investigated pH-range (pH 5 to pH 9). No half-lives were calculated. No hydrolysis studies were carried out with metabolites, because no relevant metabolites appeared.	
5.3.1 Reliability	*1*	
5.3.2 Deficiencies	*No*	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	11-11-2008.	
Materials and Methods	The applicant's version is acceptable. ***Hydrolysis of Methyl Nonyl Ketone (MNK) was conducted in a test at a nominal test concentration of 1 mg/L in four aqueous buffer solutions: pH 5, 100 mM acetate buffer; pH 7, 100mM TRIS (hydroxymethyl)aminomethane; pH 7, 10 Mm (HEPES) (N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid); and pH 9, 100Mm borate buffer. The radiochemical purity of the [^{14}C]-labelled MNK was > 98.1%. The solutions were incubated at 25° C in the dark under sterile conditions. To determine the percent of methyl nonyl ketone in each test sample, HPLC was used. LSC was used to determine the total activity of the test compound at each sample point. Sampling times were 0, 1, 3,7,14, 21 and 30 days after treatment (DAT).*** **Comments:** The test protocol deviated from the OECD guideline 111 in terms of pH values used (pH 5, 7 and 9) and temperature (1 instead of 2).	
Results and discussion	It is adopted applicant's version. No significant degradation of parent compound was observed in any of the buffered test systems during the 30-day study. The rate constants (slope of degradation rate curves) were not significantly different than 0 and yielded infinite half-lives. The degradation rates for each test solution were calculated assuming first-order kinetics. The results of the TLC and MS analysis confirmed that MNK remained stable. The overall ^{14}C-mass balance indicated no significant los of ^{14}C-activity from any of the test systems. The mean mass balance was 95.1%, 95.4%, 95.3% and 96.1% of IMD for the pH 5, pH 7 (TRIS), pH 7 (HEPES) and pH 9 tests systems, respectively (see Table 3).	
Conclusion	It is adopted applicant's version. It can be concluded that MNK is hydrolytically stable in the whole investigated pH range.	
Reliability	This study is classified with a reliability of 2.	
Acceptability	This study is considered acceptable because it does not present important deficiencies.	
Remarks	The Doc.III of this study was not provided by the Applicant although it was requested by RMS. As a consequence of this, RMS completes the Applicant's evaluation following strictly the information containing in the study.	

Table 1: Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
5	100 mM	0.2 M acetic acid and 0.2 M Sodium acetate
7	100 mM	0.2 M HCl and 0.2 M TRIS (hydroxymethyl)aminomethane
7	10 mM	0.2 M HCl and 0.2 M HEPES (N-2-hydroxyethylpiperazine-N´-2-ethanesulfonic acid)
9	100 mM	0.2 M boric acid and 0.2 M borax solution

Table 2: Description of test solution

Criteria	Details
Purity of water	Deionised water
Preparation of test medium	
Test concentrations (mg a.i./L)	1 ppm
Temperature (°C)	25
Controls	
Identity and concentration of co-solvent	
Replicates	

Table 3. Recovery of radioactivity in % TAR during hydrolysis of ^{14}C-Methyl nonyl ketone

Time (d)	pH 5	pH 7 (TRIS buffer)	pH 7 (HEPES buffer)	pH 9
0	100	100	100	100
1	91.6	94.7	94.3	95.3
3	87.5	93.2	95.3	90.3
7	96.8	97.7	96.6	100.8
14	98	93.3	95.3	95.4
21	95.3	95.8	89.6	94.4
30	96.5	93.1	96.3	96.7
Mean ± sd	**95.1 ± 4.2**	**95.4 ± 2.6**	**95.3 ± 3.1**	**96.1 ± 3.5**

Section IIIA 7.1.1.2 Biotic
Annex Point IIA 7.6.1.1 **IIIA 7.1.1.2.1 Ready Biodegradability**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: February 25, 2002 - March 28, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Pet and Garden Manufacturing Ltd.	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, test method was based on OECD 301B	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	MNK TECH (Metyl Nonyl Ketone)	
3.1.1	Lot/Batch number	0550109421006	
3.1.2	Specification	Please, refer to point 3.1.3	
3.1.3	Purity	99.5%	
3.1.4	Further relevant properties	MNK is extremely insoluble in water (0.018 g/l)	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	No	
3.2	**Reference substance**	Yes, Sodium acetate	
3.2.1	Initial concentration of reference substance	10 mg total organic Carbon [C]/l	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Please refer to Table A 7.1.1.2.1-1	

Section IIIA 7.1.1.2 **Biotic**
Annex Point IIA 7.6.1.1 **IIIA 7.1.1.2.1 Ready Biodegradability**

3.3.2	Test system	Please refer to Table A 7.1.1.2.1-2
3.3.3	Test conditions	Please refer to Table A 7.1.1.2.1-3
3.3.4	Method of preparation of test solution	Insoluble substances were first weighed onto microcopic coverslips which were then introduced directly to the bioreactor. Soluble and emulsifiable substances were washed in to the bioreactor using approximately 10 ml of distilled water.
3.3.5	Initial TS concentration	10 mg total organic Carbon [C]/l
3.3.6	Duration of test	29 days
3.3.7	Analytical parameter	CO_2 evolution determined as dissolved inorganic Carbon (DIS)
3.3.8	Sampling	Sampling occurred on days 2, 4, 7, 10, 14, 18, 23, 28 and 29
3.3.9	Intermediates/ degradation products	Not identified
3.3.10	Nitrate/nitrite measurement	No
3.3.11	Controls	Two control vessels contained inoculated mineral salts medium. Two reference vessels contained inoculated mineral salts medium and Sodium acetate. An additional mixture containing Sodium acetate, inoculated mineral salts medium and MNK was established in order to assess the potential inhibitory effects of the test substance on the activity of the microbial inoculum.
3.3.12	Statistics	The amount of carbon dioxide produced was expressed as a percentage of the organic carbon in the test material. Test and reference values were corrected for inoculum blank.
		4 RESULTS
4.1	**Degradation of test substance**	
4.1.1	Graph	Please refer to Figure A 7.1.1.2.1-1
4.1.2	Degradation	MNK attained 23.5% degradation after 29 days. Please refer to Tables A 7.1.1.2.1-4 and A 7.1.1.2.1-5
4.1.3	Other observations	The Toxicity Control attained 74% degradation after 29 days, indicating that MNK is not inhibitory to the activity of the microbial inoculum. Please, refer to Tables A 7.1.1.2.1-4 and A 7.1.1.2.1-5.
4.1.4	Degradation of TS in abiotic control	No abiotic control
4.1.5	Degradation of reference substance	Sodium acetate attained 77% degradation after 29 days. Please refer to Tables A 7.1.1.2.1-4 and A 7.1.1.2.1-5
4.1.6	Intermediates/ degradation	Not applicable

Section IIIA 7.1.1.2 **Biotic**
Annex Point IIA 7.6.1.1 **IIIA 7.1.1.2.1 Ready Biodegradability**

products

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The ready biodegradability of MNK was assessed in a CO_2 evolution test. MNK was added to two vessels containing mineral salts medium inoculated with activated sludge at a nominal test concentration of 10 mg/Carbon/l. Two control vessels and the reference substance Sodium acetate were also investigated. An additional mixture containing Sodium acetate and MNK was established in order to assess the potential of the test substance for microbial inhibition. Carbon dioxide was trapped in Sodium hydroxide and measured as Dissolved Inorganic Carbon (DIC).

This study was conducted according to OECD guideline 301B 'Ready Biodegradability: CO_2 evolution test' and is described under point 3.

5.2 Results and discussion

MNK achieved 23.5% degradation after 29 days. Substances are considered to be readily biodegradable in the test if CO_2 production is equal to or greater than 60% of the t heretical value within 10 days of the level achieving 10%. MNK cannot therefore be considered readily biodegradable. However, because of the stringencies of this type of test, poorly soluble substances that fail to show the required rate of biodegradation are not necessarily poorly degradable under environmental conditions. Biodegradation may progress at a higher rate where the substance, at a concentration within its limit of aqueous solubility, is exposed to a larger and more diverse microbial population.

Sodium acetate achieved 77% degradation after 29 days.

The toxicity Control achieved 74% degradation after 29 days, indicating that MNK is not inhibitory to the activity of the microbial inoculum.

5.3 Conclusion

The validity Criteria for the test were fulfilled (please refer to Table A 7.1.1.2.1-6). MNK is not readily biodegradable.

5.3.1 Reliability

1

5.3.2 Deficiencies

No

Section IIIA 7.1.1.2 **Biotic**
Annex Point IIA 7.6.1.1 **IIIA 7.1.1.2.1 Ready Biodegradability**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28-02-2007
Materials and Methods	It is adopted applicant's version. The ready biodegradability of MNK was assessed in a CO_2 evolution test. MNK was added to two vessels containing mineral salts medium inoculated with activated sludge at a nominal test concentration of 10 mg TOC/l. Two control vessels and the reference substance Sodium acetate were also investigated. An additional mixture containing Sodium acetate and MNK was established in order to assess the potential of the test substance for microbial inhibition. Carbon dioxide was trapped in Sodium hydroxide and measured as Dissolved Inorganic Carbon (DIC). **Comments:** In the test report (section 2.3, Principle of the test medod) is mentioned: "*A measured volume of inoculated mineral medium, containing a known concentration of the test substance (10 to 20 mg TOC/l) as the nominal source of organic carbon, is aerated by the passage of carbon dioxide free air at a controlled rate in the dark at 22 ± 2°C*". The exact concentration of MNK expressed as mg TOC/l was not reported in the study. However, assuming a 3 litres inoculated mixture (according to OECD guideline), and taking into account that quanty of MNK in the replicates was equivalent to 30 mg TOC, the concentration of MNK (as the sole source of organic carbon) was assumed to be 10 mg TOC/l.
Results and discussion	It is adopted applicant's version (revised). MNK achieved 23.5% degradation after 29 days. Substances are considered to be readily biodegradable in the test if CO_2 production is equal to or greater than 60% of the theoretical value within 10 days of the level achieving 10%. MNK cannot therefore be considered readily biodegradable. Sodium acetate achieved 77% degradation after 29 days. The toxicity Control achieved 74% degradation after 29 days, indicating that MNK is not inhibitory to the activity of the microbial inoculum. **Comments:** The CO_2 evolution method (guideline OECD 301 B) is not the most suitable method for determination of ready biodegradability for compounds which are volatile such as MNK (the vapour preassure of MNK was determined to be 11.8 Pa at 20°C). Moderately volatile chemicals may be tested by the DOC Die-Away method if there is sufficient gas space in the test vessels (which should be suitably stopperd). In this case, an abiotic control must be set up to allow for any physical loss. Also, the volatile chemicals may be tested by other methods such as Closed Bottle (guideline OECD 301 D), CO_2 in sealed vessels -Headspace test- (guideline OECD 310).
Conclusion	It is adopted applicant's version (because environmental is the most conservative assumption).

Section IIIA 7.1.1.2
Annex Point IIA 7.6.1.1

Biotic
IIIA 7.1.1.2.1 Ready Biodegradability

	MNK is not readily biodegradable under the test conditions.
Reliability	This study is classified with a reliability of 3.
Acceptability	This study is considered acceptable although it presents important deficiencies.
Remarks	

Table A 7.1.1.2.1-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Source	[redacted]
Sampling site	[redacted]
Preparation of inoculum for exposure	The sludge was used directly upon collection. It was passed through a 500 µm sieve, centrifuged (at 4000 rpm for 5 minutes) and re-suspended in mineral media. This process was repeated. The solids content of the centrifuged sludge was determined by loss on drying (at 105ºC).
Pretreatment	No
Initial cell concentration	30 mg/l suspended solids

Table A 7.1.1.2.1-2: Test system

Criteria	Details
Culturing apparatus	Bioreactor
Number of culture flasks/concentration	Seven. Two vessels were used for test, reference and control tests. One vessel was used for toxicity tests.
Measuring equipment	Carbon dioxide was measured as Dissolved Inorganic Carbon (DIC) using a Tekmar-Dohrmann Phoenix 8000 (UV-Persulfate Analyser).
Test performed in closed vessels due to significant volatility of TS	No

Table A 7.1.1.2.1-3: Test conditions

Criteria	Details
Additional substrate	No
Test temperature	22 ± 2°C
Suspended solids concentration	30 mg/l
Other relevant criteria	No

Table A 7.1.1.2.1-4: Cumulative Inorganic carbon produced in MNK, Sodium acetate and toxicity Control

Time (days)	Cumulative inorganic carbon from test (mg)						
	Sodium acetate			MNK			Toxicity Control
	1	2	Average	1	2	Average	1
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	6.89	10.53	8.71	1.14	1.06	1.10	5.80
4	9.41	16.52	12.96	2.77	2.76	2.76	9.25
7	16.41	20.24	18.32	6.00	4.83	5.41	12.75
10	18.14	21.74	19.94	6.93	6.83	6.88	*
14	19.42	22.93	21.17	7.23	6.96	7.09	16.95
18	19.82	23.63	21.72	7.45	7.11	7.28	18.72
23	19.80	24.45	22.12	7.66	7.21	7.43	19.81
28	19.58	24.88	22.23	7.78	7.23	7.50	20.55
29	19.36	25.10	22.23	7.68	7.20	7.44	21.45
29	20.17	25.97	23.07	7.86	6.25	7.05	22.08

* Sample lost

Table A 7.1.1.2.1-5: Percentage degradation of MNK, Sodium acetate and toxicity Control

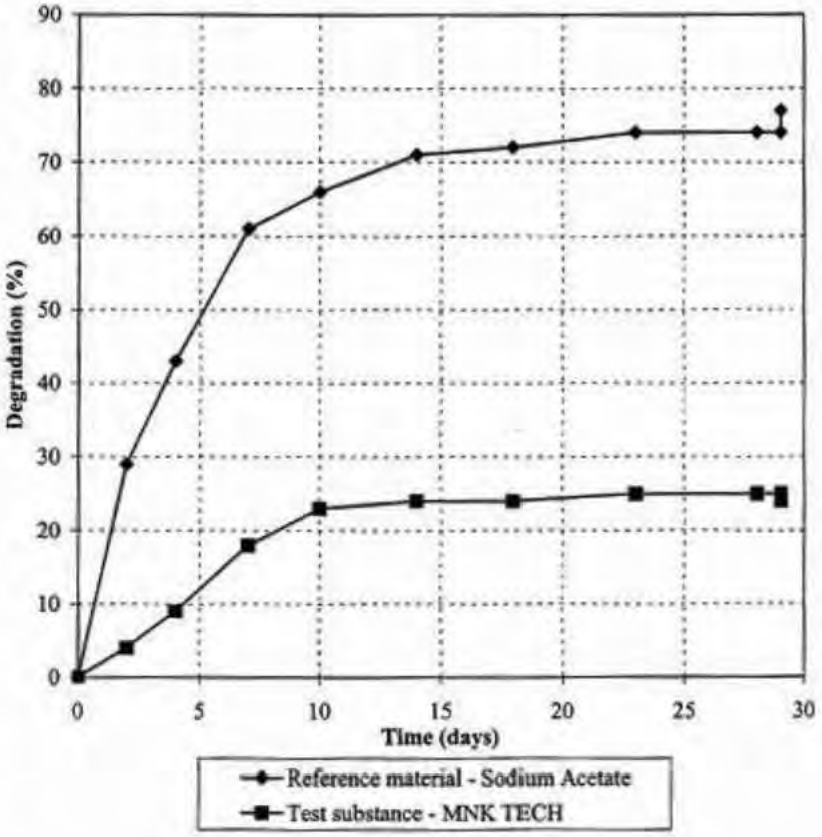
Time (days)	Percentage degradation (%)						
	Sodium acetate			MNK			Toxicity Control
	1	2	Average	1	2	Average	1
0	0	0	0.0	0	0	0.0	0
2	23	35	29.0	4	4	4.0	19
4	31	55	43.0	9	9	9.0	31
7	55	67	61.0	20	16	18.0	43
10	60	72	66.0	23	23	23.0	*
14	65	76	70.5	24	23	23.5	57
18	66	79	72.5	25	24	24.5	62
23	66	81	73.5	26	24	25.0	66
28	65	83	74.0	26	24	25.0	68
29	65	84	74.5	26	24	25.0	71
29	67	87	77.0	26	21	23.5	74

* Sample lost

Table A 7.1.1.2.1-6: Pass levels and validity criteria for tests on ready biodegradability

	Fulfilled
Pass levels	
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	No
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	No
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%	Yes
Percentage of removal of reference substance reaches pass level by day 14	Yes

Figure A 7.1.1.2.1-1: Percentage degradation of MNK and Sodium acetate



Section A 7.2.1 **Aerobic degradation in soil, initial study**
Annex Point/TNsG

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1 REFERENCE

1.1 Reference
A 7.2.1

[redacted]

1.2 Data protection Yes

1.2.1 Data owner McLaughlin Gormley King Company (MGK)

1.2.2. Companies with letter of access Pet and Garden Manufacturing Ltd.

1.2.3 Criteria for data protection Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes
EPA guideline N 162-1

2.2 GLP Yes
(only where required)

2.3 Deviations No

Section A 7.2.1 **Aerobic degradation in soil, initial study**
Annex Point/TNsG

3 MATERIALS AND METHODS

3.1 Test material ^{14}C-Methyl nonyl ketone

3.1.1 Lot/Batch number

3.1.2 Specification

3.1.3 Description

3.1.4 Purity 98.1%

3.1.5 Stability

4 RESULTS

4.1 Contact time 1 month

4.2 Degradation Half-life: 4.07 days at 25° C

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods-discussion

The aerobic soil metabolism study with ^{14}C-methyl nonyl ketone was conducted under dark conditions on sandy loam soil. This study was conducted in an environmental chamber regulated at 25 ± 1 °C and the moisture level was adjusted between 70-75% field capacity. The concentration of ^{14}C-methyl nonyl ketone in the test system after dosing was determined to be 10.24 ppm (mean of triplicate Day 0 samples).

Soil samples collected throughout the study were extracted and analyzed for residues of ^{14}C-methyl nonyl ketone by LSC and HPLC analysis. Samples were collected at 0, 4, 8, 16, 24 and 36 hours, and at day 2, 3, 4, 7, 14 and 30.

5.2 Results and discussion

The extractable residues markedly decreased during the study, obtaining a maximum concentration of 99.4% of IMD at hour 0, and declining to a minimum of 1.94% of the IMD at the Day 14 sample point. The bound ^{14}C-residues in the soil increased from 0.59% of IMD at Day 0 to 58.2 of IMD at the Day 1 and then gradually decreased to 38.3% of the IMD at the study's termination point. The level of ^{14}C-volatile degradation products was 3.21% of the IMD at 4 hours and increased to 60.7% of the IMD at the Day 30 sample point.

The majority of the ^{14}C-volatile activity (> 80%) was found in the KOH traps. The ^{14}C-activity found in the KOH traps was confirmed to be $^{14}CO_2$ by barium chloride precipitate analysis. The majority of the remaining ^{14}C-volatile activity (19%) was found in the foam plug traps. The ^{14}C-activity found in the foam plug traps was confirmed to be ^{14}C-methyl nonyl ketone by HPLC.

The mean ^{14}C-mass accountability for the study was 96.5% of the IMD through 1 month.

The half-life determinations were calculated by using first-order kinetics. The concentration of the parent compound decreased from 98.9% of the IMD at day 0 to 0.4% of the IMD at the study's termination. An initial rapid degradation

Section A 7.2.1 **Aerobic degradation in soil, initial study**
Annex Point/TNsG

half-life (day 0 to day 1.5) was calculated to be 0.374 days (correlation = 0.994). The half-life for the period following the rapid degradation (day 1.5 to 1 month) was calculated to be 6.19 days (correlation = 0.882). This accounted for an overall net half-life (day 0 through 1 month) of 4.07 days (correlation = 0.807). The presence of ^{14}C-methyl nonyl ketone was confirmed by MS.
The major degradation product was $^{14}CO_2$, accounting for 48.7% of the IMD at the study's termination. In addition to the evolution of the $^{14}CO_2$, six degradation products were observed in the soil extracts. The degradation products did not exceed 10% of the IMD at any sample point. Attempts were made to isolate and propose structures for metabolites, which were observed at a concentration exceeding 0.01 ppm. Structure elucidation is based on LC/MS analysis.

Based on the structures of the parent compound and its metabolites observed during the study, it can be theorized that the test compound will further metabolize to $^{14}CO_2$ following the classical β-oxidation sequence of fatty acids.

5.3 Conclusion The half-life of MNK was determined 4.07 days

5.3.1 Reliability 1

5.3.2 Deficiencies No

Section A 7.2.1 Aerobic degradation in soil, initial study
Annex Point/TNsG

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

8-11-2008

Materials and Methods

It is adopted applicant's version (revised).

The aerobic soil metabolism was investigated applying ^{14}C-Methyl Nonyl Ketone labelled in the position 7 of the chain to sandy loam soil (*see Table 1*) at approximately 10 mg MNK/kg soil used. The radiochemical purity of the [^{14}C]-labelled MNK was 98.1%. The soil was kept under aerobic conditions in the dark at 25 ± 1 ºC and approximately 75% of field capacity. Soil samples collected throughout the study were extracted and analyzed for residues of ^{14}C-Methyl Nonyl Ketone by Liquid Scintillation Counting (LSC) and High Performance Liquid Chromatographic (HPLC) analysis. Samples were collected at 0, 4, 8, 16, 24 and 36 hours, and at day 2, 3, 4, 7, 14, and 1 month.

Results and discussion

It is adopted applicant's version (revised).

The extractable residues markedly decreased during the study, obtaining a maximum concentration of 99.4% of Initial Measured Dose (IMD) at hour 0, and declining to a minimum of 1.94% of the IMD at the day 14 sample point (*see Table 2*). The bound ^{14}C-residues in the soil increased from 0.59% of IMD at day 0 to 58.2% of IMD at the day 1 and then gradually decreased to 38.3% of the IMD at the study's termination point. The level of ^{14}C-volatile degradation products was 3.21% of the IMD at 4 hours and increased to 60.7% of the IMD at the sample point.

The majority of the ^{14}C-volatile activity (> 80%) was found in the KOH traps. The ^{14}C-activity found in the KOH traps was confirmed to be $^{14}CO_2$ by barium chloride precipitate analysis. The majority of the remaining ^{14}C-volatile activity (11.78%) was found in the foam plug traps. The ^{14}C-activity found in the foam plug traps was confirmed to be ^{14}C-Methyl Nonyl Ketone by HPLC/MS.

The mean ^{14}C-mass accountability for the study was 96.5% of the IMD through 1 month.

The half-life determinations were calculated by using first-order kinetics. The concentration of the parent compound decreased from 98.9% of the IMD at day 0 to 0.4% of the IMD at the study's termination (*see Table 3*). An initial rapid degradation half-life (day 0 to day 1.5) was calculated to be 0.374 days (correlation = 0.994). The half-life for the period following the rapid degradation (day 1.5 to 1 month) was calculated to be 6.19 days (correlation = 0.882). This accounted for an overall net half-life (day 0 through 1 month) of 4.07 days (correlation = 0.807).

The major degradation product was $^{14}CO_2$, accounting for 48.7% of the IMD at the study's termination. In addition to the evolution of the $^{14}CO_2$, six degradation products were observed in the soil extracts. The degradation products did not exceed 3% of the IMD at any sample point. Attempts were made to isolate and propose structures for metabolites, which were observed at a concentration exceeding 0.01 ppm. Structure elucidation is based on LC/MS analysis. The identified metabolites were: 4-hydroxy-2-undecanone; 10-hydroxy-2-undecanone; 2,4-undecanone; 2,10-undecanone and 4-hydroxypentanoic acid. Based on the structures of the parent compound and its metabolites observed during the study,

Section A 7.2.1 **Annex Point/TNsG**	**Aerobic degradation in soil, initial study**

it can be theorized that the test compound will further metabolize to CO_2 following the classical β-oxidation sequence (*see Fig 1*).

Comments:

A half-life of 1.22 days was calculated from a preliminary aerobic metabolism study based on the results of the HPLC analysis of soil extracts. However, the data for this preliminary investigation are not attached with the definitive study. Therefore, this information was only taken into account as complementary information that supports the estimated DT_{50} in the definitive study.

Conclusion

Ii is adopted applicant's version

Methyl Nonyl Ketone is rapidly degraded in soil. Its overall net half-life (Day 0 through 1 month) at 25ºC was estimated to be of 4.07 days.

Reliability

This test is assessed with reliability indicator of 2

Acceptability

This test is considered acceptable since it does not present important deficiencies.

Remarks

The Doc.III of this study was not provided by the Applicant although it was requested by RMS.

As a consequence of this, RMS completes the Applicant's evaluation following strictly the information containing in the study.

Table 1. Soil specifications

Characteristic	Origin (Arizona, USA)
Textural class	Sandy loam
pH	8.5
% Organic matter	0.7
CEC (mval/100 g)	19.8
Bulk density (g/ml)	1.16
Particles size distribution	Weight %
Sand Silt Clay	58.7 28.0 13.3
1/3 Bar moisture percentage	21.2
Total plate count (colonies/g) at the beginning the test	2.0×10^7
Total plate count (colonies/g) at the end the test (30 DAT)	7.9×10^5
Fungal plate count (colonies/g) at the beginning the test	2.0×10^4
Fungal plate count (colonies/g) at the end the test (30 DAT)	$< 10 \times 10^4$

Table 2. Distribution of radioactivity expressed as % IMD after the application of MNK to soil and incubation under aerobic conditions

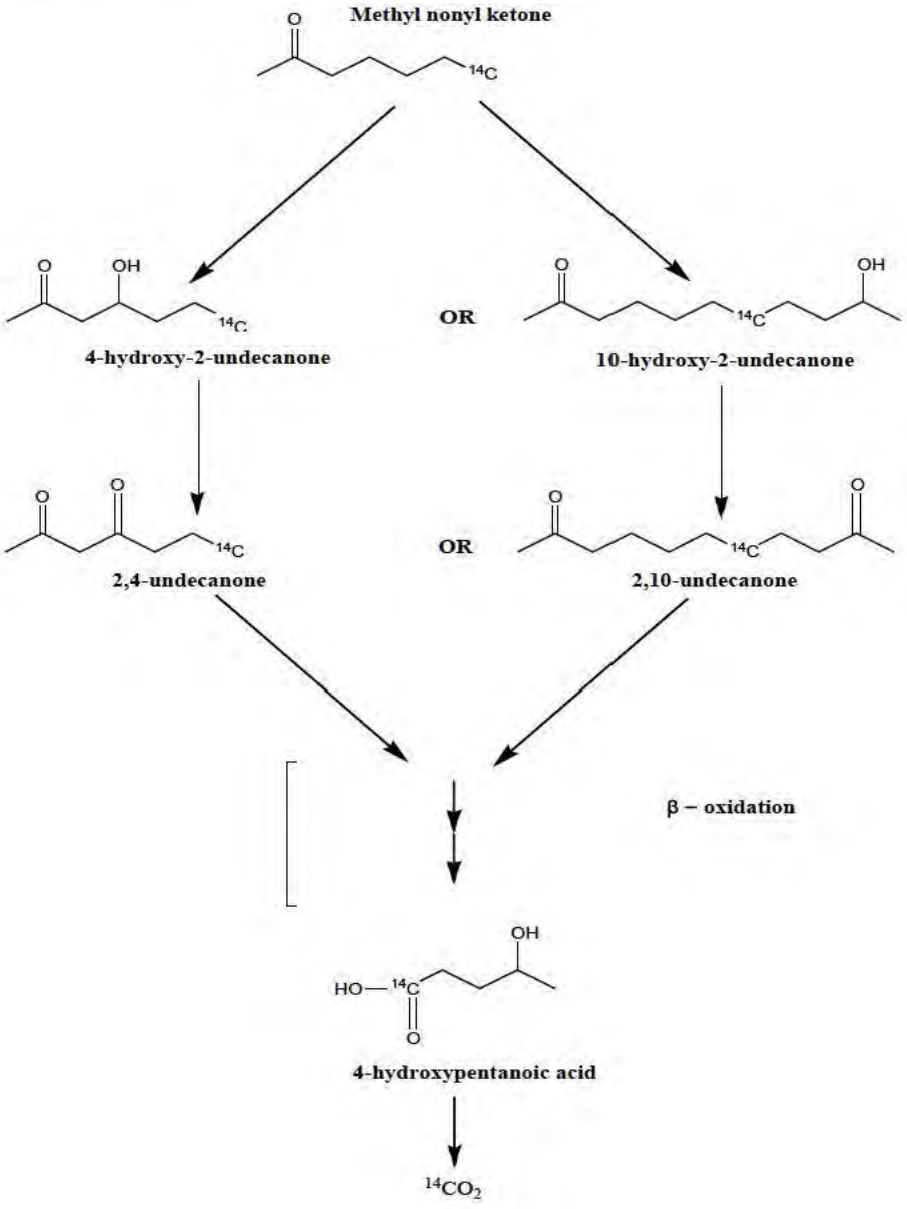
Analysis time	Extract residue	Bound residue	Others*	CO_2	MNK volatilized	Total
0	99.4	0.59	0.00	0.00	0.00	100
hour	77.3	9.80	0.01	0.20	3.00	90.8
8 hour	70.4	27.6	0.01	0.50	6.55	106.2
16 hour	31.9	43.6	0.02	4.48	8.77	90.3
1	20.8	58.2	0.02	4.77	8.88	94.5
36 hour	12.3	54.5	0.08	20.25	9.68	98.4
2	10.9	47.2	0.1	26.05	10.11	94.3
3	14.4	44.5	0.12	29.45	10.71	100.4
4	6.61	45.6	0.13	30.82	10.88	94.5
7	4.01	38.3	0.14	38.49	11.26	92.9
14	1.94	32.9	0.20	46.55	11.57	94.0
1 month	2.27	38.3	0.21	48.67	11.78	101.7

*Others = Ethylene glycol trap + H_2SO_4 trap

Table 3. Distribution of MNK and its metabolites in soil extracts following the treatment of ^{14}C-MNK at an application rate of approx. 10 mg/kg soil. The results are expressed as % IMD

Analysis time	MNK	Met. 1	Met. 2	Met. 3	Met. 4	Met. 5	Met. 6
0	98.93	0.000	0.000	0.00	0.00	0.00	0.05
4 hour	74.89	0.114	0.000	0.08	0.01	0.66	1.15
8 hour	66.55	0.302	0.000	0.02	0.00	1.27	1.58
16 hour	26.27	0.913	0.000	0.00	0.00	0.88	2.15
1	14.22	1.214	0.101	0.14	0.00	0.56	2.35
36 hour	6.94	1.106	0.000	0.04	0.03	0.24	2.28
2	6.19	1.109	0.000	0.08	0.00	0.22	1.58
3	10.91	0.697	0.000	0.16	0.00	0.09	2.22
4	3.73	0.716	0.000	0.12	0.03	0.00	1.71
7	1.52	0.889	0.000	0.24	0.00	0.00	1.23
14	0.57	0.604	0.000	0.00	0.00	0.11	0.56
1 month	0.37	0.739	0.000	0.00	0.00	0.07	1.04

Figure 1. **Proposed degradation pathways of MNK in soil**



Section IIIA 7.2.3 Adsorption and Mobility in Soil, further studies

Annex Point XII.1.2 **IIIA 7.2.3.1 Adsorption and desorption studies**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: March 4, 2002 – March 8, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Pet and Garden Manufacturing Ltd.	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes, test method was based on OECD guideline 121	
2.3	**GLP**	Yes	
2.4	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.2	**Test material**	MNK TECH (Methyl Nonyl Ketone)	
3.2.1	Lot/Batch number	0550109421006	
3.2.2	Specification	Please refer to point 3.1.3 to 3.1.5.	
3.2.3	Purity	99.5%	
3.2.4	Specific activity	Not applicable	
3.2.5	Radiolabeling	Not applicable	
3.2.6	Further relevant properties	MNK is extremely insoluble in water (0.018 g/l)	
3.2.7	Method of analysis	HPLC was performed on MNK and the reference substances. A chromatography system consisting of an RI and UV detector was used to calculate retention times. Calibration graphs were generated for log k against log K_{oc} for the reference compounds and from this, K_{oc} values for MNK could be deduced. Please, refer to Figure A 7.2.3.1-1.	
3.3	**Degradation products**	Not applicable	
3.3.1	Method of analysis for degradation products	Not applicable	
3.4	**Reference substance**	Yes, please refer to Table A 7.2.3.1-1	
3.4.1	Method of analysis for reference substance	HPLC with UV and RI detection	

Section IIIA 7.2.3 **Adsorption and Mobility in Soil, further studies**

Annex Point XII.1.2 **IIIA 7.2.3.1 Adsorption and desorption studies**

3.5	**Soil types**	Not applicable-HPLC method used
3.6	**Testing procedure**	
3.6.1	Test system	Tests were conducted in a Perkin Elmer Quaternary chromatography system consisting of an RI and UV detector.
3.6.2	Test solution and Test conditions	HPLC-grade methanol and distilled water were added to a pH 7.0 buffer at a ratio of 55:45. This solvent was then degassed. MNK was then dispersed into the solvent.
3.7	**Test performance**	
3.7.1	Preliminary test	Not applicable
3.7.2	Screening test: Adsorption	Please, refer to point 3.6.4
3.7.3	Screening test: Desorption	Not applicable
3.7.4	HPLC-method	According to "OECD-HPLC- method"[1]: Yes HPLC grade methanol and distilled water, buffered to pH 7.0, were used to prepare the eluting solvent. The mixture was degassed before use. The test material, MNK, was dispersed in this solvent and injected into the chromatography system in duplicate (0.13g in 10 ml per injection). Reference substances were also injected into the system. Please refer to Table A 7.2.3.1-1 for details on quantities injected. HPLC was performed on MNK and the reference substances. A chromatography system consisting of an RI and UV detector was used to calculate retention times. Calibration graphs were generated for log k against log K_{oc} for the reference compounds and from this, K_{oc} values for MNK could be deduced.
3.7.5	Other test	Not applicable
		4 RESULTS
3.2	**Preliminary test**	Not applicable
3.3	**Screening test: Adsorption**	Not applicable
3.4	**Screening test: Desorption**	Not applicable
3.5	**HPLC method**	Four peaks were detected with UV and one with RI. The Log K_{oc} was determined by extrapolation versus calibration graphs generated for reference compounds of known K_{oc}. Please, refer to Table A 7.2.3.1-2 and Figure A 7.2.3.1-1.

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

Section IIIA 7.2.3 **Adsorption and Mobility in Soil, further studies**

Annex Point XII.1.2 **IIIA 7.2.3.1 Adsorption and desorption studies**

4.5	**Calculations**	
4.5.1	Ka, Kd	Not documented
4.5.2	Ka_{oc}, Kd_{oc}	K_{oc} values ranged from 95 to 74,131, with an average of 16,177.2. Please, refer to Table A7.2.3.1-2
4.6	**Degradation product(s)**	Not evaluated

5 APPLICANT'S SUMMARY AND CONCLUSION

5.2	**Materials and methods**	In an adsorption study, the binding potential of MNK on soil and sewage sludge was studied by HPLC. MNK was eluted in 55:45 methanol: buffer pH 7.0 and the K_{oc} determined by extrapolation versus calibration graphs generated for reference compounds of known K_{oc}. This study was conducted according to OECD guideline 121 and is described under point 3.
5.3	**Results and discussion**	K_{oc} values ranged from 95 to 74,131 with an average of 16,177.2. According to the McCall Classification System and the SSLRC Mobility Classification Scheme, MNK is of low mobility and therefore has a tendency to adsorb to soils.
5.3.1	Adsorbed a.s. [%]	Not evaluated
5.3.2	K_a	Not evaluated
5.3.3	K_d	Not evaluated
5.3.4	K_{oc}	K_{oc} values for adsorption of MNK ranged from 95 to 74131 with an average of 16,177.2. Please refer to Table A 7.2.3.1-2.
5.3.5	Ka/Kd	Not evaluated
5.3.6	Degradation products (% of a.s.)	Not applicable
5.4	**Conclusion**	According to the adsorption constants obtained from this study by Drake R. M., which ranged from 95 to 74131, it would appear that MNK is slightly immobile and has a tendency to adsorb to sediments and soil. In a leaching-adsorption/desorption study, documented by the US EPA (1995), MNK was observed to be slightly immobile in Sodium azide-sterilised sandy loam, clay loam and silt loam soils (Kads =18; K_{oc}=2,480). MNK was observed to be relatively immobile in soil and have a low potential to leach into groundwater or move offsite into surface water. Please note: There seems to be some discrepancy between the K_{oc} values obtained by Drake, R.M. The K_{oc} values of 95 and 74131 appear to be outliers while the middle three values are more similar to each other and give an average K_{oc} of 2220, which is quite similar to values documented by the US EPA. The US EPA leaching-adsorption/desorption method is considered more reliable than the HPLC method employed by Drake, R.M. and thus the K_{oc} of 2480 will be referenced throughout this dossier and in the risk assessment carried out in Doc. IIB Section 3.3.

Section IIIA 7.2.3	**Adsorption and Mobility in Soil, further studies**
Annex Point XII.1.2	**IIIA 7.2.3.1 Adsorption and desorption studies**

5.4.1	Reliability	1
5.4.2	Deficiencies	No

Section IIIA 7.2.3 Adsorption and Mobility in Soil, further studies

Annex Point XII.1.2 **IIIA 7.2.3.1 Adsorption and desorption studies**

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1-03-2007
Materials and Methods	It is adopted applicant's version. In an adsorption study, the binding potential of MNK on soil and sewage sludge was studied by HPLC (OECD guideline 121). HPLC grade methanol and distilled water, buffered to pH 7.0, were used to prepare the eluting solvent. The mixture was degassed before use. The test material, MNK, was dispersed in this solvent and injected into the chromatography system in duplicate (0.13g in 10 ml per injection). Reference substances were also injected into the system. A chromatography system consisting of an RI and UV detector was used to calculate retention times. Calibration graphs were generated for log k against log K_{oc} for the reference compounds and from this, K_{oc} values for MNK could be deduced.
Results and discussion	It is adopted applicant's version (revised). According to the McCall Classification System and the SSLRC Mobility Classification Scheme, MNK is a compound with low/slightly mobility and therefore has a tendency to adsorb to soils and sediments. A K_{oc} value of 2,656 l/kg was estimated (*see Comments*). **Comments:** The adsorption constants obtained by Drake ranged from 95 to 74,131, since four peaks were detected with UV detector. The peaks were not identified during the test, so all of them were considered that corresponded to MNK because the test substance had a purity of 99.5%. However, according to physical-chemical properties of this substance only one of four peaks detected with UV detector should be corresponded with MNK, so the rest of peaks should be regarded as impurities. This same conclusion can be deduced from the results of chromatography with RI detector. Only one peak was detected by HPLC with RI detector. Like in the latter case this sole peak was considered that corresponded to MNK (the compound in major proportion), and on the other hand it was assumed the impurities could not be detected due to poor detection limit of the RI detectors. The Applicant provided additional study about adsorption/desorption of MNK on soil (*see Doc. III-A7.2.3.1b*). This substance was observed to be relatively immobile in soil and have a low potential to leach into groundwater or move offsite into surface water. MNK was observed to be slightly immobile in Sodium azide-sterilised sandy loam, clay loam and silt loam soils. The arithmetic mean of K_{oc} values was estimated to be 2,490 l/kg. This study was also evaluated by US-EPA* obtaining the same results as the RMS. Therefore, taking into consideration all above mentioned, the RMS decided to take as K_{oc} the average value derived from the peak detected with RI detector and from the peak detected with UV detector with a similar retention time to the peak detected with RI detector. With this approach, it was determined a K_{oc} value of 2,656 l/kg, comparable to K_{oc} value of 2,490 l/kg obtained in soil/sediment adsorption/desorption study of MNK (Doc. III-A7.2.3.1b).

Section IIIA 7.2.3 Adsorption and Mobility in Soil, further studies

Annex Point XII.1.2 **IIIA 7.2.3.1 Adsorption and desorption studies**

	*The summary of this study is included in the evaluation of U.S-EPA, Reregistration Eligibility Decision (RED): Methyl Nonyl Ketone. This document was provided by the Applicant with the Dossier. Nevertheless, it can be also downloaded from the official website of the Agency.
Conclusion	MNK is slightly mobile and has a tendency to adsorb to sediments and soil. Although a K_{oc} value was estimated (see the latter section, Results and discussion), this value is only used to supports the latter conclusion. For the risk assessment the coefficients derived from Doc. III-A7.2.3.1b were used.
Reliability	This study is classified with a reliability of 3.
Acceptability	This study is considered acceptable although it presents important deficiencies.
Remarks	

Table IIA 7.2.3.1-1: Reference substances used to generate calibration graphs

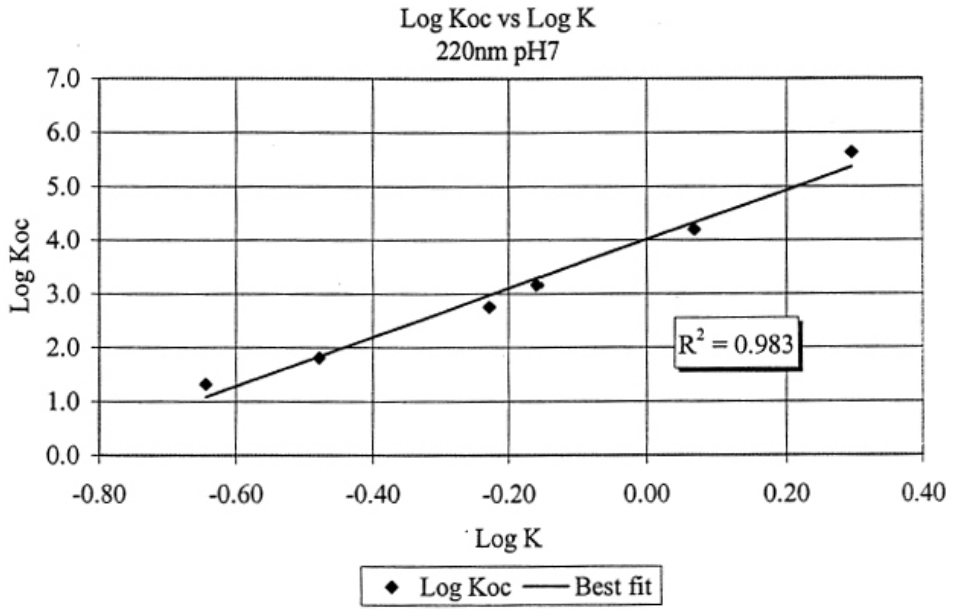
Compound	Log K_{oc} (from literature)	% Purity	Weight of Standard (mg)*	Amount injected (µg)
Phenol	**1.32**	**99+**	**8.6**	**8.6**
Atrazine	**1.81**	**98**	**9.6**	**9.6**
Naphthalene	**2.75**	**99+**	**9.0**	**9.0**
1,2,3-Trichlorobenzene	**3.16**	**99**	**8.8**	**8.8**
Dichlofop-methyl	**4.20**	**99.9**	**12.8**	**12.8**
DDT	**5.63**	**98**	**7.5**	**7.5**
Sodium Nitrate (t_0)	**-**	**>99.5**	**48.8**	**48.8**

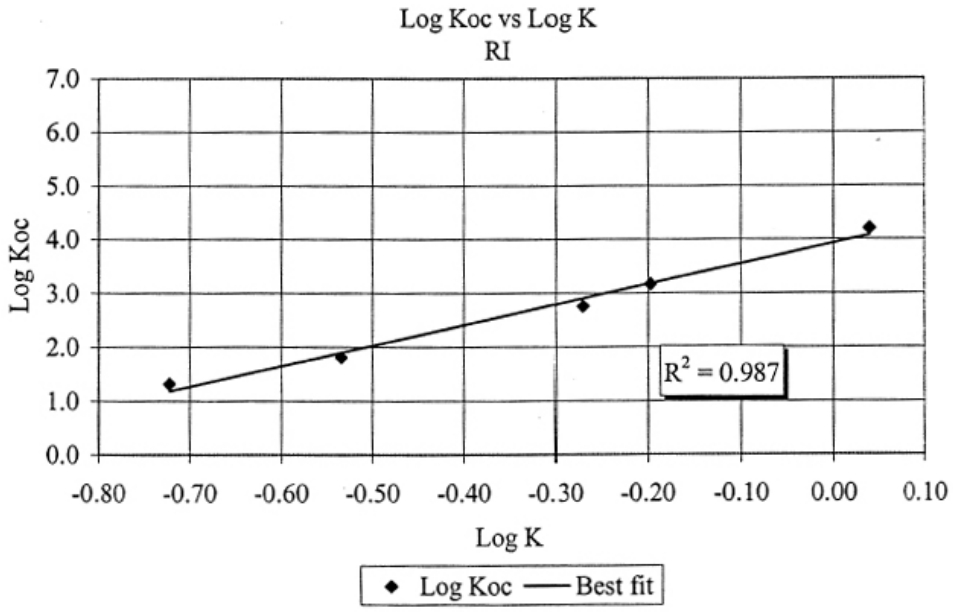
***In 20 ml of non-buffered mobile phase. On mixing the standards a precipitate was formed which settled rapidly. The standard was filtered using a 0.2 µm syringe filter prior to injection. The amount injected values represent the nominal values.**

Table IIA 7.2.3.1-2: Adsorption constants for MNK as determined by HPLC at pH 7 and 220 nm

Component	Detector	Run 1 Log K_{oc}	Run 2 Log K_{oc}	Mean Log K_{oc}	K_{oc}
Peak 1	**UV**	**2.04**	**1.98**	**2.01**	**95**
	RI	**-**	**-**	**-**	**-**
Peak 2	**UV**	**3.12**	**3.14**	**3.13**	**1349**
	RI	**-**	**-**	**-**	**-**
Peak 3	**UV**	**3.47**	**3.49**	**3.48**	**3020**
	RI	**3.36**	**3.36**	**3.36**	**2291**
Peak 4	**UV**	**4.87**	**4.87**	**4.87**	**74131**
	RI	**-**	**-**	**-**	**-**

Figure IIA 7.2.3.1-1: Calibration graphs





Section A 7.2.3.1 Annex Point/TNsG	Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106

Official use only

1 REFERENCE

1.1 Reference	[redacted]
1.2 Data protection	Yes
1.2.1 Data owner	McLaughlin Gormley King Company (MGK)
1.2.2 Companies with letter of access	Pet and Garden Manufacturing Ltd.
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	
2.2 GLP *(only where required)*	Yes
2.3 Deviations	No

3 MATERIALS AND METHODS

3.1 Test material	
3.1.1 Lot/Batch number	
3.1.2 Specification	^{14}C-Methyl Nonyl ketone
3.1.3 Description	
3.1.4 Purity	98.1 %
3.1.5 Stability	
3.1.6 Method	Adsorption and desorption of ^{14}C-Methyl Nonyl ketone were measured using a batch equilibrium procedure to determine the K adsorption/desoprtion and Koc values in four American soils. The definitive study was conducted at a soil to water ratio 1:10 for soils sand, sandy loam, and clay loam, and a soil to water ratio of 1.20 was used for soil silt loam. An 8-ml aliquot of each ^{14}C-test solution with nominal concentrations of 0, 1.0, 3.0, 4.0, and 5.0 µg/ml was added to the respective soils. They were shaken for 24 h at 25 ±1 °C on a mechanical shaker.

4 RESULTS

4.1 Log K_{OC}	The Koc values were 1972, 3323, 2914 and 1752 with a mean of 2490

Section A 7.2.3.1 Annex Point/TNsG	**Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106**

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods-discussion

Adsorption and desorption of ^{14}C-Methyl Nonyl ketone were measured using a batch equilibrium procedure to determine the K adsorption/desoprtion and Koc values in four American soils. The definitive study was conducted at a soil to water ratio 1:10 for soils sand, sandy loam, and clay loam, and a soil to water ratio of 1.20 was used for soil silt loam. An 8-ml aliquot of each ^{14}C-test solution with nominal concentrations of 0, 1.0, 3.0, 4.0, and 5.0 µg/ml was added to the respective soils. They were shaken for 24 h at 25 ±1 °C on a mechanical shaker.

5.2 Results and discussion

The adsorption constants for the four soils were 4.93, 21.6, 10.2 and 36.8. The Koc values were 1972, 3323, 2914 and 1752 with a mean of 2490

A subsequent desorption was determined with water and saturated $Ca(NO_3)_2$ solution.

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 5-11-2008

Materials and Methods

It was adopted applicant's version (revised).

Adsorption and desorption of ^{14}C-Methyl Nonyl ketone were measured using a batch equilibrium procedure to determine the K adsorption/desoprtion and Koc values in four American soils. The radiochemical purity of the [^{14}C]-labelled MNK was > 98.1%. Liquid Scintillation Counting (LSC) analysis was employed to measure the test material concentrations in the aqueous phases. The amount of ^{14}C-test material remaining adsorbed on the soil was determined by extraction-radioanalysis.

The adsorption/desorption study was conducted in sterile conditions at 25 ± 1ºC in the dark with ^{14}C-MNK and four soils (sand, clay loam, sandy loam and silt loam). The nominal concentration levels for all soil types were 5.0, 4.0, 3.0, 1.0 and 0 ppm.

The constants of the Freundlich isotherm were evaluated by determination of the adsorption equilibrium concentrations in water and soil.

Comments (with respect to OECD guideline 106):

pH does not vary between 4 and 8, and the organic carbon content ranges from 0.5 to 4.2 percent (Table 1).

It is used a $Ca(NO_3)_2$ solution (0.01 m) as aqueous solvent phase to improve centrifugation and minimise cation exchange.

The soil:solution ratio (in w/w) is very high.

Section A 7.2.3.1 Annex Point/TNsG	Adsorption and desorption in accordance with the new test guideline EC C18 or the corresponding OECD 106
	Sodium azide was used as a chemical inhibitor for sterilization of soils.
Results and discussion	It was adopted applicant's version (revised).
	The mean percent of compound adsorbed to the test soils during the study was 28%, 63.1%, 43.8%, and 59.6% for soils sand, clay loam, sandy loam and silt loam, respectively. The desired range for the Freundlich Model is 20% to 80%. All four of the soils were in this range, implying that MNK adequately fit the Freundlich isotherms (Table 2).
	The mean ^{14}C-mass balance of the test compound and the san, clay loam, sandy loam and silt loam was 95%, 99%, 90.1% and 92.2%, respectively.
	High Performance Liquid Chromatography (HPLC) was used to test the stability of the test compound under the test conditions. MNK was identified as the main ^{14}C-component in the aqueous adsorption phases and soil extracts.
	A comparison with the soil data revealed appositive correlation between adsorption constant (K_A) and the organic carbon content of the soil. The regression coefficient r^2 is a function of the approximated linear regression line:
	$$K_A = 15.62 \times \% C + 5.30 \ (r^2 = 0.903)$$
	The K_{oc} value was 1,972; 3,323; 2,914 and 1,752 L/kg for soils sand, clay loam, sandy loam and silt loam, respectively. The arithmetic mean value of K_{oc} was 2,490 L/kg. The values of K_{oc} were calculated with the help of the given percentage of organic carbon contents of the soils using the following equation:
	$$k_{oc} = k_A \times \left(\frac{100}{\% C} \right)$$
Conclusion	The K_{oc} values denote an slight/low mobility of MNK in soils
Reliability	This test is assessed with reliability indicator of 2.
Acceptability	This test is considered acceptable since it does not present important deficiencies.
Remarks	The Doc.III of this study was not provided by the Applicant although it was requested by RMS. As a consequence of this, RMS completes the Applicant's evaluation following strictly the information containing in the study.

Table 1. Soil specifications

Soil type	Sand	Clay loam	Sandy loam	Silt loam
pH	7.4	6.4	8.5	7.4
% Organic matter	0.5	1.3	0.7	4.2
CEC (mval/100 g)	0.3	19.6	19.8	22.7
Bulk density (g/cm^3)	1.64	1.38	1.16	1.08
Field capacity at 1/3 Bar moisture (%)	1.87	28.33	21.2	35.92
	Weight %	Weight %	Weight %	Weight %
Sand	92	22	58.7	24
Silt	4	40	28.0	52
Clay	4	38	13.3	24

Table 2. Adsorption and desorption parameters of MNK for soils

	Sand	Clay loam	Sandy loam	Silt loam
Adsorption in H_2O				
k_A	4.93	21.6	10.2	36.8
n	1.30	1.61	1.61	1.65
r	0.971	0.9559	0.819	0.978
Desorption with $Ca(NO_3)_2$				
K_D	34.6	51.4	10.2	82.1
n	1.16	1.46	3.37	1.35
r	0.988	0.885	0.489	0.998

K_A = Adsorption constant of a.i. using as solvent water
K_D = Desoprtion constant of a.i. using as aqueous solvent a $Ca(NO_3)_2$ solution

Section IIIA 7.3.1 Fate and behaviour in air
Annex Point IIIA VII.5 **IIIA 7.3.1 Phototransformation in air (Atkinson calculation)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Date of experimental work: May 31, 2005	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Pet and Garden Manufacturing Ltd.	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	**Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.**	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Not applicable	
2.3	**GLP**	Not applicable	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Not applicable	
3.3	**Testing procedure**		
3.2.1	Test system	A computer modelling program, AOP (Atmospheric Oxidation Program), was used to estimate the atmospheric half-life of Methyl Nonyl Ketone (MNK). The program estimated the rate constant for the atmospheric gas phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It can also estimate the rate constant for the gas phase reaction between ozone and olefinic/acetylenic compounds. The rate constants were used by the program to calculate the atmospheric half-life for MNK based upon average concentrations of hydroxyl radicals and ozone.	
		4 RESULTS	
4.2	**Rate constant (k)**	AOP estimated the overall rate constant for the gas phase reaction between hydroxyl radicals (OH) and MNK to be 13.8254×10^{-12} cm^3/molecule-sec. MNK does not react with ozone.	
4.3	**DT_{50}**	The atmospheric half-life (DT_{50}) of MNK, as a result of gas-phase reactions between MNK and photochemically produced atmospheric hydroxyl radicals, was estimated to be 9.28 hours (0.774 days based on 12-hour days).	

Section IIIA 7.3.1
Annex Point IIIA VII.5

Fate and behaviour in air

IIIA 7.3.1 Phototransformation in air (Atkinson calculation)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.2 Materials and methods

The rate constants and half-lives for reactions of MNK with OH radicals in the atmosphere were estimated using the Atmospheric Oxidation Program (AOP). Using the weighted global average OH radical concentration in the troposphere, the half-life of these processes was calculated.

5.3 Results and discussion

5.2.1 Rate constant (k) The overall rate constant for the gas phase reaction between hydroxyl radicals (OH) and MNK was estimated to be 13.8254×10^{-12} cm^3/molecule-sec.

5.2.2 DT_{50} The atmospheric half-life (DT_{50}) of MNK, as a result of gas-phase reactions between MNK and photochemically produced atmospheric hydroxyl radicals, was estimated to be 9.28 hours.

5.4 Conclusion

The computer program AOP has estimated the overall rate constant for the gas-phase reaction of hydroxyl radicals as 13.8254×10^{-12} cm^3/molecule-sec. The atmospheric half-life of MNK, as a result of gas phase reactions with photochemically produced atmospheric hydroxyl radicals, is rapid at 9.284 hours (0.774 days based on 12 hour days).

5.4.1 Reliability 1

5.3.2 Deficiencies No

Section IIIA 7.3.1 Fate and behaviour in air
Annex Point IIIA VII.5 **IIIA 7.3.1 Phototransformation in air (Atkinson calculation)**

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	6-03-2007
Materials and Methods	It is adopted applicant's version (revised) The rate constants and half-lives for reactions of MNK with OH radicals in the atmosphere were estimated using the Atmospheric Oxidation Program (AOP). The program estimated the rate constant for the atmospheric gas phase reaction between photochemically produced hydroxyl radicals (weighted global average) and organic chemicals. It can also estimate the rate constant for the gas phase reaction between ozone and olefinic/acetylenic compounds. The rate constants were used by the program to calculate the atmospheric half-life for MNK based upon average concentrations of hydroxyl radicals and ozone.
Results and discussion	It is adopted applicant's version (revised) The overall rate constant for the gas phase reaction between hydroxyl radicals (HO·) and MNK was estimated to be 13.83×10^{-12} cm^3/(molecule·sec). The atmospheric half-life (DT_{50}) of MNK, as a result of gas-phase reactions between MNK and photochemically produced atmospheric hydroxyl radicals, was estimated to be 1.16 days.
Conclusion	The atmospheric half-life (DT_{50}) of MNK, as a result of gas-phase reactions between MNK and photochemically produced atmospheric hydroxyl radicals, is estimated to be 1.16 days.
Reliability	This study is classified with a reliability of 2.
Acceptability	This study is considered acceptable although it does not present important deficiencies.
Remarks	

Section IIIA 7.3
Annex Point IIIA VII.5

Fate and behaviour in air, further studies
IIIA 7.3.2 Distribution in the environment

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted]
		Date of experimental work: April 12, 2006
1.2	**Data protection**	Yes
1.2.1	Data owner	Guaber UK Limited
1.2.2	Companies with letter of access	Not applicable
1.2.3	Criteria for data protection	**Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.**
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable
2.2	**GLP**	Not applicable
2.3	**Deviations**	Not applicable
	3 MATERIALS AND METHODS	
3.1	**Test material**	Not applicable
3.2	**Testing procedure**	
3.2.1	Test system	The Mackay Level I fugacity model was used to simulate the equilibrium distribution of a fixed quantity of conserved (i.e. non-reacting) Methyl Nonyl Ketone (MNK) in a closed environment at equilibrium. No degrading reactions, advective processes or intermedia transport processes (e.g. wet deposition or sedimentation) were considered. Three types of chemicals are considered in this model – Type 1: chemicals that partition into all media, Type 2: involatile chemicals and Type 3: chemicals with zero or near-zero solubility. MNK was assessed under Type 1. Physical-chemical properties and partition coefficient data were input values used by the model to derive environmental properties such as volume, density and organic matter. These parameters were then used to quantify MNK behaviour in an evaluative environment. Please refer to Table A7.3.2-1 and A7.3.2-2 for input parameters and environmental properties generated by the model. Distribution was simulated for the following homogenous environmental media (or compartments): air, water, soil, sediment, suspended sediment, fish and aerosols.

Section IIIA 7.3
Annex Point IIIA VII.5

Fate and behaviour in air, further studies
IIIA 7.3.2 Distribution in the environment

		4 RESULTS
4.1	**Fugacity**	2.91×10^{-7} Pa
4.2	**Distribution**	MNK was predicted to partition predominantly to air (99.9%). Insignificant amounts are anticipated to be distributed to aerosols in the atmosphere (0.0002%), to soil (0.00002%), to water (0.000001%), to sediment (0.0000003%), to suspended sediment (0.00000001%) and to fish (0.0000000009%). Please refer to Table A7.3.2-3 and Figure A7.3.2-1.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The Mackay Level I fugacity model was used to simulate the equilibrium distribution of MNK in a closed environment at equilibrium. The physical-chemical properties, partition coefficient data and user-defined volumes and densities were used to quantify the behaviour of MNK for the following homogenous environmental compartments: air, water, soil, sediment, suspended sediment, fish and aerosols.
5.2	**Results and discussion**	
5.3.1	Fugacity	2.91×10^{-7} Pa
5.3.2	Distribution	MNK was predicted to partition predominantly to air (99.9%). Insignificant amounts are anticipated to be distributed to aerosols in the atmosphere (0.0002%), to soil (0.00002%), to water (0.000001%), to sediment (0.0000003%), to suspended sediment (0.00000001%) and to fish (0.0000000009%).
5.3	**Conclusion**	Methyl Nonyl Ketone has a fugacity value of 2.91×10^{-7} Pa and is found to partition predominantly to air.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section IIIA 7.3 Fate and behaviour in air, further studies
Annex Point IIIA VII.5 **IIIA 7.3.2 Distribution in the environment**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	6-3-2007
Materials and Methods	It is adopted applicant's version (revised) The Mackay Level I fugacity model was used to simulate the equilibrium distribution of a fixed quantity of conserved (*i.e.* non-reacting) Methyl Nonyl Ketone (MNK) in a closed environment at equilibrium. No degrading reactions, advective processes or intermedia transport processes (*e.g.* wet deposition or sedimentation) were considered. The physical-chemical properties, partition coefficient data and user-defined volumes and densities were used to quantify the behaviour of MNK for the following homogenous environmental compartments: air, water, soil, sediment, suspended sediment, fish and aerosols. Three types of chemicals are considered in this model – Type 1: chemicals that partition into all media, Type 2: involatile chemicals and Type 3: chemicals with zero or near-zero solubility. MNK was assessed under Type 1. **Comment:** Physical-chemical properties and partition coefficient data of the Table A7.3.2-1 are wrong. Therefore, all calculations were done again with the correct data (see Table A7.3.2-4).
Results and discussion	It is adopted applicant's version (revised) MNK was predicted to partition predominantly to air (98.7537%) due to its high VP and low water solubility, and with much lower amounts anticipated to be distributed to aerosols in the atmosphere (0.0010%), to soil (0.7461%), to water (0.3450%), to sediment (0.1492%), to suspended sediment (0.0047%) and to fish (0.000379%). **Comment:** The new calculations with the correct data are shown in Table A7.3.2-5 and Figures A7.3.2-2.
Conclusion	Methyl Nonyl Ketone has a fugacity value of 2.83 x 10^{-7} Pa and is found to partition predominantly to air.
Reliability	This study is classified with a reliability of 2.
Acceptability	This study is considered acceptable although it does not present important deficiencies.
Remarks	

Table A7.3.2-1: Physical and partition coefficient input data for Level I Fugacity Model

Input Parameter	Value
Molecular mass (g/mol)	170.29
Data temperature (°C)	25
Log K_{ow}	9.05×10^{-4}
Water solubility (g/m^3)	5448
Vapour pressure (Pa)	2270
Melting point (°C)	4488
Amount of chemical (kg)	2000

Table A7.3.2-2: Environmental properties for MNK as generated by the Level I Fugacity model

Environmental properties	Air	Water	Soil	Sediment	Suspended sediment	Fish	Aerosol
Volume, V (m^3)	1.00×10^{14}	2.00×10^{11}	9.00×10^{9}	1.00×10^{8}	1.00×10^{6}	2.00×10^{5}	2000
Density (kg/m^3)	1.185	1000	2400	2400	1500	1000	2000
Organic carbon (g/g)	-	-	0.02	0.04	0.2	-	-

Table A7.3.2-3: Phase properties and composition of MNK in the relevant environmental compartments

Model output	Air	Water	Soil	Sediment	Suspended sediment	Fish	Aerosol
Z ($mol/m^3.Pa$)	4.03×10^{-4}	2.08×10^{-9}	6.80×10^{-7}	1.36×10^{-6}	4.25×10^{-6}	1.73×10^{-6}	4.76×10^{1}
VZ (mol/Pa)	4.03×10^{10}	4.16×10^{2}	6.12×10^{3}	1.36×10^{2}	4.25	3.45×10^{-1}	9.53×10^{4}
Concentration (mol/m^3)	1.17×10^{-10}	6.06×10^{-16}	1.98×10^{-13}	3.96×10^{-13}	1.24×10^{-12}	5.03×10^{-13}	1.39×10^{-5}
Amount (%)	99.9998	1.03×10^{-6}	1.52×10^{-5}	3.37×10^{-7}	1.05×10^{-8}	8.56×10^{-10}	2.36×10^{-4}

Figure A7.3.2-1: Distribution of MNK in the environment as predicted by the Mackay Level 1 Fugacity Model

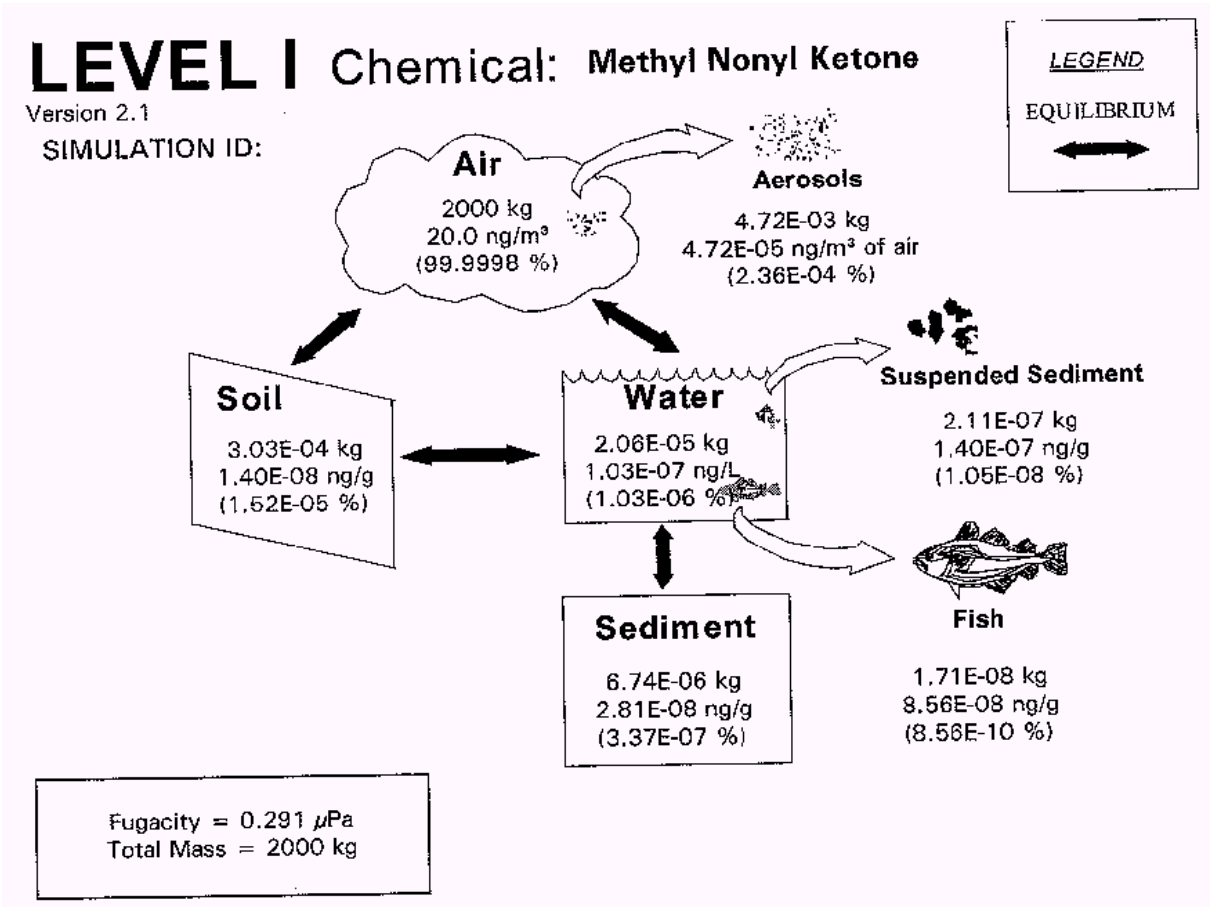


Table A7.3.2-4: Physical-chemical and partition coefficient input data for Level I Fugacity Model

Input Data

MNK
Chemical Type 1

Emissions
2000 kg
11745 mol

Chemical Properties

Molecular Mass (g/mol)	170.29	Water Solubility (mol/m³)	8.46E-03
Data Temperature (°C)	20	Henry's Law Constant (Pa.m³/mol)	1395
Log Kow	4.34	Vapour Pressure (Pa)	11.8
Water Solubility (g/m³)	1.44	Melting Point (°C)	12.2

Environmental Properties

PHASE	AIR	WATER	SOIL	SEDMT.	SUSP. SEDMT.	FISH	AEROSOL
Volume (m³)	1.00E+14	2.00E+11	1.00E+09	1.00E+08	1.00E+06	2.00E+05	2000
Density (kg/m³)	1.206	1000	2400	2400	1500	1000	2000
Organic Carbon Content (g/g)	-	-	0.02	0.04	0.2	-	-
Fish Lipid Content (g/g)	-	-	-	-	-	0.05	-

OK Help

Partition Coefficients

MNK
Chemical Type 1

Note that all amounts are dimensionless, except where noted

Log Octanol-Water Partition Coefficient	4.34
Octanol-Water Partition Coefficient	21979
Organic C-Water Partition Coefficient (L/kg)	9011
Air-Water Partition Coefficient	0.573
Soil-Water Partition Coefficient	433
Soil-Water Partition Coefficient (L/kg)	180
Sediment-Water Partition Coefficient	865
Sediment-Water Partition Coefficient (L/kg)	360
Suspended Sediment-Water Partition Coefficient	2703
Suspended Sediment-Water Partition Coefficient (L/kg)	1802
Fish-Water Partition Coefficient	1099
Fish-Water Partition Coefficient (L/kg)	1099
Aerosol-Air Partition Coefficient	5.08E+05

OK Help

Table A7.3.2-5: Phase properties and composition of MNK in the relevant environmental compartments

Results

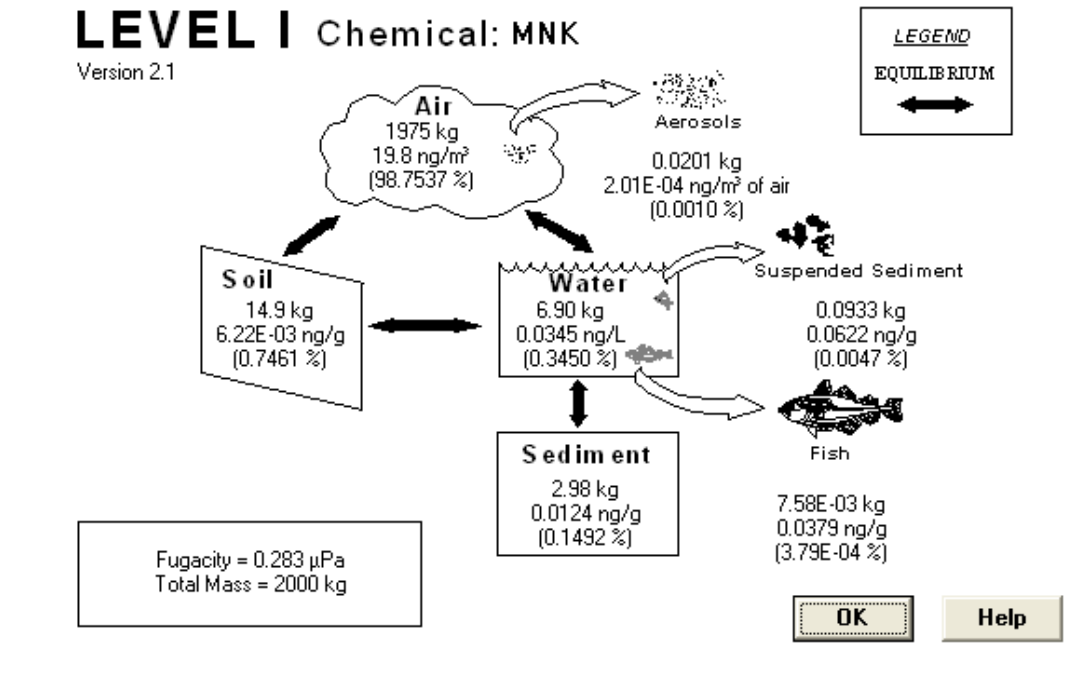
MNK
Chemical Type 1

Fugacity Pa	2.83E-07
Total of VZ Products	4.15E+10

PHASE	AIR	WATER	SOIL	SEDMT.	SUSP. SEDMT.	FISH	AEROSOL
Volume, V (m³)	1.00E+14	2.00E+11	1.00E+09	1.00E+08	1.00E+06	2.00E+05	2000
Density (kg/m³)	1.206	1000	2400	2400	1500	1000	2000
Organic Carbon Content (g/g)	-	-	0.02	0.04	0.2	-	-
Fish Lipid Content (g/g)	-	-	-	-	-	0.05	-
Z Value (mol/m³.Pa)	4.10E-04	7.17E-04	3.10E-01	6.20E-01	1.94E+00	7.88E-01	2.09E+02
VZ (mol/Pa)	4.10E+10	1.43E+08	3.10E+08	6.20E+07	1.94E+06	1.58E+05	4.17E+05
Concentration (mol/m³)	1.16E-10	2.03E-10	8.76E-08	1.75E-07	5.48E-07	2.23E-07	5.90E-05
Concentration (g/m³)	1.98E-08	3.45E-08	1.49E-05	2.98E-05	9.33E-05	3.79E-05	1.00E-02
Concentration (µg/g)	1.64E-05	3.45E-08	6.22E-06	1.24E-05	6.22E-05	3.79E-05	5.02E-03
Amount (kg)	1.98E+03	6.90E+00	1.49E+01	2.98E+00	9.33E-02	7.58E-03	2.01E-02
Amount (mol)	1.16E+04	4.05E+01	8.76E+01	1.75E+01	5.48E-01	4.45E-02	1.18E-01
Amount (%)	98.7537	0.3450	0.7461	0.1492	0.0047	3.79E-04	0.0010

OK Help

Figure A7.3.2-2: Distribution of MNK in the environment as predicted by the Mackay Level I Fugacity Model



Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes US-EPA 72-1
2.2	**GLP**	Yes
2.3	**Deviations**	no

3 MATERIALS AND METHODS

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

3.1	**Test material**	
3.1.1	Lot/Batch number	
3.1.2	Specification	Methyl Nonyl Ketone
3.1.3	Purity	100 %
3.1.4	Composition of Product	
3.1.5	Further relevant properties	
3.1.6	Method of analysis	Gas liquid chromatography (GLC)
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	—
3.3	**Reference substance**	Not reported
3.3.1	Method of analysis for reference substance	
3.4	**Testing procedure**	Non-entry field
3.4.1	Dilution water	
3.4.2	Test organisms	Bluegill
3.4.3	Test system	Flow-through
3.4.4	Test conditions	**Bluegill (Lepomis Macrochirus), mean body length 34 (± 5) mm; mean body weight 1.0 (± 0.42) g. Test design: Flow-through (96 hours); 20 fish per aquarium (loading about 0.09 g fish/L/day) and per concentration Concentrations: Control, vehicle blank (dimethylformamide), 0.38, 0.75, 1.5, 3.0 and 6.0 mg a i./L (nominal).** Temperature 22 ±1 °C; pH 8.2 - 8.3; oxygen content 8.4 – 8.5 mg/L; hardness about 172-178 mg/L (as $CaCO_3$); 16:8 hours light:dark.
3.4.5	Duration of the test	96 hours
3.4.6	Test parameter	Mortality and symptoms
3.4.7	Sampling	Yes
3.4.8	Monitoring of TS concentration	Yes
3.4.9	Statistics	Probit analysis, Moving average method, Binomial method

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

4 RESULTS

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

4.1	**Limit Test**	
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	Non-entry field
4.2.1	Initial concentrations of test substance	**Control, vehicle blank (dimethylformamide), 0.38, 0.75, 1.5, 3.0 and 6.0 mg a.i./L (nominal).**
4.2.2	Actual concentrations of test substance	
4.2.3	Effect data (Mortality)	Biological results: MNK caused mortality to the rainbow trout at measured concentrations > 1.6 mg a.i./L.
4.2.4	Concentration / response curve	
4.2.5	Other effects	Behavioural symptoms such as tumbling, narcotic-like state and swimming near the bottom were monitored in the 1.6 mg a.s./L measured concentration and above during the study.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	
4.3.2	Nature of adverse effects	
4.4	**Test with reference substance**	
4.4.1	Concentrations	
4.4.2	Results	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	**Bluegill (Lepomis Macrochirus), mean body length 34 (± 5) mm; mean body weight 1.0 (± 0.42) g.** **Test design: Flow-through (96 hours); 20 fish per aquarium (loading about 0.09 g fish/L/day) and per concentration Concentrations: Control, vehicle blank (dimethylformamide), 0.38, 0.75, 1.5, 3.0 and 6.0 mg a i./L (nominal).** Temperature 22 ±1 °C; pH 8.2 - 8.3; oxygen content 8.4 – 8.5 mg/L;

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

		hardness about 172-178 mg/L (as $CaCO_3$); 16:8 hours light:dark. DURATION OF THE TEST: 96 h
5.2	**Results and discussion**	Analytical measurements of MNK were made from samples collected at 0 and 96 hours. The measured concentrations averaged 0.21, 0.38, 0.74, 1.6 and 3.1 mg/L, respectively. During the test, there was an oily surface film observed in the mixing cell of the diluter. From the data collected during this study, the 96-hours LC_{50} was determined to be 2.1 mg/L (95% C.I. = 1.6 to 3.1 mg/L) based on the mean measured concentrations of MNK. The abnormal/behavioural effects, mortality, surfacing, loss of equilibrium, fish on the bottom of the test chamber, laboured respiration, dark discoloration, vertical orientation and/or quiescence, were observed in the 1.6 and 3.1 mg/L test concentrations during the study. Therefore, the 96-hours no effect concentration of MNK toxicity to bluegill was 0.74 mg/L based on the lack of sublethal responses at this concentration.
5.2.1	LC_0	NOEC: 0.74 mg/L
5.2.2	LC_{50} (96 h)	2.1 mg/L (95% C.I. = 1.6 to 3.1 mg/L)
5.2.3	LC_{100}	
5.3	**Conclusion**	
5.3.1	Other Conclusions	
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Section A7.4.1.1 **Acute toxicity to fish**

Annex Point IIA7.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
DATE	6-11-2008
MATERIALS AND METHODS	It is adopted applicant's version. Methyl Nonyl Ketone was tested on Bluegill (*Lepomis macrochirus*) in a flow-through system during 96 hours at nominal concentrations of 0, 0.38, 0.75, 1.5, 3.0 and 6.0 mg /L. The purity of MNK was 100%. The bluegill used as the control group during this study had a mean wet weight of 1.0 g (±0.42 g) and a mean standard length of 34 mm ((±5 mm) when measured at the end of the test. Test conditions: Temperature 21 - 22 °C; pH 8.2 - 8.3; oxygen content 8.4 - 8.5 mg/L; 16:8 hours light:dark.
RESULTS AND DISCUSSION	It is adopted applicant's version. Analytical measurements of MNK were made from samples collected at 0 and 96 hours by GLC. The measured concentrations averaged 0.21, 0.38, 0.74, 1.6 and 3.1 mg/L, respectively. Overall, these measured concentrations represented 52 (±2%) of the nominal concentrations. During the test, there was an oily surface film observed in the mixing cell of the diluter. From the data collected during this study, the 96-hours LC_{50} was determined to be 2.1 mg/L (95% C.I. = 1.6 to 3.1 mg/L) based on the mean measured concentrations of MNK (see Table 5). The abnormal/behavioural effects, mortality, surfacing, loss of equilibrium, fish on the bottom of the test chamber, laboured respiration, dark discoloration, vertical orientation and/or quiescence, were observed in the 1.6 and 3.1 mg/L test concentrations during the study. Therefore, the 96-hours no effect concentration of MNK toxicity to bluegill was 0.74 mg/L based on the lack of sublethal responses at this concentration (see Table 6).
CONCLUSION	96-h LC_{50} value: 2.1 mg/L (95% C.I. = 1.6 to 3.1 mg/L). 96-h NOEC value: 0.74 mg/l (Table 7).
RELIABILITY	This study is classified with a reliability of 2.
ACCEPTABILITY	This study is considered acceptable because it does not present important deficiencies.
REMARKS	The Doc.III of this study was not provided by the Applicant although it was requested by RMS. As a consequence of this, RMS completes the Applicant's evaluation following strictly the information containing in the study.

Table 1:Dilution water

Criteria	Details
Source	Prepared from deionised water
Alkalinity	
Hardness	172 - 178 mg/L (as $CaCO_3$)
pH	7.8 - 8.1
Oxygen content	-
Conductance	
Holding water different from dilution water	No

Table 2:Test organisms

Criteria	Details
Species/strain	Bluegill (*Lepomis macrochirus*)
Source	
Wild caught	No
Age/size	mean body length 34 (± 5) mm mean body weight 1.0 (± 0.42) g.
Kind of food	Hatched brine shrimp, D magna and/or a commercially available fish food
Amount of food	
Feeding frequency	
Pretreatment	Adaptation
Feeding of animals during test	no

Table 3:Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	7.2 tank volume replacements flowing through each chamber every day
Volume of test vessels	45 l
Volume/animal	
Number of animals/vessel	20
Number of vessels/ concentration	
Test performed in closed vessels due to significant volatility of TS	

Table 4:Test conditions

Criteria	Details
Test temperature	21 - 22°C
Dissolved oxygen	8.4 - 8.5 mg/l
pH	8.2 - 8.3
Adjustment of pH	Yes
Aeration of dilution water	
Intensity of irradiation	
Photoperiod	16:8

Table 5: Mortality

Mean measured test conc. (mg/l)	Number of fish	Mortality (cumulated) after:			
		24 h	48 h	72 h	96 h
0	20	0	0	0	0
vehicle blank	20	0	0	0	0
0.21	20	0	0	0	0
0.38	20	0	0	0	0
0.74	20	0	0	0	0
1.6	20	0	1	1	1
3.1	20	7	13	18	20

Table 6: Symptoms

Mean measured test conc. (mg/l)	Number of fish	Symptoms after:			
		24 h	48 h	72 h	96 h
0	20	20 N	20 N	20 N	20 N
vehicle blank	20	20 N	20 N	20 N	20 N
0.21	20	20 N	20 N	20 N	20 N
0.38	20	20 N	20 N	20 N	20 N
0.74	20	20 N	20 N	20 N	20 N
1.6	20	2 SUR/DK/VO 2 OB/VO;16 Q	2 SUR/DK/VO/LR 16 Q;2 VO/OB	2 SUR/DK/VO/Q 7 Q;10 OB/VO/Q	2 SUR/DK/Q; 10 OB/Q
3.1	20	7 OB/DK/LR/VO/Q; 5 OB/DK/LOE/Q; 1 OB/Q	1 SUR/DK/LR/LOE; 5 OB/LOE/LR/DK; 1 OB/DK/VO	1 SUR/LOE/LR/Q; 1 OB/LOE/LR/Q;	---

Explanation of symptoms:
N: Normal
SUR: Surfacing
LOE: Loss of equilibrium
OB: Fish on bottom of the test chambers
Q: Quiesence
LR: Labored respiration
DK: Dark discoloration
VO: Vertical orientation
Number behind the symbol for symptom = number the affected fish.

Table 7: Effect data

	24 h [mg/l][1]	% C.L.	48 h [mg/l][2]	% C.L.	72 h [mg/l][3]	% C.L.	96 h [mg/l][3]	% C.L.
LC_{50}	> 3.1	-	2.7	2.3-3.4	2.3	2.1-2.6	2.1	1.6-3.1

[1] Probit method
[2] Moving average method
[3] Binomial method

Table 8:Validity criteria for acute fish test according to OECD Guideline 203

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥ 80% of initial concentration during test		**X**
Criteria for poorly soluble test substances	**n.a.**	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 ***Daphnia magna***

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Dates of experimental work: February 12, 2002 - February 14, 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Guaber UK Limited	
1.2.1	Companies with letter of access	Not applicable	
1.2.2	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes OECD 202 Guideline for Testing of Chemicals, *Daphnia* sp., Acute Immobilisation Test	
2.3	**GLP**	Yes	
2.4	**Deviations**	Yes, the study deviates from OECD 202 Guideline in the following respect: 1. The validity criteria according to OECD 202 test guideline which states the concentration of test substance should be ≥80% of initial concentration during the test was not fulfilled. However, this deviation is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Methyl Nonyl Ketone (MNK Tech)	
3.2.1	Lot/Batch number	ECO120136	
3.2.2	Specification	As given in section 2	
3.2.3	Purity	99.5% (refer to Certificate of Analysis in Report No. ENV5982/120136)	
3.2.4	Composition of Product	Not applicable	
3.2.5	Further relevant properties	None	
3.2.6	Method of analysis	Not applicable	
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.1.2-1	

Section A7.4.1.2 Acute toxicity to invertebrates
Annex Point IIA7.2 ***Daphnia magna***

3.4	**Reference substance**	Potassium dichromate
3.4.1	Method of analysis for reference substance	Not documented
3.5	**Testing procedure**	
3.5.1	Dilution water	Details are given in Table A7.4.1.2-2
3.5.2	Test organisms	*Daphnia magna*, details are given in Table A7.4.1.2-3
3.5.3	Test system	Details are given in Table A7.4.1.2-4
3.5.4	Test conditions	Details are given in Table A7.4.1.2-5
3.5.5	Duration of the test	48 hours
3.5.6	Test parameter	Immobility
3.5.7	Sampling	Observations were made at 24 and 48 hours after test initiation.
3.5.8	Monitoring of TS concentration	Yes Interval: 0, 24 and 48 hours
3.5.9	Statistics	The EC_{50} value was determined according to the Maximum Likelihood-Probit method, while the NOEC and LOEC were determined according to Fisher's Exact Test.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2.1	Concentration	Not applicable
4.2.2	Number/ percentage of animals showing adverse effects	Not applicable
4.2.3	Nature of adverse effects	Not applicable

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 ***Daphnia magna***

4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Please refer to Table A7.4.1.2-8
4.2.2	Actual concentrations of test substance	Test substance specific analysis was carried out and it has been shown that Methyl Nonyl Ketone disappears from solution over time. Recovery of MNK from solutions is variable with typical recovery rates at 0 hours of 47 to 80%. After 24 hours recovery is shown to have dropped significantly to only 0.5 to 7.0%. Similar values were obtained for the replacement solutions at 24 hours, with initial recovery of 38 to 91% of the nominal value, which dropped to 2 to 5% after the solutions had aged for a further 24 hours. The recovery of Methyl Nonyl Ketone in mg/l and the percentage recovery of Methyl Nonyl Ketone of nominal test concentrations are given in Table A7.4.1.2-8 and Table A7.4.1.2-9, respectively.
4.2.3	Effect data (Immobilization)	Please refer to Table A7.4.1.2-6. The EC_{50} value for each of the 24 hour time-points can be found in Table A7.4.1.2-7.
4.2.4	Concentration / response curve	Not documented
4.2.5	Other effects	None
4.3	**Results of controls**	Please refer to Table A7.4.1.2-6.
4.4	**Test with reference substance**	
4.4.1	Concentrations	Not documented
4.4.2	Results	24 hour EC_{50}: 1.3 mg/l 48 hour EC_{50} : 0.9 mg/l 48 hour NOEC: 0.56 mg/l 48 hour 100% mortality: 3.2 mg/l

5 APPLICANT'S SUMMARY AND CONCLUSION

5.2	**Materials and methods**	The test system was semi-static and *Daphnia magna* was chosen as the test organism. The test was conducted in accordance with OECD test guideline 202: *Daphnia* sp., Acute Immobilization Test and is described under point 3.
5.3	**Results and discussion**	Please refer to Table A7.4.1.2-6 and Table A7.4.1.2-7 The 48-hour EC_{50} value of Methyl Nonyl Ketone to *Daphnia magna* was estimated to be 2.3 mg/l (as determined by the Maximum Likelihood-Probit method), within 95% confidence limits of 1.6 mg a.s./l and 3.9 mg a.s./l. The highest no observed effect concentration (NOEC) after 48 hours was 0.5 mg/l. The lowest test concentration that immobilized all twenty invertebrates within 48 hours could not be determined since there was only 75% immobilization at the highest test concentration (i.e. 4.0 mg/l). None of the controls *Daphnia* were trapped at the surface nor were they

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 ***Daphnia magna***

		immobilized, thus fulfilling the validity criteria of the study, which states that control immobilization must not exceed 10% at the end of the test.
5.3.1	EC_0	Not documented
5.3.2	EC_{50}	2.3 mg/l
5.3.3	EC_{100}	Not documented
5.4	**Conclusion**	Under the conditions of this study, the estimated 48-hour EC_{50} value was determined to be 2.3 mg a.s./l. The 48-hour NOEC (immobilization) value for Methyl Nonyl Ketone with *Daphnia magna* was determined to be 0.50 mg/l. The 48-hour LOEC (immobilization) value for Methyl Nonyl Ketone with *Daphnia magna* was determined to be 1.0 mg/l.
5.4.1	Reliability	1
5.4.2	Deficiencies	No

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2-03-2007
Materials and Methods	It was adopted applicant's version (revised). The test was conducted in accordance with OECD test guideline 202: *Daphnia* sp., Acute Immobilization Test. Neonates of Waterflea (*Daphnia magna STRAUSS*), collected from in house culture, with less than 24 hours were exposed to different concentrations of MNK (purity 99.5%) in semi-static test during 48 hours. A control plus six test concentrations of MNK were tested: 0.13, 0.25, 0.5, 1.0, 2.0 and 4.0 mg a.i./L. A further control solution (solvent control) was tested containing Dimethyl Formamide (DMF) at a concentration of 1 ml DMF/L. It was recorded the immobility (and other effects) after 24 and 48 hours in all replicates. In the study was used dechlorinated main tap water with a typical hardness of 240 mg/l $CaCO_3$. The water was adjusted to 20 ± 1 °C, aerated until the dissolved oxygen concentration had reached a minimum of 60% air saturation and the pH stabilized between 7.0 and 8.0. The photoperiod was 16:8 hours light:dark with light intensity of 370 flux. **Comments: (with respect to OECD guideline 202)** Test performed was not conducted in closed vessels (or headspace method) in spite of significant volatility of MNK (VP 11.8 Pa at 20 °C).
Results and discussion	It was adopted applicant's version (revised). Test substance specific analysis was carried out and it has been shown that Methyl Nonyl Ketone disappears from solution over time. Recovery of MNK from solutions is variable with typical recovery rates at 0 hours of 47 to 80%. After 24 hours recovery is shown to have dropped significantly to only 0.5 to 7.0%. Similar values were obtained for the replacement solutions at 24 hours, with initial recovery of 38 to 91% of the nominal value, which dropped to 2 to 5% after the solutions had aged for a further 24 hours. The recovery of Methyl Nonyl Ketone in mg/l and the percentage recovery of Methyl Nonyl Ketone of nominal test concentrations are given in Table A7.4.1.2-8 and Table A7.4.1.2-9, respectively. **Comment:** MNK disappears from solution over time since is a volatile substance (VP 11.8 Pa at a T = 20 °C) reason why after 24 and 48 hours the recoveries expressed as the percentage of initial concentration applied are very low in spite of the renewal of the medium. Due to this fact, the results of the study must not be expressed as nominal concentrations if not that according to "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures" of OECD must be expressed as the geometric mean exposure concentration during the test. In this case the concentrations to derive the curve dose-response were established as the arithmetic mean of the geometric means of the measured concentration (see Table A7.4.1.2-8) during the two periods in which test is divided: 0-24 hrs (initial period without change of test medium) and 24-48 hrs (renewal of medium).

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Annex Point IIA7.2 ***Daphnia magna***

Nominal concentration (mg/l)	Time/geometric mean concentration (mg/l)		Arithmetic mean of concentrations (1) and (2)
	0-24 hrs geometric mean concentration (1)	24-48 hrs geometric mean concentration (2)	
0.13	$2.45 \cdot 10^{-4}$	$2.65 \cdot 10^{-4}$	$2.55 \cdot 10^{-4}$
0.25	$3.61 \cdot 10^{-4}$	$3.87 \cdot 10^{-3}$	$2.12 \cdot 10^{-3}$
0.5	$2.19 \cdot 10^{-2}$	$1.15 \cdot 10^{-2}$	$1.67 \cdot 10^{-2}$
1.0	$5.23 \cdot 10^{-2}$	$3.67 \cdot 10^{-2}$	$4.45 \cdot 10^{-2}$
2.0	$8.83 \cdot 10^{-2}$	$1.22 \cdot 10^{-1}$	$1.05 \cdot 10^{-1}$
4.0	$3.42 \cdot 10^{-2}$	$9.86 \cdot 10^{-2}$	$6.64 \cdot 10^{-2}$ (*)

(*)This value was eliminated on establishing the curve dose-response because was regarded as an outlier

The 48-hour EC_{50} value of Methyl Nonyl Ketone to *Daphnia magna* was estimated to be 0.23 mg/l (as determined by the Maximum Likelihood-Probit method), within 95% confidence limits of 0.09 mg a.s./l and 8.47 mg a.s./l.

Remark:

The results of other freshwater aquatic invertebrate toxicity study* also characterize MNK as a highly toxic substance to aquatic invertebrates. The EC_{50} derived from an acute toxicity test with MNK (purity: 97.9%) performed on *Daphnia magna* under flow-through conditions was estimated to be 0.54 mg/l.

*The summary of this study is included in the evaluation of U.S-EPA, Reregistration Eligibility Decision (RED): Methyl Nonyl Ketone. This document was provided by the Applicant with the Dossier. Nevetheless, it can be also downloaded from the official website of the Agency.

Conclusion Under the conditions of this study, the estimated 48-hour EC_{50} value was determined to be 0.23 mg a.s./l.

Reliability This study is classified with a reliability of 2.

Acceptability This study is considered acceptable because it does not present important deficiencies.

Remarks

Table A7.4.1.2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	DMF (solvent control) Dilution water (negative control)
Concentration of vehicle	Not documented
Vehicle control performed	Yes, the vehicle control group was exposed to 1 ml/l DMF.
Other procedures	None documented

Table A7.4.1.2-2: Dilution water

Criteria	Details
Source	Dechlorinated mains tap water
Alkalinity	Not documented
Hardness	240 mg/l $CaCO_3$
pH	7.0 – 8.0
Ca / Mg ratio	Not documented
Na / K ratio	Not documented
Oxygen content	60 % air saturation
Conductance	Not documented
Holding water different from dilution water	No

Table A7.4.1.2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	[redacted]
Age	Less than 24 hours
Kind of food	Green algae *(Chlorella vulgaris)*
Amount of food	1 mg organic carbon per litre of culture water
Feeding frequency	Once each working day
Pretreatment	Daphnids were cultured under semi-static conditions
Feeding of animals during test	No

Table A7.4.1.2-4: Test system

Criteria	Details
Test Type	Semi-static
Renewal of test solution	Test solutions were renewed after 24 hours
Volume of test vessels	50 ml
Volume/animal	5 ml/ daphnid
Number of animals/vessel	5 daphnids/vessel
Number of vessels/ concentration	4 / concentration
Test performed in closed vessels due to significant volatility of TS	Yes, to limit aerial contamination and reduce evaporative losses, a transparent Perspex sheet was placed over the tops of the dishes.

Table A7.4.1.2-5: Test conditions

Criteria	Details
Test temperature	20 ± 1°C
Dissolved oxygen	98 - 100% ASV
pH	7.7 – 8.0
Adjustment of pH	No adjustment of pH was performed
Aeration of dilution water	Dilution water was aerated until the dissolved oxygen concentration reached a minimum of 60% ASV.
Quality/Intensity of irradiation	350 lux
Photoperiod	Illumination of 16 hours light and 8 hours dark

Table A7.4.1.2-6: Immobilisation and water quality data

Test-Substance Concentration (nominal) [mg/l]	Immobile *Daphnia*				Oxygen [%ASV] 48 h	pH 48 h	Temperature [°C] 48 h
	Number		Percentage				
	24 h	48 h	24 h	48 h			
0 control	0	0	0	0	100	7.8	20.0
DMF control	0	0	0	0	100	7.9	20.0
0.13	0	0	0	0	100	7.7	20.0
0.25	0	0	0	0	100	7.8	20.0
0.50	0	3	0	15	100	7.8	20.0
1.0	1	6	5	30	100	7.8	20.0
2.0	3	6	15	30	100	7.8	20.0
4.0	1	15	5	75	100	7.8	20.0

Table A7.4.1.2-7: Effect data

5.4.2.1.1.1.1.1 TIME (HOURS)	**EC_{50} (mg/l)**	**95% Confidence Limits (mg/l)**
24	> 4.0	Not possible to determine
48	2.3	1.6 -3.9

Table A7.4.1.2-8: Recovery of MNK TECH in mg/l

Nominal concentration (mg/l)	**Time**			
	0 hrs (fresh)	**24 hrs (aged)**	**24 hrs (renewed)**	**48 hrs (aged)**
0	0	0	0	0
0.13	0.06	0	0.07	0
0.25	0.13	0	0.15	0.01
0.5	0.30	0.04	0.33	0.02
1.0	0.76	0.06	0.84	0.04
2.0	1.59	0.07	1.83	0.09
4.0	2.93	0.02	1.52	0.08

Table A7.4.1.2-9: Percentage recovery of MNK TECH of nominal test concentrations

Nominal concentration (mg/l)	**Time**			
	0 hrs (fresh)	**24 hrs (aged)**	**24 hrs (renewed)**	**48 hrs (aged)**
0	-	-	-	-
0.13	46.8	3.1	52.3	3.1
0.25	52.4	2.0	60.8	4.0
0.5	59.7	7.0	66.0	4.2
1.0	76.4	5.5	83.9	4.0
2.0	79.4	3.5	91.4	4.7
4.0	73.3	0.5	38.0	2.0

Table A7.4.1.2-10: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

Criteria	**Fulfilled**
Immobilisation of control animals <10%	Yes
Control animals not staying at the surface	Yes
Concentration of dissolved oxygen in all test vessels >3 mg/l	Yes
Concentration of test substance ≥80% of initial concentration during test	No

Criteria for poorly soluble test substances	Yes

Section A7.4.1.3 Growth inhibition test on algae

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			Official use only
		1 REFERENCE	
1.2	**Reference**	[redacted]	
		Dates of experimental work: February 4, 2002 - February 7, 2002.	
1.3	**Data protection**	Yes	
1.3.1	Data owner	Guaber UK Limited	
1.3.2	Companies with letter of access	Not applicable	
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	**Yes**	
		OECD 201 Guideline for Testing of Chemicals, Alga, Growth Inhibition Test	
2.3	**GLP**	Yes	
2.4	**Deviations**	Yes, the study deviates from OECD 201 Guideline in the following respect: 1. The validity criteria which states the concentration of test substance should be ≥80% of initial concentration during the test was not fulfilled. However, this deviation is not considered to compromise the scientific validity of the study.	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Methyl Nonyl Ketone (MNK Tech)	
3.2.1	Lot/Batch number	ECO120136	
3.2.2	Specification	As given in section 2	
3.2.3	Purity	99.5% (refer to Certificate of Analysis in Report No. ENV5982/120136)	
3.2.4	Composition of Product	Not applicable	
3.2.5	Further relevant properties	None	
3.2.6	Method of analysis	Not documented	
3.3	**Preparation of TS solution for poorly**	Details are given in Table A7.4.1.3-1.	

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Annex Point IIA7.3

	soluble or volatile test substances	
3.4	**Reference substance**	Potassium dichromate
3.4.1	Method of analysis for reference substance	Not documented
3.5	**Testing procedure**	
3.5.1	Culture medium	The composition of the nutrient medium is shown in Table A7.4.1.3-2. The culture medium was prepared using deionized water, which was filtered and sterilized by autoclaving at 120°C for 30 minutes. The sterile nutrient stock solutions were then added and the pH adjusted to 8.0 ± 0.2.
3.5.2	Test organisms	*Selenastrum capricornutum* (Printz), details are given in Table A7.4.1.3-3.
3.5.3	Test system	Details are given in Table A7.4.1.3-4.
3.5.4	Test conditions	Details are given in Table A7.4.1.3-5.
3.5.5	Duration of the test	72 hours
3.5.6	Test parameter	The 72 hour EC_{50} with respect to growth inhibition.
3.5.7	Sampling	Cell densities were determined at 24, 48 and 72 hours.
3.5.8	Monitoring of TS concentration	No
3.5.9	Statistics	The EC_{50} values for algal growth were determined using the biomass integral and the growth rate calculation, according to the OECD Guideline for Testing of Chemicals reference 201 "Alga, Growth Inhibition Test" (1984).
		4 RESULTS
4.2	**Limit Test**	Not performed
4.2.1	Concentration	
4.2.2	Number/ percentage of animals showing adverse effects	
4.3	**Results test substance**	
4.3.1	Initial concentrations of test substance	0 (control), 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 mg/l ; solvent control of 1 ml/l DMF
4.3.2	Actual concentrations of test substance	Not determined
4.3.3	Growth curves	Refer to Figure A7.4.1.3-1.
4.3.4	Concentration / response curve	Refer to Figure A7.4.1.3-2 and Figure A7.4.1.3-3.

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Annex Point IIA7.3

4.3.5	Cell concentration data	Data are provided in Table A7.4.1.3-6.
4.3.6	Effect data (cell multiplication inhibition)	The 72 hour E_rC_{50} and E_bC_{50} values were estimated to be 14.3 mg/l and 2.9 mg/l, respectively. The NOEC was not determined.
4.3.7	Other observed effects	None documented
4.4	**Results of controls**	The mean cell density of the control was 207.50×10^4 cells per ml at 72 hours. The mean cell density of the solvent control was 211.56×10^4 cells per ml at 72 hours.
4.5	**Test with reference substance**	
4.5.1	Concentrations	0 (control), 0.18, 0.32, 0.56, 1.0 and 1.8 mg/l.
4.5.2	Results	Data are provided in Table A7.4.1.3-9 for 48 and 72 hour percent inhibition by biomass integral and growth rate (E_rC_{50} and E_bC_{50} values). The 72 hour E_rC_{50} and E_bC_{50} values were estimated to be 0.88 mg/l and 0.58 mg/l, respectively. Data are provided in Table A7.4.1.3-10.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.2	**Materials and methods**	*Selenastrum capricornutum* was used as the test organism. The test was conducted according to OECD guideline 201 and is described under point 3.
5.3	**Results and discussion**	The analytical data given in IIIA 7.4.1.2, Table A7.4.1.2-9 demonstrates that recovery of MNK from solution is low and variable with typical recovery rates at 24 hours of less than 75%. No test substance specific analysis was carried out as it was shown that MNK disappears from solution over time. After 48 hours recovery has been shown to have dropped significantly to only 0.5 to 7.0%. Therefore, it was considered that recovery rates for MNK at the end of the test (72 hours) would not produce meaningful data, and that all toxicity values should be based upon nominal concentrations. Mean cell densities at 24, 48 and 72 hours are given in Table A7.4.1.3-6; calculated percent values for the levels of inhibition of biomass and growth rate are given in Table A7.4.1.3-7. Values from the reference test with potassium dichromate (positive control) are given in Table A7.4.1.3-9.
5.3.1	NOE_rC	Not determined
5.3.2	E_rC_{50}	14.3 mg/l
5.3.3	E_bC_{50}	2.9 mg/l
5.4	**Conclusion**	The cell density of the control increased by a factor of 208, which fulfils the validity criteria of the test, which states that the density shall have increased by a factor of at least 16 within the 72-hour test period. Under the conditions of this study, the 72-hour EC_{50} of Methyl Nonyl Ketone to *Selenastrum capricornutum* was estimated as 2.9 mg/l using the biomass integral and 14.3 mg/l by growth rate calculation. The 48-hour EC_{50} was <2.5 mg/l by biomass integral and 7.3 mg/l by growth

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

		rate.
5.4.1	Reliability	1
5.4.2	Deficiencies	No

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5-03-2007
Materials and Methods	It is adopted applicant's version (revised) *Selenastrum capricornutum* at initial cell concentration of 10000 cells/ml was incubated with MNK (purity 99.5%) in growth medium (prepared according to OCDE guideline 201) at 22 °C and light intensity of 7,000 lux. A preliminary study had identified the 72 hrs-EC_{50} as being approximately 18 mg/l whereby tested ranges of concentrations were: a control, 2.5, 5.0, 10.0, 20.0, 40.0 and 80.0 mg a.s./L. Additionally, two control solutions were tested: one of them containing Dimethyl Formamide as solvent control (at a concentration of 1 ml DMF/L) and the other containing potassium dichromate as positive control. The culture medium was prepared using deionized water, which was filtered and sterilized by autoclaving at 120°C for 30 minutes. The sterile nutrient stock solutions were then added and the pH adjusted to 8.0 ± 0.2. The calculation of the biomass and algal growth was made using a haemocytometer and microscope after 24, 48, 72 hrs. Additional information: TEST ORGANISMS - Strain: Selenastrum capricornutum CCAP 278/4 - Source/supplier: [redacted] - Number of Replicates: 3. - Number of controls: 6. - Number of solvent controls: 3. STOCK AND TEST SOLUTION AND THEIR PREPARATION - Procedure: prepared from 80 mg/L stock solution. - Solvent/dispersant: Dimethyl Methyl Formamide (DMF). **Comments: (with respect to OECDE guideline 201)** Test performed was not conducted in closed vessels (or headspace method) in spite of significant volatility of MNK (Vp 11.8 Pa at 20 °C). In the test is indicated the following: *"Samples were taken at each exposure concentration at the start and end of the definitive test, for verification. Where present, algal cells were removed from the samples by centrifugation for 20 minutes at 3,000 rpm prior to analysis with samples being stored frozen".* However, the appendix with analytical verification of the test substance concentration is not enclosed with the study.
Results and discussion	It is not adopted applicant's version The analytical data given in study IIIA 7.4.1.2 demonstrates that recovery of MNK from solution is very low with typical recovery rates at 24 hours ranges from 0.5 to 7%. No test substance specific analysis was carried out as it was shown that MNK disappears from solution over time. After 48 hours recovery has been shown to have dropped significantly to only 2.0 to 4.7% (despite of renewal of the medium after the first 24 hrs).

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

	Therefore, MNK disappears from solution over time since is a volatile substance (Vp 11.8 Pa at T 20 ºC). Due to this fact, the results of the study must not be expressed as nominal concentrations if not that according to "Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures" of OECD must be expressed as the geometric mean exposure concentration during the test. However, as the appendix with analytical verification of the test substance concentration is not enclosed with the study, the measured concentrations are not available to estimate the geometric mean to derive EC_{50}. So, according to the above mentioned is rejected the use of the nominal concentration and hence the results and conclusions obtained by Applicant are considered as not valid.
Conclusion	The results and conclusions obtained by Applicant are considered as not valid, as are based on nominal concentrations in spite of the fact that MNK is a volatile substance, reason why the results should be based on measured concentrations (not available in the study).
Reliability	This study is classified with a reliability of 4.
Acceptability	This study is not considered acceptable because it presents important deficiencies and hence, the acute toxicity test in algae should be conducted again.
Remarks	

Table A7.4.1.3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Dimethyl formamide (DMF)
Concentration of vehicle	Not documented
Vehicle control performed	Yes, the vehicle control group was exposed to 1 ml/l DMF.
Other procedures	None documented

5.4.2.1 Table A7.4.1.3-2: Composition of algal nutrient medium

Nutrient	Final concentration in culture medium (mg/l)
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.6
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnCl_2$	0.003
$FeCl_3.6H_2O$	0.08
$CoCl_2.6H_20$	0.0015
$Na_2MoO_4.2H_2O$	0.007
$CuCl_2.2H_2O$	0.00001
$Na_2EDTA.2H_2O$	0.1
$NaHCO_3$	50

Table A7.4.1.3-3: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum* (Printz)
Strain	CCAP 278/4
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	A pre-culture growing in exponential phase under the following conditions: Temperature: 23 ± 2°C Illumination: 6000-10,000 lux (continuous white light) Shaking: 200 rpm
Pretreatment	None documented
Initial cell concentration	1×10^4 cells/ml

Table A7.4.1.3-4: Test system

Criteria	Details
Volume of culture flasks	250 ml
Culturing apparatus	Not documented
Light quality	Continuous illumination of white light: 6000- 10,000 lux
Procedure for suspending algae	Shaking at 200 rpm
Number of vessels/ concentration	3 replicates per test concentration, 6 control flasks and three DMF control flasks
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3-5: Test conditions

Criteria	Details
Test temperature	22 °C
pH	0 hours: 8.0 - 8.1 72 hours: 7.1 - 9.5
Aeration of dilution water	Not documented
Light intensity	7000 lux
Photoperiod	Continuous illumination

Table A7.4.1.3-6: Cell concentration data

Concentration nominal (mg/l)	Mean cell density measurements (cells/ml x 10^4)		
	24 hours	48 hours	72 hours
0	8.11	56.61	207.50
2.5	2.67	30.00	144.55
5.0	1.67	10.45	64.67
10.0	0.89	5.56	30.33
20.0	0.78	1.55	15.56
40.0	0.89	0.44	1.89
80.0	0.67	1.22	0.56
0 (DMF control)	4.78	42.22	211.56

Table A7.4.1.3-7: Percent inhibition by biomass integral and growth rate

Nominal concentration (mg/l)	Percent inhibition by biomass integral		Percent inhibition by growth rate	
	48 hours	72 hours	48 hours	72 hours
2.5	54	38	16	7
5.0	84	75	42	22
10.0	94	89	60	36
20.0	100	95	79	49
40.0	100	100	100	88
80.0	100	100	97	100
DMF control	30	9	7	-1

Table A7.4.1.3-8: EC_{50} values by biomass integral (E_bC_{50} value) and growth rate (E_rC_{50} value) for Methly Nonyl Ketone

Period of exposure (hours)	E_bC_{50} value (mg/l)	E_rC_{50} value (mg/l)
0 to 48	< 2.5	7.3
0 to 72	2.9	14.3

Table A7.4.1.3-9: Percent inhibition by biomass integral and growth rate (Reference test with Potassium Dichromate)

Nominal concentration (mg/L)	Percent inhibition by biomass integral		Percent inhibition by growth rate	
	48 hours	72 hours	48 hours	72 hours
0.18	0	-1	-2	1
0.32	0	-5	-4	-1
0.56	52	55	15	16
1.0	89	94	56	64
1.8	97	99	100	87

Table A7.4.1.3-10: EC_{50} values by biomass integral (E_bC_{50} value) and growth rate (E_rC_{50} value) for reference substance (Potassium Diochromate)

Period of exposure (hours)	E_bC_{50} value (mg/l)	E_rC_{50} value (mg/l)
0 to 48	0.59	0.86
0 to 72	0.58	0.88

Table A7.4.1.3-11: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**
Concentration of test substance ≥80% of initial concentration during test	**No**

Criteria for poorly soluble test substances	**Yes**

Figure A7.4.1.3-1: Growth curves for control and nominal test concentrations of Methyl Nonyl Ketone

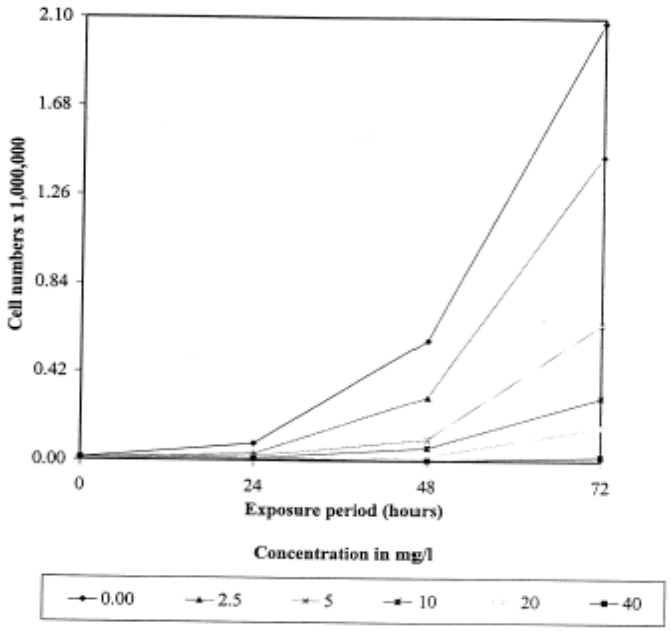


Figure A7.4.1.3-2: Estimation of E_bC_{50} values for Methyl Nonyl Ketone

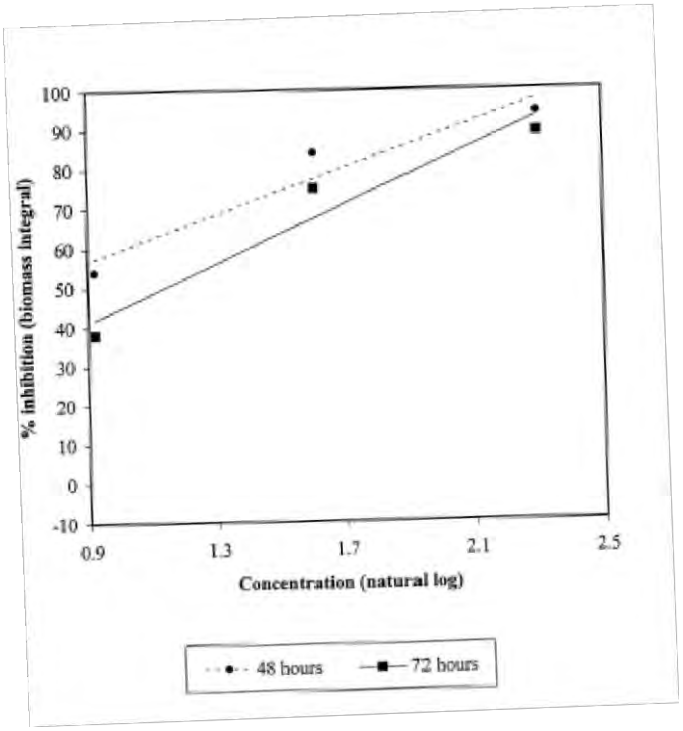
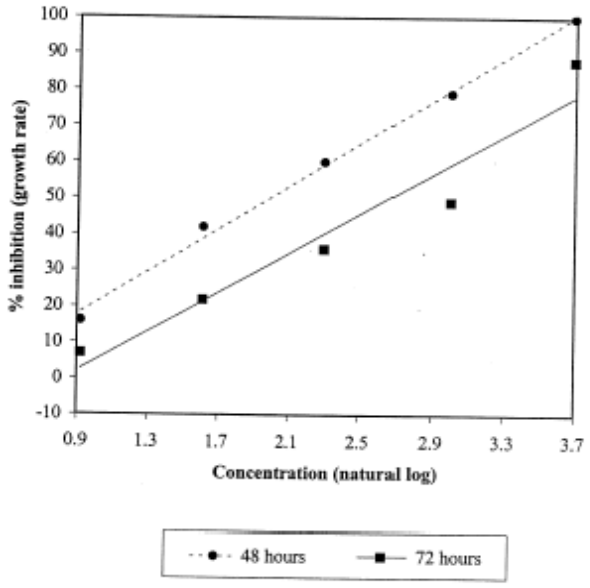


Figure A7.4.1.3-3: Estimation of E_rC_{50} values for Methyl Nonyl Ketone



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1 REFERENCE

1.2	**Reference**	[redacted]
		Dates of experimental work: April 18, 2008 - May 20, 2008.
1.3	**Data protection**	Yes
1.3.1	Data owner	Spotless UK Limited
1.3.2	Companies with letter of access	Not applicable
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.2	**Guideline study**	**Yes the test was carried out in accordance with OECD Guideline number 201 for Testing of Chemicals, "Alga, Growth Inhibition Test" .**
2.3	**GLP**	Yes
2.4	**Deviations**	Yes, the study deviates from OECD 201 Guideline in the following respects: 1. The validity criteria which states the concentration of test substance should be ≥80% of initial concentration during the test was not fulfilled. 2. The pH drift in the control and two of the test samples was equal to or greater than the 1.5 units recommended. However, the deviations are not considered to compromise the scientific validity of the study.

3 MATERIALS AND METHODS

3.2	**Test material**	Methyl Nonyl Ketone
3.2.1	Lot/Batch number	0550609421036
3.2.2	Specification	As given in section 2
3.2.3	Purity	99.6%
3.2.4	Composition of Product	Not applicable
3.2.5	Further relevant properties	Vapour pressure 3Pa at 20ºC and 50.8Pa at 25ºC. Volatility of the test substance led to the test being carried out in a closed system

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3.2.6	Method of analysis	Not documented
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Details are given in Table A7.4.1.3-1.
3.4	**Reference substance**	Yes, Potassium dichromate
3.4.1	Method of analysis for reference substance	Not documented
3.5	**Testing procedure**	
3.5.1	Culture medium	The composition of the nutrient medium is shown in Table A7.4.1.3-2. The culture medium was prepared using purified water, which was filtered and sterilized by autoclaving at 120°C for 30 minutes. The sterile nutrient stock solutions were then added and the pH adjusted to 8.0 ± 0.2 using 6 mmol/L HEPES-buffer.
3.5.2	Test organisms	*Pseudokirchneriella subcapitata (Formerly Selenastrum capricornutum)* (Printz), details are given in Table A7.4.1.3-3.
3.5.3	Test system	Details are given in Table A7.4.1.3-4.
3.5.4	Test conditions	Details are given in Table A7.4.1.3-5.
3.5.5	Duration of the test	72 hours
3.5.6	Test parameter	The 24 hour EC_{50} with respect to algal growth inhibition.
3.5.7	Sampling	Cell densities were determined at 24, 48 and 72 hours. A small volume was withdrawn at these time points and algal biomass was determined by fluorescence measurement At the completion of the test phase the shape of the algal cells were inspected visually
3.5.8	Monitoring of TS concentration	Yes, at the commencement of the test without algae and at completion of the test with algae
3.5.9	Statistics	The EC_{50} values for algal growth were determined using the biomass integral and the growth rate calculation, according to the OECD Guideline for Testing of Chemicals reference 201 "Alga, Growth Inhibition Test" (1984).
		4 RESULTS
4.2	**Limit Test**	Not performed
4.2.1	Concentration	Not applicable
4.2.2	Number/ percentage of animals showing adverse effects	Not applicable

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4.3	**Results test substance**	
4.3.1	Initial concentrations of test substance	0 (control), 0*(1:100 dilution), 0.38 (1:32 dilution), 1.4 (1:10 dilution), 3.6 (1:3.2 dilution) and 12.0mg/L (Undiluted filtrate). * Not detectable by analytical methods
4.3.2	Actual concentrations of test substance	0 (control), 0*, 0.38, 1.4, 3.6 and 12.0mg/L. * Not detectable by analytical methods
4.3.3	Growth curves	Refer to Figure A7.4.1.3-1.
4.3.4	Concentration / response curves	Refer to Figure A7.4.1.3-2 and Figure A7.4.1.3-3.
4.3.5	Cell concentration data	Data are provided in Table A7.4.1.3-6.
4.3.6	Effect data (cell multiplication inhibition)	Data are provided in Table A7.4.1.3-7 for 72 hour percent inhibition by growth rate. The 24 hour E_rC_{50} and E_yC_{50} values were estimated to be 1.9 mg/L and 1.4 mg/L, respectively. The NOEC was determined to be 0.38 mg/L. Data are provided in Table A7.4.1.3-8.
4.3.7	Other observed effects	No remarkable observations were made concerning the test media. All media were clear solutions throughout the test period.
4.4	**Results of controls**	The mean biomass of the control was 268.20 relative fluorescence units per ml at 72 hours. In the control mean biomass increased by a factor of 242 over 72 hours therefore fulfilling validity criteria. 10000 algal cells/mL, corresponds to approx 1.1×10^3 relative fluorescence units
4.5	**Test with reference substance**	
4.5.1	Concentrations	Not documented
4.5.2	Results	Data are provided in Table A7.4.1.3-9 for 72 hour E_rC_{50} and E_yC_{50} values.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.2	**Materials and methods**	*Pseudokirchneriella subcapitata* was used as the test organism. The test was conducted according to OECD guideline 201 and is described under point 3. The validity criteria which states the concentration of test substance should be 80% of initial concentration during the test was not fulfilled. The pH drift in the control and two of the test samples was equal to or greater than the 1.5 units recommended.
5.3	**Results and discussion**	As the test item is a volatile substance, the test was performed in a closed test system. At the start of the test, the analytically determined concentrations of the

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test item in the test media were 0.38 mg/L (dilution 1:32), 1.4 mg/L (dilution 1:10), 3.6 mg/L (dilution 1:3.2) and 12 mg/L (undiluted filtrate). At the end of the test, the concentrations of the test item in the test media could not be determined.

During the test period of 72 hours, the highest inhibitory effect on the growth of the algae was determined during the first day of the test. A lag phase was determined in the algal cultures exposed to the test item. During the second and third day of the test, a recovery of the algal growth was determined in the test item treatments with the exception of the highest test concentration in which the algal growth was completely inhibited. The lag phase in the exposed algal cultures may indicate recovery after initial toxic stress or reduced exposure due to loss of the test item (although the test was performed in a closed test system).

Therefore, the algal growth inhibition determined during the first day of the test was taken into account for the evaluation of the study and the biological results were related to the initial measured concentrations of the test item in the test media.

The test item had a significant inhibitory effect on the growth of the algae (growth rate and yield) after the test period of 24 hours at the initial measured concentration of 1.4 mg/L (dilution 1:10) and at all higher test concentrations. Thus, this concentration was determined to be the LOEC. The NOEC was determined to be 0.38 mg/L, since up to and including this test concentration (dilution 1:32) the growth rate and yield of the algae after 24 hours were not significantly lower than in the control.

5.3.1	NOE_rC	0.38 mg/L
5.3.2	E_rC_{50}	1.9 mg/L
5.3.3	E_yC_{50}	1.4 mg/L

5.4 Conclusion

The cell density of the control increased by a factor of 242, which fulfils the validity criteria of the test, which states that the density shall have increased by a factor of at least 16 within the 72-hour testperiod.

Under the conditions of this study, the 24-hour EC_{50} of Methyl Nonyl Ketone to *Pseudokirchneriella subcapitata* was estimated as 1.4 mg/L using the biomass integral and 1.9 mg/L by growth rate calculation.

5.4.1	Reliability	1
5.4.2	Deficiencies	Yes, The validity criteria which states the concentration of test substance should be 80% of initial concentration during the test was not fulfilled despite the study being carried out based on the recommendations of the OECD guidance document on aquatic toxicity testing of difficult substances and mixtures (2000) and the international standard ISO 14442 (1999). The pH drift in the control and two of the test samples was equal to or greater than the 1.5 units recommended.

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	Evaluation by Competent Authorities Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15-07-2008
Materials and Methods	It is adopted applicant's version (revised) The algal, Pseudokirchnerriella subcapitata, at initial concentration of 10,000 cell/ml was incubated with MNK in a growth medium at a temperature of 23-24°C and at a mean measured light intensity of approx. 7,700 lux. The purity of the technical MNK was 99.6%. The composition of the nutrient medium is shown in Table A7.4.1.3-2. The culture medium was prepared using purified water, which was filtered and sterilized by autoclaving at 120°C for 30 minutes. The sterile nutrient stock solutions were then added and the pH adjusted to 8.0 ± 0.2 using 6 mmol/L HEPES-buffer. As MNK is a volatile substance, the test was performed in a closed system (modified guideline OECD 201). The tests flasks were completely filled with the test medium and were sealed with glass stoppers to avoid losses of MNK due to volatilization. The test method was based on the recommendations of the OECD *"Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures"* and the International Standard ISO 14442. As the solubility of MNK in the test water is below 100 mg/L, a dispersion of the MNK was prepared in the test water and stirred for 24 hours in order to dissolve a maximum amount of MNK. After the stirring period, the dispersion was filtered through a membrane filter and the undiluted filtrate (saturated solution) was used as the highest concentration and as stock solution for the preparation of the lower concentrated test media. The following treatments were tested in parallel to a control: Dilution 1:100, 1:32, 1:10, 1:3.2 and the undiluted filtrate (saturated solution). Cell densities were determined at 24, 48 and 72 hours. A small volume was withdrawn at these time points and algal biomass was determined by fluorescence measurement At the completion of the test phase the shape of the algal cells was inspected visually. **Comments:** The validity criteria which states the concentration of test substance should be 80% of initial concentration during the test was not fulfilled despite the study being carried out based on the recommendations of the OECD guidance document on aquatic toxicity testing of difficult substances and mixtures (2000) and the international standard ISO 14442 (1999). The pH drift in the control and two of the test samples was equal to or greater than the 1.5 units recommended.
Results and discussion	It is adopted applicant's version (revised) At the start of the test, the concentrations of MNK (determined by HPLC) in the test media were 0.38 mg/L (dilution 1:32), 1.4 mg/L (dilution 1:10), 3.6 mg/l (dilution 1:3.2) and 12 mg/L (undiluted filtrate). At the end of the test, the concentrations of MNK in the test media could not be determined. The samples from the dilution of 1:100 were not analyzed, since this concentration was below

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	the NOEC determined in the test. During the test period of 72 hours, the highest inhibitory effect on the growth of the algae was determined during the first day of the test. A lag phase was determined in the algal cultures exposed to MNK. During the second and third day of the test, a recovery of the algal growth was determined in the MNK treatments. The lag phase in the exposed algal cultures may indicate recovery after initial toxic stress or reduced exposure due to loss of MNK (although the test was performed in a closed test system). Therefore, the algal growth inhibition determined during the first day of the test was taken into account for the evaluation of the study and the results were related to initial measured concentrations of MNK in the test media *(see Table A7.4.1.3-8).* MNK had a significant inhibitory effect on the growth of the algae (growth and biomass) after the test period of 24 hours at the initial measured concentration of 1.4 mg/L and at all higher test concentrations (results of Dunnett's tests, one sided, $\alpha = 0.05$). Thus, this concentration was determined to be the LOEC. The NOEC was determined to be 0.38 mg/L, since up to and including this concentration the growth rate and biomass of the algae after 24 hours were not significantly lower than in the control. The microscopic examination of the algal cells at the end of the test showed no difference between the algae growing in the concentration of 3.6 mg/L and the algal cells in the control. MNK did not affect the shape and size of the algal cells up to at least this concentration.
Conclusion	Based on the first day of the study, the NOEC was determined to be 0.38 mg a.s./L. The LOEC was determined to be 1.4 mg a.s./L, and the EC_{50} for the inhibition of the growth rate and biomass were 1.9 mg a.s./L and 1.4 mg a.s./L, respectively.
Reliability	This study is classified with a reliability of 3.
Acceptability	This study is considered acceptable although it does not fulfil the validity criteria which states the concentration of test substance should be 80% of initial concentration during the test.
Remarks	

Table A7.4.1.3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Test water
Concentration of vehicle	Not applicable
Vehicle control performed	No
Other procedures	The test was performed in a closed system. Test flasks were completely filled and fitted with glass stoppers.

5.4.2.1 Table A7.4.1.3-2: Composition of algal nutrient medium

Nutrient	Final concentration in culture medium (mg/L)
NH_4Cl	15
$MgCl_2.6H_2O$	12
$CaCl_2.2H_2O$	18
$MgSO_4.7H_2O$	15
KH_2PO_4	1.6
H_3BO_3	0.185
$MnCl_2.4H_2O$	0.415
$ZnCl_2$	0.003
$FeCl_3.6H_2O$	0.064
$CoCl_2.6H_20$	0.0015
$Na_2MoO_4.2H_2O$	0.007
$CuCl_2.2H_2O$	0.00001
$Na_2EDTA.2H_2O$	0.1
$NaHCO_3$	250
HEPES-buffer	1430

Table A7.4.1.3-3: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata* (Printz)
Strain	61.81 SAG
Source	[redacted]
Laboratory culture	Yes
Method of cultivation	A pre-culture growing in exponential phase under the following conditions: Temperature: 23 ± 1°C Illumination: 7700 Lux (range: 7100 to 8100 Lux continuous white light) Magnetic stirring
Pretreatment	None documented
Initial cell concentration	10,000 cells/ml

Table A7.4.1.3-4: Test system

Criteria	Details
Volume of culture flasks	50 ml
Culturing apparatus	Not documented
Light quality	Continuous illumination of white light: 7700 Lux (range: 7100 to 8100 Lux)
Procedure for suspending algae	Magnetic stirring
Number of vessels/ concentration	3 replicates per test concentration, 6 control flasks
Test performed in closed vessels due to significant volatility of TS	Yes, each flask was completely filled and fitted with a glass stopper to prevent loss of volatile test substance.

Table A7.4.1.3-5: Test conditions

Criteria	Details
Test temperature	23 °C to 24 °C
pH	0 hours: 8.2 72 hours: 8.1 – 10.2
Aeration of dilution water	Not documented
Light intensity	7700 lux
Photoperiod	Continuous illumination

Table A7.4.1.3-6: Cell concentration data

Concentration nominal (mg/L)	Biomass of algae*(relative fluorescence units)		
	24 hours	48 hours	72 hours
0 (Control)	5.5	49.1	268.2
0.38	5.6	40.8	217.0
1.4	3.3	24.8	152.8
3.6	1.4	6.2	48.5
12	0.3	0.0	0.4

*: The biomass was determined by fluorescence measurement (duplicate measurements per replicate) and is given as relative fluorescence units (x 10^3). At the start of the test, the initial cell density was 10000 algal cells/mL, corresponding to 1.1 x 10^3 relative fluorescence units).

Table A7.4.1.3-7: Percent inhibition by growth rate

Concentration nominal (mg/L)	Percent inhibition by growth rate		
	24 hours	48 hours	72 hours
0 (Control)	0	0	0
0.38	-0.5	9.0	-4.9
1.4	33.7	6.9	-5.0
3.6	83.5	33.6	-20.3
12	172.2*	387.2*	27.9

* Biomass was lower than the start value resulting in a calculated inhibition higher than 100%

Table A7.4.1.3-8 Results of test based on the interval 0-24 hours and related to initial measured concentrations

Parameter	Growth rate	Biomass
EC_{10} (mg/L) 95% Confidence interval	0.79 (0.72-0.86)	0.62 (0.39-0.79)
EC_{20} (mg/L) 95% Confidence interval	1.07 (0.99-1.13)	0.82 (0.59-0.98)
EC_{50} (mg/L) 95% Confidence interval	1.9 (1.8-2.0)	1.4 (1.2-1.6)
NOEC (mg/L)	0.38	0.38
LOEC (mg/L)	1.4	1.4

Table A7.4.1.3-9: Percent inhibition by biomass integral and growth rate (Reference test with Potassium Dichromate)

Period of exposure (hours)	E_rC_{50} value (mg/L)
0 to 72	1.20

Table A7.4.1.3-10: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	Fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**Yes**
Concentration of test substance ≥80% of initial concentration during test	**No**

Criteria for poorly soluble test substances	**Yes**

Figure A7.4.1.3-1: Growth Curves of the Algae over the Test Duration of 72 hours

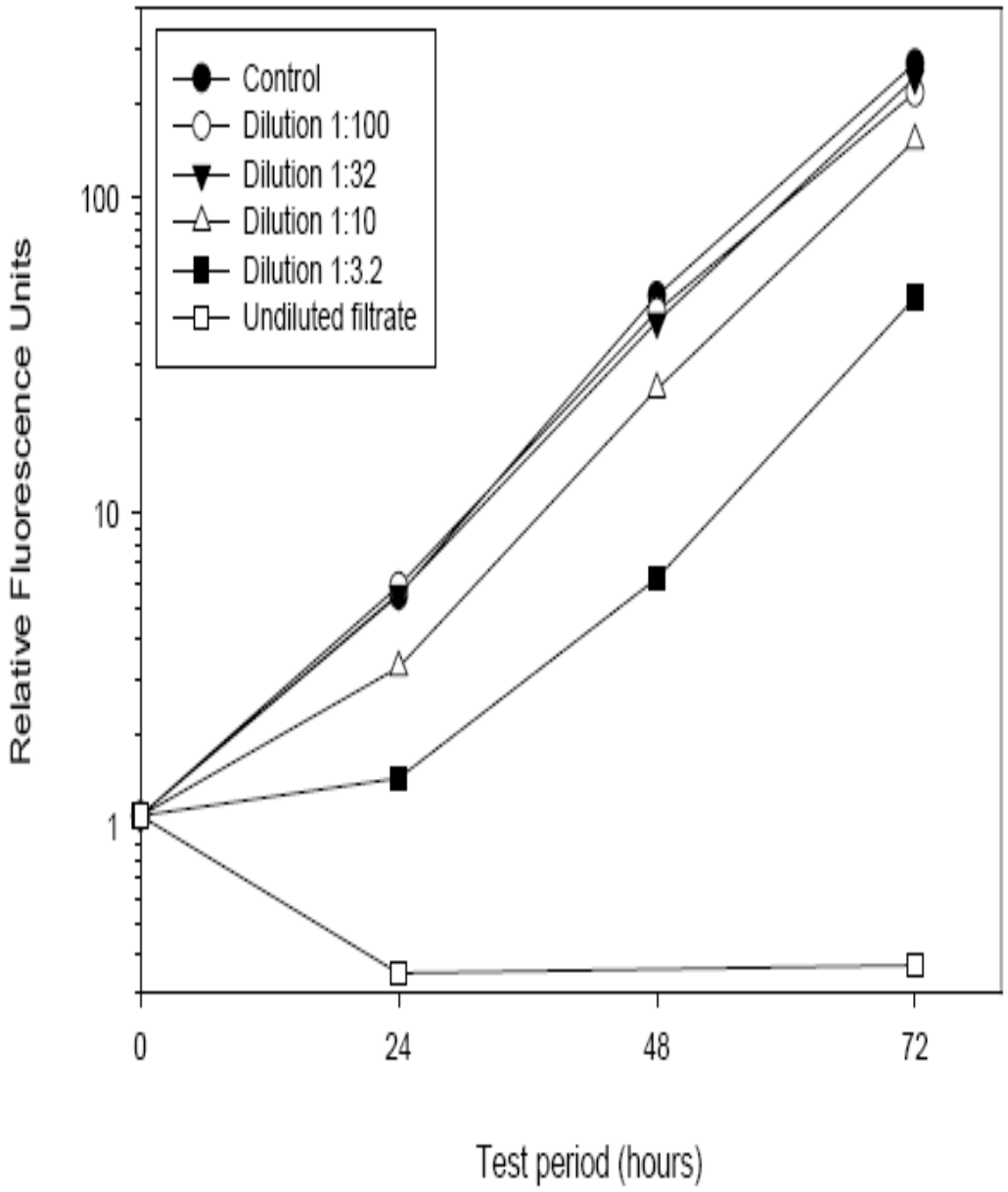


Figure A7.4.1.3-2: Concentration-Effect Relationship of Growth Rates after 24 Hours

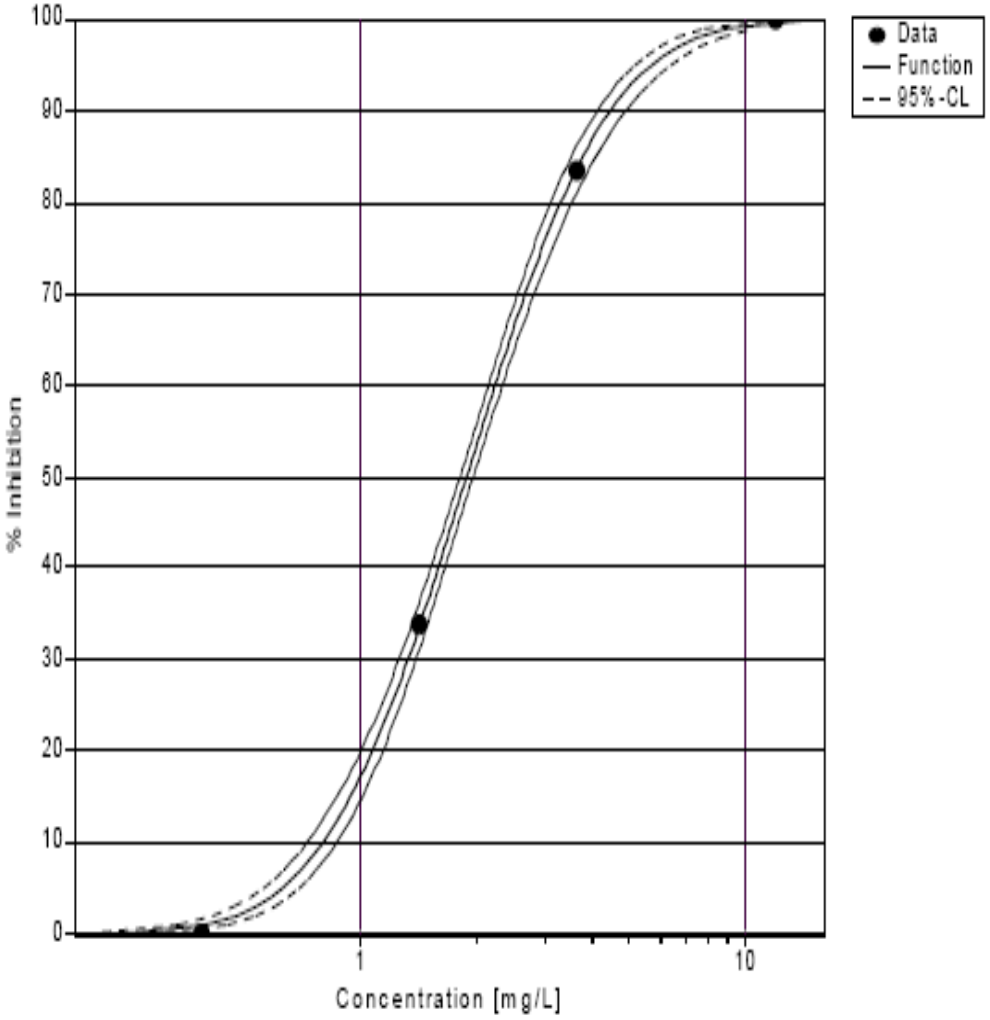
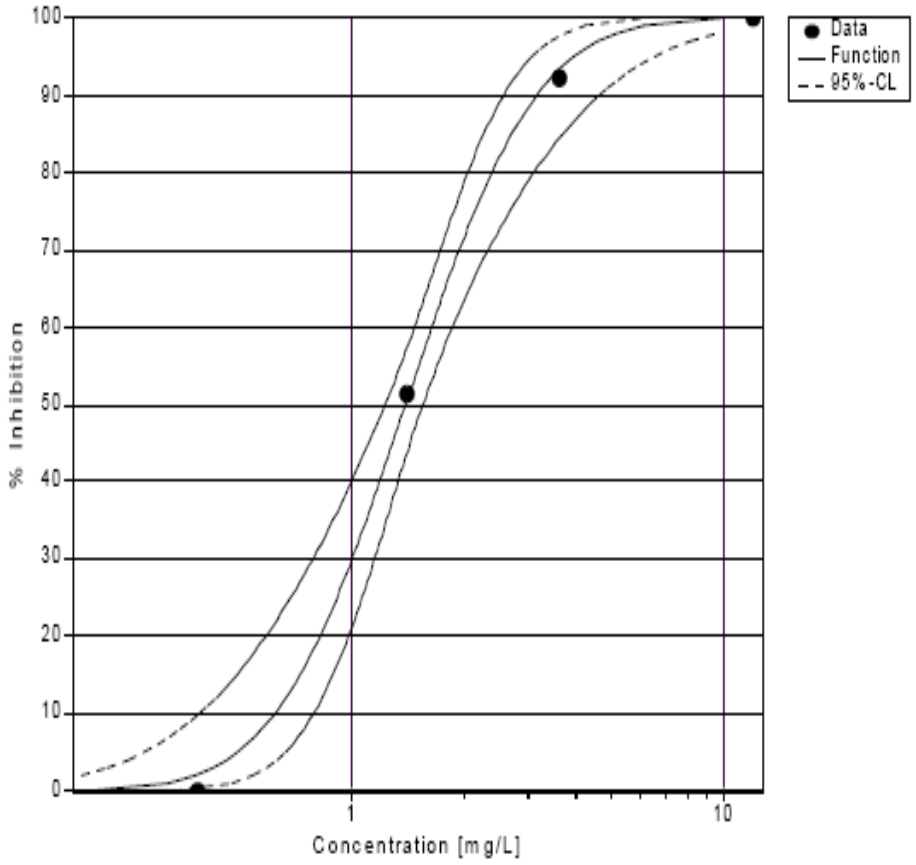


Figure A7.4.1.3-3: Concentration-Effect Relationship of Yield after 24 Hours



Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]	
		Date of experimental work: February 20, 2002.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Guaber UK Limited	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	**Yes**	
		OECD Guideline 209 for Testing of Chemicals, Activated Sludge, Respiration Inhibition Test.	
2.3	**GLP**	Yes	
2.4	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.2	**Test material**	Methyl Nonyl Ketone (MNK Tech)	
3.2.1	Lot/Batch number	ECO120136	
3.2.2	Specification	As given in section 2	
3.2.3	Purity	99.5% (refer to Certificate of Analysis in Report No. ENV5982/120136)	
3.2.4	Composition of Product	Not applicable	
3.2.5	Further relevant properties	None	
3.2.6	Method of analysis	Not applicable	
3.3	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable	
3.4	**Reference substance**	3,5-dichlorophenol (3,5-DCP)	
3.4.1	Method of analysis for reference	Not applicable	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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	substance	
3.5	**Testing procedure**	
3.5.1	Culture medium	Synthetic medium was prepared according to OECD test guideline 209
3.5.2	Inoculum / test organism	Please refer to Table A7.4.1.4-1.
3.5.3	Test system	Please refer to Table A7.4.1.4-2.
3.5.4	Test conditions	Please refer to Table A7.4.1.4-3.
3.5.5	Duration of the test	3 hours
3.5.6	Test parameter	Respiration inhibition
3.5.7	Analytical parameter	Oxygen consumption rate
3.5.8	Sampling	After a 3 hour incubation period the respiration rates were measured in each vessel.
3.5.9	Monitoring of TS concentration	No
3.5.10	Controls	Two inoculated samples without test substance.
3.5.11	Statistics	The results obtained from each test vessel were plotted as a graph of dissolved oxygen concentrations (mg/l) against time (minutes). In order to calculate the inhibitory effect of a test substance at a particular concentration, the respiration rate is expressed as a percentage of the mean of the two control respiration rates. Where possible, the EC_{50}, EC_{20} and EC_{80} values were obtained by plotting a graph of percentage respiration inhibition against the natural logarithm of the test material concentration. Linear regression analysis was done to describe the relationship between percentage respiration inhibition and test material concentration.
		4 RESULTS
4.2	**Preliminary test**	Not performed
4.2.1	Concentration	
4.2.2	Effect data	
4.3	**Results test substance**	
4.3.1	Initial concentrations of test substance	Nominal concentration range: 60, 120, 240, 480, and 960 mg/l
4.3.2	Actual concentrations of test substance	Not documented
4.3.3	Growth curves	Not applicable
4.3.4	Cell concentration data	Not applicable

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

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4.3.5	Concentration/ response curve	Refer to Figures A7.4.1.4-1 and A7.4.1.4-2.
4.3.6	Effect data	The results are presented in Table A7.4.1.4-4. An EC_{50} of 379.49 mg/l was determined. The EC_{80} and EC_{20} values were calculated as 1182.91 mg/l and 121.75 mg/l respectively.
4.3.7	Other observed effects	MNK gave a dose related response with a maximum value of 68% inhibition at the top concentration (nominally 960 mg/l). The results of the abiotic control flask (test without addition of inocula) indicated that there would be no reduction in oxygen concentration in any of the test vessels up to, and including, 960 mg/l MNK, attributable to processes in the test system other than those due to the activity of the activated sludge.
4.4	**Results of controls**	The validation criteria for the control respiration rates were satisfied. Refer to Table A7.4.1.4-4.
4.5	**Test with reference substance**	Performed
4.5.1	Concentrations	The reference substance, 3,5-DCP was applied at 5, 15, and 30 mg/l.
4.5.2	Results	The 3 hour EC_{50} for 3,5-DCP was 6.75mg/L. This result satisfied the OECD validity condition that the EC_{50} be within the range of 5 to 30 mg/l.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.2	**Materials and methods**	The study was conducted to determine the effect of Methyl Nonyl Ketone on the respiration of activated sewage sludge. The test was conducted according to OECD guideline 209 and is described under point 3.
5.3	**Results and discussion**	The validity criteria for the control respiration rates and reference material EC_{50} values were satisfied. MNK gave a dose related response with a maximum value of 68% inhibition at the top concentration (nominally 960 mg/l). The results of the abiotic control flask (test without addition of inocula) indicated that there would be no reduction in oxygen concentration in any of the test vessels up to, and including, 960 mg/l MNK, attributable to processes in the test system other than those due to the activity of the activated sludge.
5.3.1	EC_{20}	121.75 mg/l
5.3.2	EC_{50}	379.49 mg/l
5.3.3	EC_{80}	1182.91 mg/l
5.4	**Conclusion**	The validity criteria for the control respiration rates and reference material EC_{50} values were satisfied. The effect of the test material on the respiration of activated sewage sludge micro-organisms gave a 3 hour EC_{50} of 379.49 mg/l.
5.4.1	Reliability	1
5.4.2	Deficiencies	None

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5-03-2007
Materials and Methods	It is adopted applicant's version (revised). Assessment of the inhibitory effect of MNK (purity 99.5%) on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure of 180 min was determined. The inoculum from laboratory wastewater plant treating municipal sewage was brought to a concentration of 4 g/L dry substance and aerated overnight. A volume of 120 ml of the final inoculum was added to each test vessel giving a final concentration of 1.60 g/l dry weight, in each test vessel For test substance concentrations tested were: a control, 60, 120, 480, 960 mg a.i./L, and 3 concentrations of a reference substance (3,5-dichlorophenol). The test was performed at a temperature of 20±2°C, **Comments:** Test performed was not conducted in closed vessels (or headspace method) in spite of significant volatility of MNK (Vp 11.8 Pa at 20 °C). pH value after an incubation time of 3 hours is not indicated.
Results and discussion	It is adopted applicant's version (revised). The validity criteria for the control respiration rates and reference material EC_{50} values were satisfied. MNK gave a dose related response with a maximum value of 68% inhibition at the top concentration (see Table A7.4.1.4-4). The results of the abiotic control flask (test without addition of inoculums) indicated that there would be no reduction in oxygen concentration in any of the test vessels up to, and including, 960 mg/l MNK, attributable to processes in the test system other than those due to the activity of the activated sludge. The results obtained from each test vessel were plotted as a graph of dissolved oxygen concentrations (mg/l) against time (minutes). In order to calculate the inhibitory effect of a test substance at a particular concentration, the respiration rate is expressed as a percentage of the mean of the two control respiration rates. Where possible, the EC_{50}, EC_{20} and EC_{80} values were obtained by plotting a graph of percentage respiration inhibition against the natural logarithm of the test material concentration. Linear regression analysis was done to describe the relationship between percentage respiration inhibition and test material concentration. So, for the test substance the results are: EC_{20} = 121.75mg/l (nominal concentration) EC_{50} = 379.49 mg/l (nominal concentration) EC_{80} = 1182.91 mg/l (nominal concentration) NOEC = 60 mg/l (nominal concentration) These results can be observed in Figure A7.4.1.4-3. **Comment:** The results are expressed as nominal concentrations (in spite of the fact that MNK is a volatile substance) as the concentrations were not measured during the test.
Conclusion	It is adopted applicant's version.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

	The effect of the test material on the respiration of activated sewage sludge micro-organisms gave a 3-hours EC_{50} of 379.49 mg/l and a NOEC of 60 mg/l.
Reliability	This study is classified with a reliability of 2.
Acceptability	This study is considered acceptable because it does not present important deficiencies.
Remarks	

Table A7.4.1.4-1: Inoculum / Test organism

Criteria	Details
Nature	Activated sewage sludge
Species	Not applicable
Strain	Not applicable
Source	[redacted]
Sampling site	[redacted]
Laboratory culture	No, sewage was freshly obtained
Method of cultivation	Not documented
Preparation of inoculum for exposure	The sludge was centrifuged and washed with dechlorinated tap water 3 times before use. The pellet was resuspended in dechlorinated tap water to give a dry weight level of 4.0g/l in the final inoculum. The inoculum was aerated on a magnetic stirrer overnight at room temperature.
Pretreatment	A volume of 120 ml of the final inoculum was added to each test vessel giving a final concentration.
Initial cell concentration	1.60 g/l dry weight

Table A7.4.1.4-2: Test system

Criteria	Details
Culturing apparatus	250 ml (nominal) BOD bottles
Number of culture flasks/concentration	2 control flasks, 1 flask at each test concentration and at each reference standard concentration.
Aeration device	Aquarium type pump
Measuring equipment	Oxygen electrode with a built-in stirrer
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.4-3: Test conditions

Criteria	Details
Test temperature	20.0 ± 2 °C
pH	7.88 (at test initiation)
Aeration of dilution water	Yes
Suspended solids concentration	1.6 g/l dry weight

Table A7.4.1.4-4: Respiration rates and percentage respiration inhibition

Treatment	Respiration rate (R) (mg O_2/l/h)	Percentage respiration inhibition (I)
Control (1)	31.39	-
Abiotic 960 mg/L	0.14	100
DCP 5 mg/L	21.86	39
DCP 15 mg/L	7.90	78
DCP 30 mg/L	3.29	91
60 mg/L	36.78	-2
120 mg/L	28.05	22
240 mg/L	22.89	37
480 mg/L	12.75	65
960 mg/L	11.46	68
Control (2)	40.72	-

Mean control respiration rate = 36.05 (mg O_2/L/h)
Control 1 valid range (±15%) = 26.68 to 36.10 (mg O_2/L/h)
Control 2 valid range (±15%) = 34.61 to 46.83 (mg O_2/L/h)

Figure A7.4.1.4-1: The effect of MNK on activated sludge oxygen consumption (mg/l)

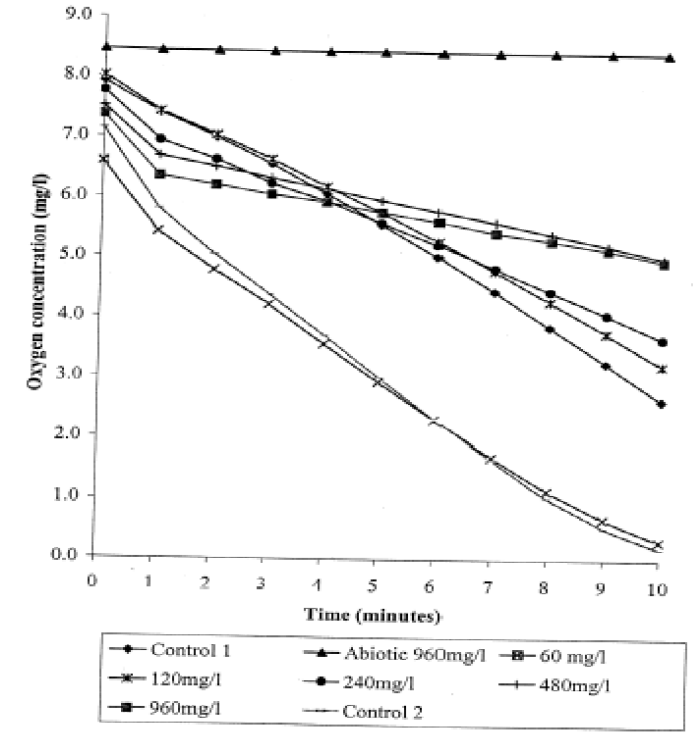


Figure A7.4.1.4-2: The effect of 3,5-DCP on activated sludge oxygen consumption (mg/l)

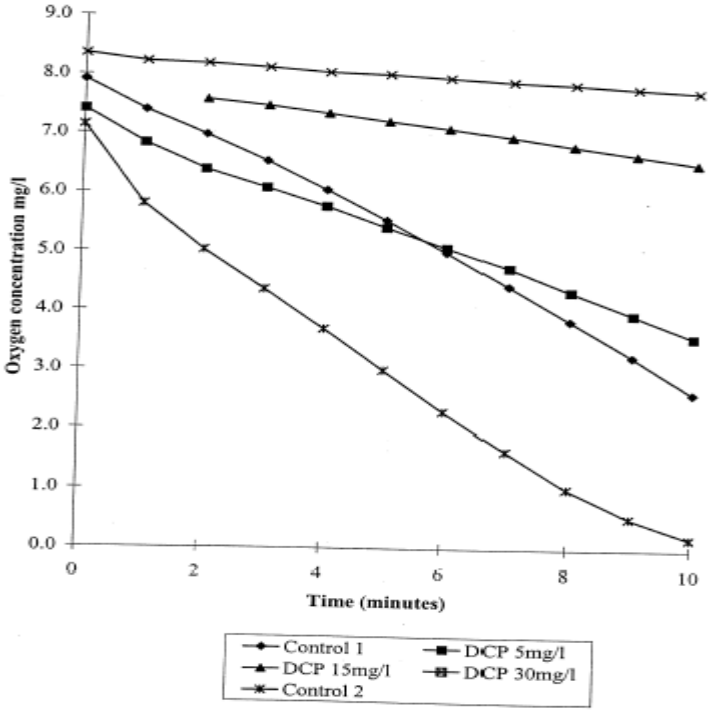
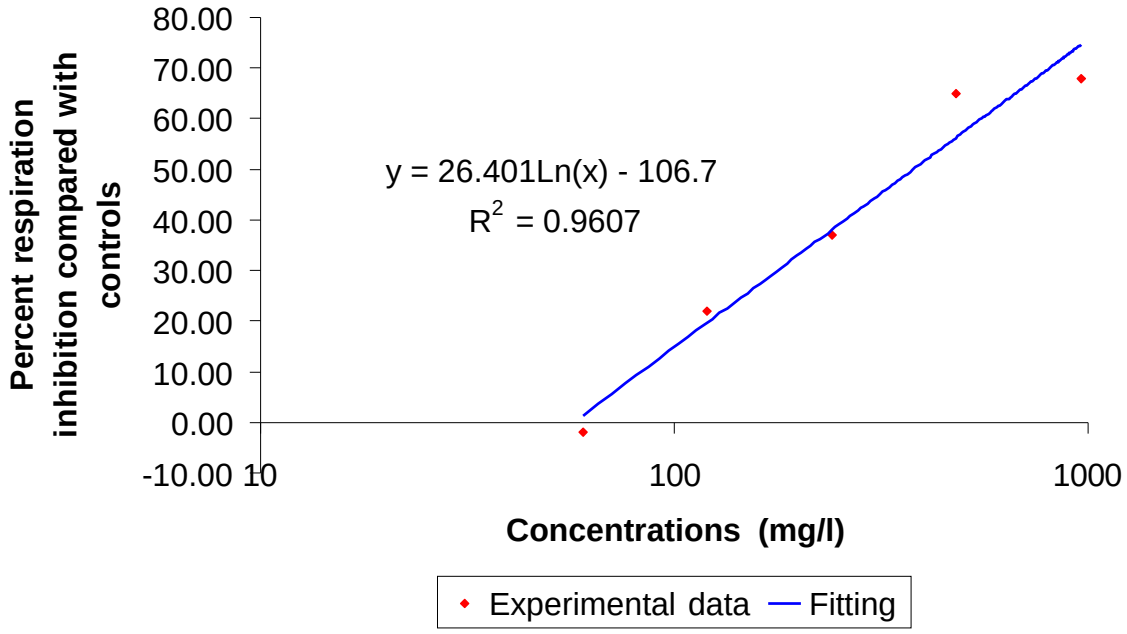


Figure A7.4.1.4-3: Inhibition of activated sludge respiration by MNK



Section A8 Measures necessary to protect man, animals and the environment

Official use only

Subsection (Annex Point)		
8.1		**Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA8.1)**
8.1.1	**Methods and precautions concerning placing on the market**	Vapet® Get Off™ Spray is recommended for use by non-professionals. Specific training is not required for the use of Vapet® Get Off™ Spray. Methyl Nonyl Ketone is produced in systems with appropriate control measurements in place to exclude release of the active substance. The production area also has extraction.
8.1.2	**Methods and precautions concerning production, handling and use of the active substance and its formulations**	Engineering Control: None required Hygiene Measures: None required Protective Equipment: In production, it is recommended that suitable gloves and eye and face protection is worn in addition to overalls and safety work boots generally worn by all operatives within the production area. Protection of Bystanders: None required. The recommended uses have no potential for food, feeding stuff or drinking water contact, and since Methyl Nonyl Ketone will not persist in the environment due to its high volatility, the general public will not be exposed above the thresholds of concern. Therefore, the overall secondary exposure is considered negligible. Precautionary Methods Against Environmental Exposure: Methyl Nonyl Ketone is produced in systems with appropriate control measurements in place to exclude release of the active substance. Production times are relatively short and at ambient temperatures. The usual precautionary measures for handling chemicals should be observed.
8.1.3	**Methods and precautions concerning storage of the active substance and its formulations**	Store in accordance with local/regional and international regulations. To maintain quality: store in closed containers at ambient temperatures; storage temperature preferred between 15-25°C.
8.1.4	**Methods and precautions concerning transport of the active substance and its formulations**	Not classified as a Dangerous Goods for Transport.

Section A8 Measures necessary to protect man, animals and the environment

			Official use only
8.1.5	**Methods and precautions concerning fire of the active substance and its formulations**	Suitable Extinguishing Media: Foam, dry chemical powder or carbon dioxide. Unsuitable Extinguishing Media: Water jet Unusual Fire and Explosion Hazards: None Protective Equipment: No specific recommendations. Special Exposure Hazards: Vapours may cause coughing. Other Information: Cool closed containers with water.	
8.2		**In case of fire, nature of reaction products, combustion gases, etc. (IIA8.2)** No typical hazardous decomposition products are known.	
8.3		**Emergency measures in case of an accident (IIA8.3)**	
8.3.1	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment if available**	In general: In all cases of doubt, or when symptoms persist, seek medical attention. If inhaled: Move to fresh air, rest, half upright position, loosen clothing. Oxygen or artificial respiration if there is difficulty in breathing. Seek medical advice after significant exposure. If on skin: Remove contaminated clothing. Wash skin thoroughly with soap and water or use recognised skin cleanser. Launder clothes before reuse. Wash immediately with soap and water. If in eyes: Rinse immediately and for as long as possible with plenty of water. Eyelids should be held away from the eyeball to ensure thorough rinsing. Seek medical advice if irritation persists. If swallowed: Do not induce vomiting because of risk of aspiration. Seek medical advice. Note to physician: Liquid splashes in the eyes may cause irritation and reversible damage.	
8.3.2	**Emergency measures to protect the environment**	Minimise spread – products containing Methyl Nonyl Ketone should not be dumped, spilled, rinsed or washed into sewers or public waterways. When off loading, ensure vehicle is in a bunded area, and products containing Methyl Nonyl Ketone should be stored in a bunded area. If in a worst case situation, Methyl Nonyl Ketone were to contaminate water in excessive volumes, as a consequence of its low water solubility and high vapour pressure it will form a layer on top of water bodies and will volatilise rapidly.	

Section A8 Measures necessary to protect man, animals and the environment

			Official use only
8.4		**Possibility of destruction or decontamination following release in or on the following: (a) Air; (b) Water, including drinking water; (c) Soil (IIA8.4)**	
8.4.1	**Possibility of destruction or decontamination following release in the air**	Collect as much of the substance as possible in a clean container for reuse or disposal. Cover the remainder with inert absorbent (e.g. vermiculite) for disposal. Avoid contact with eyes. Wear suitable gloves and eye/face protection.	
8.4.2	**Possibility of destruction or decontamination following release in water, including drinking water**	There is no readily available method for neutralisation or decontamination of water. As Methyl Nonyl Ketone is practically insoluble in water and has a high vapour pressure, it will form a layer on top of water bodies and will volatilise rapidly. Any chemical or additive, which could be used to decontaminate water, is likely to be more harmful than Methyl Nonyl Ketone itself. The following precautions can be taken to avoid contamination: Minimise spread – products containing Methyl Nonyl Ketone should not be dumped, spilled, rinsed or washed into sewers or public waterways. When off loading, ensure vehicle is in a bunded area, and products containing Methyl Nonyl Ketone should be stored in a bunded area. If in a worst case situation, Methyl Nonyl Ketone were to contaminate water in excessive volumes, as a consequence of its low water solubility and high vapour pressure it will form a layer on top of water bodies and will volatilise rapidly.	
8.4.3	**Possibility of destruction or decontamination following release in or on soil**	Collect as much of the substance as possible in a clean container for reuse or disposal. Cover the remainder with inert absorbent (e.g. vermiculite) for disposal. Avoid contact with eyes. Wear suitable gloves and eye/face protection.	
8.5		**Procedures for waste management of the active substance for industry or professional users e.g. possibility of re-use or recycling, neutralisation, conditions for controlled discharge, and incineration (IIA8.5)**	
8.5.1	**Possibility of re-use or recycling**	Empty containers are not for re-use.	
8.5.2	**Possibility of neutralisation of effects**	None.	
8.5.3	**Conditions for controlled discharge including leachate qualities on disposal**	None.	
8.5.4	**Conditions for controlled incineration**	As Methyl Nonyl Ketone does not have a halogen content greater than 60%, an investigation into the pyrolytic behaviour under controlled conditions is not required.	

Section A8 Measures necessary to protect man, animals and the environment

		Official use only
	The recommended means of safe disposal is by controlled incineration at an approved chemical waste facility. No compounds of concern are generated as a result of incineration.	
8.6	**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIA8.6)** According to the data presented in Section A 7.5, undesired and unintended side effects for non-target organisms and vertebrates are not expected to be caused by Methyl Nonyl Ketone.	
8.7	**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of groundwater against pollution caused by certain dangerous substances (IIA8.7*)*** None.	

Evaluation by Competent Authorities

Evaluation by Rapporteur Member State

Date	January 2008
Comments on applicant's data	
Conclusion	RMS considered sufficient the information provided by the applicant.
Acceptability	Acceptable
Remarks	

Section 9: Classification and Labelling

Classification	Irritant
Class of danger	Xi
R-phrases	R38 Irritating to skin
S-phrases	S37 Wear suitable gloves S46 If swallowed, seek medical advice immediately and show this container or label S64 If swallowed, rinse mouth with water (only if the person is conscious)
Classification for the Environment	Dangerous for the environment
Class of danger	N
R-phrases	R50/53: Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-phrases	S29: Do not empty into drains. S35: This material and its container must be disposed of in a safe way S61: Avoid release to the environment. Refer to special instruction/safety data sheet

Classification according to the Regulation (EC) No 1272/2008 of the European Parliament and of the Council and the Globally Harmonised System of Classification and Labelling of Chemicals (hereinafter referred to as "the GHS"):

GHS Pictograms		
Signal Word	Warning	
Classification for human health	Hazard class and category: Hazard statement	Category 2; Skin Irrit. 2 H315: Causes skin irritation
Prevention precautionary statement	P264: Wash … thoroughly after handling P280: Wear protective gloves/protective clothing/eye protection/ face protection	
Response precautionary statements	P302+P352: IF ON SKIN: Wash with plenty of soap and water P321: Specific treatment (see … on this label). P332+P313: If skin irritation occurs: Get medical advice/attention. P362: Take off contaminated clothing and wash before reuse	
GHS Pictograms		
Signal Word	Warning	
Classification for the Environment	Hazard class and category: Hazard statement	Hazardous to the aquatic compartment, chronic toxicity, Category 1 H410: Very toxic to aquatic life with long lasting effects
Prevention precautionary statement	P273: Avoid release to the environment	
Response precautionary	P391: Collect spillage	

statements	
Disposal precautionary statements	P501: Dispose of contents/container to…