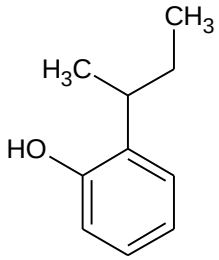
資料１－１

既存化学物質審査シート（分解性）

官報公示整理番号	3-503	CAS No.	89-72-5
判定案	良分解性		

物質名称 構造式等	名　称：２－ｓｅｃ－ブチルフェノール（官報公示整理番号 3-503：CAS 89-72-5） 分子量：150.22 分子式：$C_{10}H_{14}O$ 
既知の 分解性 情報	**既存点検結果**（経済産業公報公表　平成 13 年 5 月 10 日） **難分解性** 分解度試験（化審法テストガイドライン、OECD テストガイドライン 301C：28 日間） ＢＯＤによる平均分解度：0 %（ 0, 0, 0）（基礎呼吸区 5.8 mg、汚泥区平均 3.1 mg） HPLC による平均分解度：0 %（ 0, 0, 0）
新たに 得られた 知見	**１．分解性** **(1)易分解性** 分解度試験（OECD テストガイドライン 301D Closed Bottle 法：28 日間） ＢＯＤによる平均分解度：63 %（61, 64）（被験物質濃度 1.5mg/l） 63 %（63, 64）（被験物質濃度 3.0mg/l） ※GC 等の測定は実施せず **(2)易分解性** 分解度試験（OECD テストガイドライン 302C：28 日間） ＢＯＤによる平均分解度：90 % ※非 GLP 試験　GC 等の測定は実施せず **２．活性汚泥の阻害性** 活性汚泥阻害性試験（OECD テストガイドライン 209） 呼吸阻害率　8%（被験物質濃度 10mg/l） 78%（被験物質濃度 100mg/l） ※非 GLP 試験

分解性再評価の背景	本物質は、平成 10 年に行った既存化学物質の安全性点検(分解性試験)の結果、化審法上、難分解性となっている。 一方、OECD では、本年 10 月に本物質の有害性の評価(SIDS INITIAL ASSESSMENT)の結果を確定し、分解性については易分解と評価した。 このように、本物質の分解性については国内外で評価結果が異なっていることから、今般、OECD の評価で得られた新たな知見を含めて分解性の再評価を行う。 参考：OECD における易分解評価の理由 ①TG301D による分解性試験の結果は易分解（BOD　63%）であること。 ②TG209 による活性汚泥阻害性試験から、TG301C の被験物質濃度(100mg/l)では呼吸阻害性が認められた。このため、TG301C(既存点検)で難分解となったのは、呼吸阻害の影響によるところが大きいと見なされたこと。 ③TG302C(被験物質濃度 30mg/l))による分解性試験の結果は、易分解（BOD 90%）であること。 **分解性に関する評価結果（抜粋）** An OECD test guideline 301C test was conducted in compliance with GLP on 2-sec-butylphenol with activated sludge at a test concentration of 100 mg/L. The test result showed 0 % biodegradation by BOD after four weeks.The low degradation observed might be caused by toxicity to micro-organisms at the concentration tested. EC50 value of 2-sec-butylphenol to the micro-organisms is < 100 mg/L according to a study following OECD test guideline 209 without GLP compliance. Another study according to OECD test guideline 301D in compliance with GLP at both test concentrations of 1.5 mg/L and 3.0 mg/L showed 63 % biodegradation by BOD within a 10-day window after four weeks with an inoculum obtained from a municipal sewage treatment plant. An inherent biodegradation study with OECD test guideline 302C without GLP compliance indicated 90-91 % biodegradation by BOD after four weeks. Overall, 2-sec-butylphenol is considered to be readily biodegradable.

REPORT

Study Title

READY BIODEGRADABILITY: CLOSED BOTTLE TEST WITH 2-SEC-BUTYLPHENOL

Author

Ing. M.J.E. Desmares-Koopmans

Study completion date

17 September 2009

Test Facility

NOTOX B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Laboratory Project Identification

NOTOX Project 491505
NOTOX Substance 198198/A

1. CONTENTS

2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Guidelines (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by NOTOX.

Analysis of stability, homogeneity and concentration of the test substance under test conditions was not performed as part of this study. Information concerning stability of the test substance in vehicle was available.

NOTOX B.V.

Ing. M.J.E. Desmares-Koopmans
Study Director

Date: 17 September 2009

Ing. E.J. van de Waart, M.Sc.
Head of In Vitro & Environmental Toxicology

Date: 17/09/2009

3. QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was inspected by the NOTOX Quality Assurance Unit to confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below.
During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	30-Jun-09	30-Jun-09	30-Jun-09
	Report	09-Sep-09	09-Sep-09	09-Sep-09
Process	**Environmental Toxicology**	03-Aug-09	07-Aug-09	07-Aug-09
	Test Substance Handling			
	Exposure			
	Observations/Measurements			

Head of Quality Assurance
C.J.Mitchell B.Sc.
I.A.

P.C. Henocksa

Date: 17-SEP-2009

4. SUMMARY

Ready Biodegradability: closed bottle test with 2-sec-butylphenol.

The study procedure described in this report were based on the OECD guideline No. 301 D, 1992. In addition, the procedures were designed to meet the test methods of the Commission Regulation (EC) No 440/2008 of 30 May 2008, Part C.4-E and the ISO International Standard 10707, 1994.

2-sec-butylphenol was a clear colourless liquid with a purity of 99.6% and completely soluble at the concentrations tested. Nominal concentrations of 1.5 and 3 mg/l were tested. The Theoretical oxygen demand of 2-sec-butylphenol was calculated to be 2.77 mg O_2 / mg.

The following test setup was applied:
- Inoculum blank (only inoculum, no test substance, duplicate measurements);
- Procedure control (sodium acetate and inoculum, duplicate measurements);
- Test suspension (2-sec-butylphenol and inoculum, duplicate measurements);
- Toxicity control (sodium acetate, 2-sec-butylphenol and inoculum, duplicate measurements).

Preparation of the test media started with a stock solution of 100 mg/l. Thorough mixing was used to accelerate dissolution of the test substance in mineral medium. Exact volumes of the stock solution corresponding to the test concentrations and microbial organisms were added to the test medium. Determination of oxygen concentrations was performed at the start of the experiment (day 0) and on day 7, 14, 21 and 28.

The relative biodegradation values calculated from the O_2 measurements performed during the test period of 28 days revealed 63% biodegradation of 2-sec-butylphenol at both concentrations (mean values on day 28). Furthermore, biodegradation of at least 60% was reached within 10 days of biodegradation exceeding 10%. The toxicity control showed that 2-sec-butylphenol did not inhibit microbial activity.

Since all criteria for acceptability of the test were met, this study was considered to be valid.

In conclusion, 2-sec-butylphenol is designated as readily biodegradable.

5. INTRODUCTION

5.1. Preface

Sponsor	SI Group – Switzerland GmbH Kästeliweg 7 4133 PRATTELN Switzerland
Study Monitor	Mr. E. Knoerr Manager Regulatory Affairs Europe
Test Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. M.J.E. Desmares-Koopmans
Technical Coordinator	J.H.J.W. Kluytmans
Study Plan	Start : 10 July 2009 Completion : 31 August 2009

5.2. Aim of the study

The purpose of the study was to evaluate an organic test substance for its ready biodegradability in an aerobic, aqueous medium with microbial activity introduced by inoculation of secondary effluent during a test period of 28 days.

5.3. Guidelines

The study procedures described in this report were based on the Organization for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Section 3, Degradation and Accumulation, guideline No. 301 D: "Ready Biodegradability: Closed Bottle Test" adopted July 17, 1992.

In addition, the procedures were designed to meet the test methods prescribed by the following guidelines:

- Commission Regulation (EC) No 440/2008 of 30 May 2008, Part C: Methods for the determination of ecotoxicity, Publication No. L142, C.4. "Biodegradation: determination of the 'ready' biodegradability, C.4-E: Closed bottle test".
- ISO Standard 10707, Water Quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds - Method by analysis of biochemical oxygen demand (closed bottle test) (1994).

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data, specimens (except specimens requiring refrigeration or freezing) and the final report are retained in the NOTOX archives for a period of at least 2 years after finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. NOTOX will retain information concerning decisions made.

Those specimens requiring refrigeration or freezing will be retained by NOTOX for as long as the quality of the specimens permits evaluation but no longer than three months after finalization of the report.

NOTOX will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

5.5. Definitions

Readily biodegradable are those test substances giving a measured biochemical oxygen demand (BOD) of at least 60% of the theoretical oxygen demand (ThOD) within 28 days. This pass level must be reached within 14 days of biodegradation exceeding 10%.

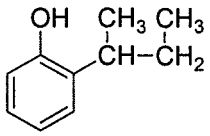
Theoretical oxygen demand (ThOD) is the amount of oxygen required to oxidize a chemical completely; it is calculated from the molecular formula and is also expressed as mg oxygen required per mg test substance.

Biochemical Oxygen Demand (BOD) is the amount of oxygen consumed by micro-organisms when metabolizing a test substance; also expressed as mg oxygen uptake per mg test substance.

6. MATERIALS AND METHODS

6.1. Test Substance

6.1.1. Test substance information

Identification	2-sec-butylphenol
Structure	
Molecular formula	$C_{10}H_{14}O$
Molecular weight	150.22
CAS Number	89-72-5
Description	Clear colourless liquid (determined at NOTOX)
Batch	Tank 1008 09.06.08
Purity	99.6%
Test substance storage	At room temperature in the dark
Stability under storage conditions	Stable
Expiry date	15 June 2010 (allocated by NOTOX, 1 year after receipt of the test substance)

6.1.2. Study specific test substance information

Stability in water	At least for 120 hours
Solubility in water	Yes, 1 g/L

6.1.3. Test concentrations and preparation of test solutions

2-sec-butylphenol was a clear colourless liquid with a purity of 99.6% and completely soluble at the concentrations tested. Nominal concentrations of 1.5 and 3 mg/l were tested. Preparation of the test media started with a stock solution of 100 mg/l by adding 24.96 mg test substance to 250 ml of mineral medium. Thorough mixing (30 minutes) was used to accelerate dissolution of the test substance in mineral medium. Exact volumes of the stock solution corresponding to the test concentrations and microbial organisms (see paragraph 6.3.) were added to the test medium.

6.2. Reference substance

6.2.1. Reference substance

Identification number	RS186
Name	Sodium acetate
Description	White powder (determined at NOTOX)
Molecular formula	CH_3COONa (taken from label)
Molecular weight	82.03 (taken from label)
Batch number	K34333668
Article number	1.06268.0250
Purity	≥99.0%
Expiry Date	28 February 2010
Certified	Yes
Storage conditions	At room temperature in the dark
Supplier	Merck, Darmstadt, Germany

6.2.2. Reference substance concentrations and preparation of test solutions

For the preparation of the test media a stock solution of 2 g/l was prepared in mineral medium. Exact volumes of the stock solution corresponding to the final sodium acetate concentration (2 mg/l) were added to the test medium of the procedure control and the toxicity control.

6.3. Test system

Source	The source of test organisms was secondary effluent freshly obtained from a municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, The Netherlands, receiving predominantly domestic sewage.
Treatment	Secondary effluent was filtered through a coarse filter paper, the first 200 ml was discarded. The filtrate was kept aerated until inoculation.
Inoculation	4 ml filtrate of secondary effluent per litre of final volume.
Reason for selection	The test has been accepted internationally (EC, OECD) for determining the 'ready' biodegradability of test substances under aerobic conditions.

6.4. Test procedure and conditions

Test duration	28 days for the inoculum blank and test suspension 14 days for the procedure and toxicity control
Test bottles	250-300 ml BOD bottles with glass stoppers.
Milli-RO / Milli-Q water	Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion-exchange cartridges (Milli-Q) (Millipore Corp., Bedford, Mass., USA).
Stock solutions of mineral components	A) 8.50 g KH_2PO_4 21.75 g K_2HPO_4 67.20 g $Na_2HPO_4.12H_2O$ 0.50 g NH_4Cl dissolved in 1 litre Milli-Q water, pH 7.4 $\pm$ 0.2 B) 22.50 g $MgSO_4.7H_2O$ dissolved in 1 litre Milli-Q water. C) 36.40 g $CaCl_2.2H_2O$ dissolved in 1 litre Milli-Q water. D) 0.25 g $FeCl_3.6H_2O$ dissolved in 1 litre Milli-Q water.
Mineral medium	1 ml of solution (A) to (D) was mixed and made up to 1 litre with Milli-RO water. The concentration of dissolved oxygen was measured for control purposes. The mineral medium was left at test temperature to obtain a saturated solution at the start of the test.
Test set up:	
- Inoculum blank	Containing only inoculum (no test substance)
- Procedure control	Containing reference substance and inoculum
- Test suspension	Containing test substance and inoculum (applicable for both test concentrations)
- Toxicity control	Containing test substance at the lowest concentration, reference substance and inoculum.
Number and groups of test bottles	Individual BOD bottles were prepared for each measuring point, except that the bottle measured at the start was also measured on day 7. Parallel groups were prepared to allow duplicate measurements of oxygen consumption at the test intervals.
Illumination	The test bottles were protected from light.

6.4.1. Measurements and recording

Determination of oxygen concentration:

- Frequency	In duplicate; immediately at the start of the experiment (day 0), and on day 7, 14, 21 and 28.
- Oxygen meter	WTW inolab Oxi 730 supplied with a WTW CellOx 325 oxygen electrode, electrolyte type ELY/G.
pH	At the start of the test.
Temperature of medium	Continuously in a vessel with Milli-RO water in the same room.

6.5. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme:
REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA): Temperature.

6.6. Interpretation

6.6.1. Data evaluation

The specific theoretical oxygen demand (ThOD) of the test substance was calculated, since the elemental composition of the test substance was known.

For the compound: $C_cH_hX_XN_nNa_{na}O_oP_pS_s$
With X_x being all halogens

ThOD without nitrification would be:

$$ThOD_{NH_3} = \frac{16\,(2c + {}^{1}\!/_{2}\,(h - x - 3n) + 3s + {}^{5}\!/_{2}\,p + {}^{1}\!/_{2}\,na - o)}{Molecular\ \ weight} \quad mg\ O_2\ /\ mg\ test\ substance$$

The biochemical oxygen demand (BOD) exerted after each time period was calculated by subtracting the mean oxygen depletion (mg O_2/l) of the inoculum blank from that exhibited by the test substance. The corrected depletion was divided by the concentration (mg/l) of the test substance. Thus the specific BOD is calculated expressed as mg oxygen per mg test substance.

$$BOD = \frac{mg\ O_2\ /\ l\ uptake\ \ (test\ substance) - mg\ O_2\ /\ l\ uptake\ \ (blank)}{mg\ test\ substance\ /\ l\ in\ vessel} = mg\ O_2\ /\ mg\ test\ substance$$

The percentage biodegradation was calculated by dividing the specific BOD by the specific ThOD. Hence, the following formula was applied:

$$\%\ degradation = \frac{BOD\ (mg\ O_2\ /\ mg\ test\ substance)}{ThOD\ (mg\ O_2\ /\ mg\ test\ substance)} \times 100\ \%$$

A figure of more than 10% biodegradation was considered as significant.
The relative biodegradation values were plotted versus time together with the relative biodegradation of the positive control. If applicable, the number of days was calculated from the attainment of 10% biodegradation until 60% biodegradation. Should this period be $\leq$ 14 days (14-day window), then the test substance is designated as readily biodegradable.

Toxicity control: if less than 25% biodegradation occurred within 14 days, the test substance can be assumed to be inhibitory.

6.6.2. Acceptability of the test

1. Oxygen depletion in the inoculum blank was below 1.5 mg O_2/l after 28 days (0.54 mg O_2/l).
2. The residual concentration of oxygen in the test bottles was > 0.5 mg/l at any time.
3. All differences of duplicate biodegradation values expressed as mg O_2/l were less than 20% (0-3%).
4. The reference substance was degraded by at least 60% (84%) within 14 days.

Since all criteria for acceptability of the test were met, this study was considered to be valid.

6.7. List of deviations

6.7.1. List of protocol deviations

There were no deviations from the protocol.

6.7.2. List of standard operating procedures deviations

Any deviations from standard operating procedures were evaluated and filed in the study file. There were no deviations from standard operating procedures that affected the integrity of the study.

7. RESULTS

7.1. Theoretical Oxygen Demand

The ThOD of 2-sec-butylphenol was calculated to be 2.77 mg O_2 / mg.
The ThOD of sodium acetate (reference substance) was calculated to be 0.78 mg O_2 / mg.

7.2. Biodegradation

Biodegradation is presented in Table 1 and Figure 1
Measured oxygen concentrations and oxygen depletions are given in Table 2 and Table 3 of Appendix I.

Table 1 Biodegradation during the test

Test medium	Concentration (mg/l)	% Biodegradation after x days [1] 0	7	14	21	28
Procedure control A [2]	2	0	77	84		
Procedure control B [2]	2	0	80	84		
Procedure control mean A and B	2	0	79	84		
Difference between duplicate values	2	0	3	0		
Test substance low A [3]	1.5	0	12	61	66	61
Test substance low B [3]	1.5	0	14	60	64	64
Test substance low mean A and B	1.5	0	13	61	65	63
Difference between duplicate values	1.5	0	2	1	2	3
Test substance high A [3]	3	0	6	58	61	63
Test substance high B [3]	3	0	7	60	62	64
Test substance high mean A and B	3	0	6	59	62	63
Difference between duplicate values	3	0	1	2	1	1
Toxicity control A	2/1.5 [4]	0	29	71		
Toxicity control B	2/1.5 [4]	0	29	73		
Toxicity control mean A and B	2/1.5 [4]	0	29	72		
Difference between duplicate values	2/1.5 [4]	0	0	1		

[1]: Calculations were performed with the mean oxygen concentrations of t=0 and the oxygen depletion as given in the Appendix. All calculations were performed without rounding off.
[2]: ThOD positive control sodium acetate: 0.78 mg O_2/mg
[3]: ThOD 2-sec-butylphenol: 2.77 mg O_2/mg
[4]: Toxicity control contains sodium acetate and 2-sec-butylphenol.

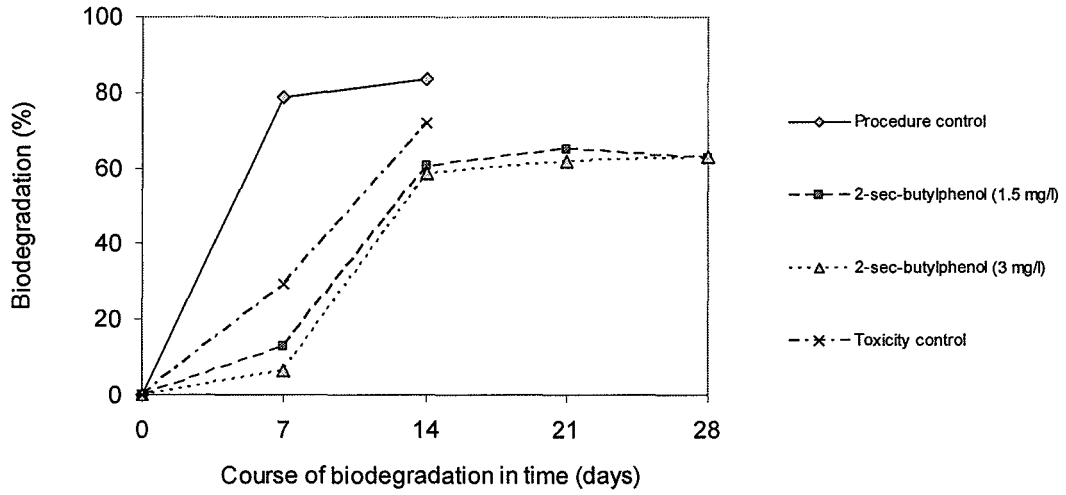


Figure 1 Course of biodegradation in time for the different test media

The relative biodegradation values calculated from the O_2 measurements performed during the test period of 28 days revealed 63% biodegradation of 2-sec-butylphenol at both concentrations (mean values on day 28). Furthermore, biodegradation of at least 60% was reached within 10 days of biodegradation exceeding 10%.

In the toxicity control more than 25% biodegradation occurred within 14 days (mean value: 72%, based on ThOD). Thus, the toxicity control showed that 2-sec-butylphenol did not inhibit microbial activity.

7.3. Test conditions

The pH values at the start of the test are given in Appendix I.
The temperature in the test room varied between 21.8 and 22.6°C.

8. CONCLUSION

2-sec-butylphenol was readily biodegradable under the conditions of the closed bottle test presently performed.

APPENDIX I TABLES

Table 2 pH at the start and oxygen concentration during the test

Series	Content	pH at t=0	Flask No.	Oxygen concentration (mg/l) after x days 0	7	14	21	28
Inoculum blank	Mineral medium, inoculum	7.6	1A	8.43	8.23	8.16	8.10	7.90
			1B	8.41	8.22	8.11	8.16	7.86
			mean	8.42	8.23	8.14	8.13	7.88
Procedure control	Mineral medium, inoculum sodium acetate (2 mg/l)	7.5	2A	8.46	7.05	6.86		
			2B	8.44	7.00	6.86		
			mean	8.45				
Test substance low	Mineral medium, inoculum 2-sec-butylphenol (1.5 mg/l)	7.5	3A	8.43	7.75	5.59	5.40	5.34
			3B	8.42	7.65	5.64	5.48	5.23
			mean	8.43				
Test substance high	Mineral medium, inoculum 2-sec-butylphenol (3 mg/l)	7.5	4A	8.42	7.74	3.34	3.05	2.67
			4B	8.44	7.67	3.20	2.97	2.60
			mean	8.43				
Toxicity control	Mineral medium, inoculum 2-sec-butylphenol (1.5 mg/l) sodium acetate (2 mg/l)	7.5	5A	8.44	6.57	4.07		
			5B	8.43	6.56	4.00		
			mean	8.44				

Table 3 Mean values of oxygen depletion at different points in time

Test medium	Concentration (mg/l)	Oxygen depletion (mg BOD/l) after x days [1] 7	14	21	28
Procedure control A	2	1.205	1.305		
Procedure control B	2	1.255	1.305		
Test substance low A	1.5	0.480	2.550	2.735	2.545
Test substance low B	1.5	0.580	2.500	2.655	2.655
Test substance high A	3	0.495	4.805	5.090	5.220
Test substance high B	3	0.565	4.945	5.170	5.290
Toxicity control A	2/1.5 [2]	1.670	4.080		
Toxicity control B	2/1.5 [2]	1.680	4.150		

[1]: Corrected for oxygen depletion in the blank control (mean value).

[2]: Toxicity control contains sodium acetate and 2-sec-butylphenol.

OECD 302C

REPORT NUMBER: S0052/E583

SAFEPHARM LABORATORIES LIMITED

ASSESSMENT OF INHERENT BIODEGRADABILITY

2-SEK-AP

MODIFIED MITI (II)

Project Number	:	47/1563.
Test Material	:	Phenol, 2-(1-methylpropyl) (Batch No.2-sek-AP/321; CAS No.89-72-5).
Date of Receipt	:	11 December 1991.
Description	:	Colourless liquid.
Storage	:	In original container at room temperature.
Inoculum	:	A mixed population of activated sewage sludge micro-organisms.
Source	:	Activated sewage was prepared by sampling 10 different sites around the UK in accordance with OECD Guideline No. 302C.
Preparation	:	The mixed sludge was fed daily with 0.1% synthetic sewage and maintained on constant aeration at 25 ± 1°C.
Usage Rate	:	Equivalent to 100 mg dry weight/l.
Reference Substance	:	Aniline.
Duration	:	28 Days.
Criteria	:	Oxygen consumption measured by direct manometer reading.
Agitation	:	By magnetic stirrers.
Temperature	:	25 ± 1°C.
Lighting	:	The test was carried out in darkness.
Observations	:	Manometer volumes were recorded daily.
Study Dates	:	12 August - 9 September 1991.

TEST CONCENTRATIONS	:	Phenol 2-(1-methylpropyl) : 30 mg/l.
		Aniline : 100 mg/l.

Day	% Biodegradation	
	Phenol 2-(1-methylpropyl)	Aniline
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	2
7	0	20
8	0	30
9	0	45
10	0	43
11	0	46
12	0	60
13	0	65
14	0	73
15	13	75
16	44	74
17	44	71
18	44	77
19	44	84
20	45	71
21	44	72
22	63	70
23	62	70
24	64	70
25	91	70
26	88	72
27	88	70
28	90	68

* All % Biodegradation values have been corrected for blank values.

Phenol 2-(1-methylpropyl) attained between 90-91% degradation within 28 days calculated from oxygen uptake and therefore, can be considered as inherently biodegradable under the strict terms and conditions of the OECD Guidelines.

Aniline attained 73% biodegradation after 14 days thereby confirming the suitability of the inoculum and culture conditions.

Total organic carbon analysis of the test media at Day 0 and Day 28 showed that Aniline attained 98% within 28 days. A Modified Miti test was carried out due to the test material inhibiting the respiration of activated sludge at concentrations in excess of 10 mg/l. A COD value of 1.31 mg O_2/l was obtained for the test material.

..... DATE 15.9.91

M. R. HORTON B.Sc.
STUDY SUPERVISOR

..... DATE 18.9.91

I. G. SEWELL B.Sc., M.Sc.
STUDY DIRECTOR

..... DATE 18.9.91

J. W. HANDLEY B.Sc., M.Sc.
HEAD OF AQUATIC TOXICOLOGY

OECD 209

REPORT NUMBER : S0052/E360

DEPARTMENT OF AQUATIC TOXICOLOGY

ACTIVATED SLUDGE RESPIRATION INHIBITION TEST

Project Number	:	47/1565
Test Material	:	Phenol, 2-(1-methylpropyl) (Batch No. 2-sek-AP/321; Cas No. 89-72-5)
Date of Receipt	:	11th December 1990
Description	:	Colourless Liquid
Method of Preparation	:	Direct dispersion in water.
Storage	:	In original container at room temperature in the dark.
Inoculum	:	A mixed population of activated sewage sludge micro-organisms.
Source	:	The aeration Stage of Severn Trent PLC Sewage Treatment Plant, Belper, Derbyshire.
Preparation	:	The sample was maintained at 21°C with continuous aeration and used on the day of collection. The pH was buffered to pH 7.0 with sodium hydroxide solution and the suspended solids level adjusted to 3.9 g/l prior to use.
Reference Substance	:	3,5-dichlorophenol.

a) Range Finding study

Date: 26th February 1991

Concentration (mg/l)		O_2 consumption rates mg/O_2/l/min 3 hour contact time	% Inhibition
Control	R_1	0.50	-
	R_2	0.43	-
Test	10	0.43	8
	100	0.10	78
	1000	0.00	100
Reference	3.2	0.42	10
	32	0.06	87

Variation between controls : ± 8%

b) Definitive Study

Date: 5th March 1991

Concentration (mg/l)		O_2 consumption rates mg/O_2/l/min 3 hour contact time	% Inhibition
Control	R_1	0.57	-
	R_2	0.56	-
	10 R_1	0.53	6
Test	10 R_2	0.58	<3>
	10 R_3	0.58	<3>
Reference	3.2	0.56	1
	32	0.08	86

<increase>

R_1 - R_3 : Replicates R_1 - R_3

Variation between controls : ±1%

RESULTS

Reference Material : EC_{50} (3 hour contact time) = 12 mg/l

Phenol, 2-(1-methylpropyl) : EC_{50} (3 hour contact time) >10 mg/l

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