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Part 1 of
M-315

CONFIDENTIAL

LSR Schedule No: PSV032
LSR Report No : 89/PSV032/0244

F.D.

PYRONIL 45: ASSESSMENT OF ITS READY BIODEGRADABILITY.

MODIFIED STURM TEST

M-315.

INDEXED

Study Director

W.R. Jenkins

To:
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Pennsylvania 19406-0018
USA

From:
Life Science Research Limited
Eye,
Suffolk, IP23 7PX

Draft: 10 April 1989
Final: 10 July 1989

TOXICOLOGY DATA
S U M M A R Y S H E T

Prepared by Pennwalt Corporation, Safety, Health & Environmental Department
900 First Avenue, King of Prussia, PA. 19406-0018

est Material: PYRONIL™45

mple Source: Pennwalt's Venture Research Laboratory, King of Prussia, PA

udy Type: Assessment of Its Ready Biodegradability. Modified Sturm Test.

sting Laboratory: Life Science Research Limited
Eye
Suffolk, IP23 7PX
England

Lorage: A report is filed in the Technical Records Center at King of Prussia under Master No. 22227. M-315. LSR Report No. 89/PSV032/0244.

r MSDS: Minimal biodegradation under aerobic conditions.

ummary of Results: The biodegradability of PYRONIL 45 was vestigated using the Closed Bottle test as a range-finding, cterial inhibition test and the modified sturm test as the efinitive study. PYRONIL 45 was not considered inhibitory to cteria during the 10 day Closed Bottle test using 2 and 10 /L. However, only 4% of the theoretical oxygen demand (ThOD) as achieved, indicating that the chemical was not readily iodegradable under the test conditions.

Results from the modified Sturm test, a stringent iodegradation test that used both carbon dioxide production and sappearance of dissolved organic carbon (DOC) as biodegradation dicators, produced biodegradation levels of only 2 to 3% of the hOD during the 28 day test. DOC measurements indicated that 'RONIL 45 was not soluble at the tested levels of 10 and 20 /L. The chemical oxygen demand (COD), using an acid dichromate eflux, represented only 69% of the ThOD, indicating that the ompound was not completely oxidized under the COD test nditions. PYRONIL 45 cannot be considered readily Lodegradable under the conditions of this test.

EPARATION DATE: November 27, 1989

PENNWALT

PYRONIL 45: ASSESSMENT OF ITS READY BIODEGRADABILITY.

MODIFIED STURM TEST

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I declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of the study. The aspects of the study conducted by Life Science Research were performed essentially in accordance with the following Good Laboratory Practice Standards or Guidelines relating to non-clinical studies:

Current OECD Good Laboratory Practice Principles

I fulfilled the responsibilities of Study Director required by these regulations.

W.R. Jenkins, B.Sc, C.Biol, M.I.Biol, M.I.F.M.
(Study Director)

Date: 10-7-89

PYRONIL 45: ASSESSMENT OF ITS READY BIODEGRADABILITY.

MODIFIED STURM TEST

LSR Schedule No : PSV032
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I have reviewed this report and concur with its contents.

D.C. Lodge, A.I.M.L.S
(Manager, Aquatic Studies)

D. C. Lodge

Date: 10/7/89

PYRONIL 45: ASSESSMENT OF ITS READY BIODEGRADABILITY

MODIFIED STURM TEST

LSR Schedule No : PSV032
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QUALITY ASSURANCE INSPECTIONS

	Dates (Day/Month/Year)		
	Inspections	Report to Study Director	Report to Management
PROTOCOL			
Inspection of protocol was made in accordance with LSR Standard Operating Procedure QAU/020. Dates for inspection of any protocol amendments in accordance with this SOP are not quoted	5.12.88	5.12.88	5.12.88
DATA			
Inspection of data generated on this type of study was made in accordance with LSR Standard Operating Procedure QAU/050	18.10.88		18.10.88
PROCEDURES			
Inspection of procedures on this type of study was made in accordance with LSR Standard Operating Procedure QAU/040	29.9.88 30.9.88 5.10.88 12.1.89 13.2.89		30.9.88 30.9.88 5.10.88 12.1.89 13.2.89

Other routine procedures used in this type of study, and facilities were inspected regularly and reports made in accordance with LSR Standard Operating Procedure QAU/040.

This report will be subject to review by the LSR Quality Assurance Unit according to the methods laid down in LSR Standard Operating Procedure QAU/060.

This review was completed on: 7 July 1989

pp D.L.M. Weller, B.Sc.
(Head of Quality Assurance)

.....

Date .....10 July 1989.....

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TABLES

1. SUMMARY

1.1 The ready biodegradability of PYRONIL 45 has been assessed by the Modified Sturm Test (EEC Procedure C5, OECD Procedure 301B). The main study was preceded by the determination of the chemical oxygen demand (COD) of the material and a ten-day bacterial inhibition assay conducted under the conditions of the Closed Bottle Test (EEC Procedure C6, OECD Procedure 301D).

1.2 The mean COD of PYRONIL 45 (0.92 mgO_2/mg), was 69% of its theoretical oxygen demand (ThOD; 1.34 mgO_2/mg), which indicated that the material was not completely oxidised under the conditions of this test.

1.3 In the bacterial inhibition assay, the degradation of the reference substance sodium benzoate achieved 63% and 66% of its ThOD (1.67 mgO_2/mg) after five and ten days in the absence of PYRONIL 45, and was not affected by its presence at 2 and 10 mg/l. This indicates that the test material was not inhibitory to the bacterial inoculum. The BOD of mixtures containing PYRONIL 45 alone at 2 and 10 mg/l after five and ten days of incubation was, at most, 4% of its ThOD indicating that the material was not readily degradable in this test.

1.4 In the Modified Sturm test, test material was added directly to inoculated mineral salts medium in sealed culture vessels to give nominal PYRONIL 45 concentrations of 10 and 20 mg/l. Each vessel was supplied with air that had been treated to remove carbon dioxide (CO_2), and degradation was assessed by measuring the CO_2 produced by each culture at intervals during a 28-day period. Control and reference vessels respectively contained inoculated medium alone, or inoculated medium plus sodium benzoate (20 mg/l). The concentrations of dissolved organic carbon (DOC) of control, reference and test cultures were also determined at the start and end of the test.

1.5 Cumulative CO_2 production from PYRONIL 45 at 10 and 20 mg/l, (1.1 and 2.3 $mgCO_2$), was equivalent to 2% and 3% respectively of its TCO_2 (1.50 $mgCO_2/mg$) on Day 28 indicating that the material was not readily degradable.

The results of DOC analysis at 10 and 20 mg/l were low at the start and end of the test (0 to 0.43 mg carbon/l), indicating that the material was not in solution. Estimates of degradation based on removal of DOC could not, therefore, be calculated.

1.6 The level of degradation of sodium benzoate after 28 days (91% based on CO_2 production, 92% based on DOC removal) and cumulative CO_2 production in the control group (20.1 $mgCO_2$) confirmed that the inoculum was viable and the test was valid.

2. INTRODUCTION

The objective of this study was to assess the biotic degradation of PYRONIL 45.

The study was preceded by the determination of the Chemical Oxygen Demand (COD) of the test material and a ten-day bacterial inhibition assay conducted, under the conditions of the Closed Bottle test detailed in Annex V, Procedure C.6. and OECD method 301D (1,2).

The investigation of ready biodegradability was conducted using the Modified Sturm Test, in accordance with Procedure C5, "Biotic Degradation-Modified Sturm Test", of Annex V of EEC Directive 79/831/EEC, published 19 September 1984 (3) and method 301B of the OECD Guidelines for Testing of Chemicals, adopted 12 May 1981 (4).

2.1 Study organisation

Location of study	:	Life Science Research Eye Suffolk IP23 7PX
Study Director	:	W.R. Jenkins, B.Sc., C.Biol., M.I.Biol., M.I.F.M.
Study timing	:	Tests reported here were carried out between 27 October and 21 December 1988.
Data storage	:	Raw data and a copy of the final report will be stored in the archives of Life Science Research.

3. MATERIALS

3.1 Test material

The test material, Pyronil 45, was received from Pennwalt Corporation, Pennsylvania, USA on 17 August 1988. The substance was a pale-yellow, clear liquid in a translucent plastic container, labelled PYRONIL (TM) 45, code 6605-57, expiry date 8 August 1989.

The inclusive weight of the test material and container on receipt was 383.63 g.

The following information was supplied by the Sponsor:

Identity	PYRONIL 45
Chemical structure	Halogenated phthalate ester
Other names	RC9927
	FR-45B
Purity	> 95%
Specific gravity	1.545 (25°C/4°C)
Empirical formula	$C_{24}H_{34}O_4Br_4$
Stability	unstable in strong acids/alkalis

3.2 Control standard for the COD test

Potassium hydrogen phthalate (Analar grade, product number 10207, batch number 4784303G), used as the control standard in the COD test was supplied by BDH Chemicals. This material was selected on the basis of its stability in aqueous solution and its complete oxidation under the conditions of this test (5).

3.3 Reference substance for the bacterial inhibition and Modified Sturm tests

Sodium benzoate (Analar grade, product number 10397, batch number 4749210G), used as the reference substance in the COD test, the bacterial inhibition test and the Modified Sturm test, was obtained from BDH chemicals.

4. METHODS

4.1 Determination of Chemical Oxygen Demand (COD)

The COD of PYRONIL 45 was determined by oxidation with an acid-dichromate mixture (in which Cr IV is reduced to Cr III) using a semimicro procedure.

A concentrated solution of the control standard, potassium hydrogen phthalate, was prepared in distilled water at a nominal COD concentration of 500 mgO_2/l on the day of the test.

Because of the low aqueous solubility of the test material, test concentrations were prepared by the direct addition of between 0.46 and 0.78 mg of PYRONIL 45, on glass cover slips, to six COD reaction vials together with water (2 ml), to give concentrations of between 230 and 390 mg/l.

Control blank vials contained distilled water, and the standard comprised the stock solution (2 ml), each in triplicate.

The reaction vials were sealed and placed in a heating block and the contents boiled under reflux at a temperature of 150°C for two hours. They were then allowed to cool to room temperature and the increase in Cr(III) was determined spectrophotometrically at a wavelength of 620nm.

4.2 Bacterial inhibition test

4.2.1 Inoculum

Secondary effluent (500 ml) was obtained on the day of the test from a trickling-filter plant at a sewage treatment works that treats predominantly domestic waste. It was maintained under aerobic conditions in the laboratory until required, and vacuum-filtered through a Whatman GFC filter paper immediately before use. The filtrate was used as the inoculum for the test (one drop filtrate/litre test medium).

4.2.2 Study design

Seven groups of BOD bottles were prepared according to the schedule shown below. Six replicate bottles were prepared in each group, care being taken to avoid the introduction of air bubbles during preparation and transfer of media. The Mineral Salts Medium (MSM; Appendix 1) was prepared in tap water that had been softened, treated by reverse osmosis and then glass distilled.

Group	Additions to MSM (mg/l)
1	No addition, MSM alone
2	Inoculum
3	Inoculum + sodium benzoate (2)
4	Inoculum + test material (2)
5	Inoculum + test material (10)
6	Inoculum + test material (2) + sodium benzoate (2)
7	Inoculum + test material (10) + sodium benzoate (2)

Test concentrations were again prepared by the direct addition of PYRONIL 45, on glass cover slips, to BOD bottles to give nominal concentrations of 2 and 10 mg/l.

Test concentrations of the reference substance were prepared from an aqueous solution (1 g/l).

The concentration of dissolved oxygen (DO) and the temperature of the contents of two of the vessels from each group was measured, using a YSI dissolved oxygen meter fitted with a self-stirring DO/temperature bottle probe, at the start of the test. Similar measurements were made on the contents of two further vessels after incubation in darkness for five days at 20°C, and on the contents of the remaining two, after ten days of incubation.

The pH of each control, test and reference mixture was measured after oxygen and temperature measurement.

The temperature of the incubator was measured at intervals during the test using a maximum-minimum thermometer.

4.3 Modified Sturm test

4.3.1 Bacterial inoculum

Activated sludge (two litres) was collected on the day of the test from a sewage treatment works that treats predominantly domestic waste, and aerated in the laboratory for four hours.

A sample of the mixed liquor (approximately 500 ml) was homogenised in a mechanical blender for two minutes and allowed to settle for 50 minutes.

An aliquot of the supernatant was passed through a Whatman GFC filter paper to remove coarse solids, and the filtrate was used as the inoculum for the test.

4.3.2 Test procedure

Four test vessels (five-litre brown glass carboys) containing mineral salts medium (MSMS; Appendix 2), bacterial inoculum (1%), and test material or sodium benzoate were prepared according to the following schedule. In each case the volume prepared was 3.5 litres.

The temperature of the test area was recorded at intervals during the test using a maximum-minimium thermometer.

4.4 Calculation of results

4.4.1 Calculation of theoretical oxygen demand (ThOD)

The ThOD of PYRONIL 45 was calculated from its empirical formula and molecular weight in the following way:

$$\text{ThOD } (mgO_2/mg) = \frac{16[2C + 1/2(H - Br) - O]}{MW}$$

where MW is the molecular weight of the material.

The ThODs of sodium benzoate and potassium hydrogen phthalate, used as the reference materials in the bacterial inhibition assay and COD test respectively, were calculated using the following equations:

sodium benzoate

$$\text{ThOD} = \frac{16\ [2C + 1/2H + 1/2Na - O]}{MW}$$

potassium hydrogen phthalate

$$\text{ThOD} = \frac{16\ [2C + 1/2H + 1/2K - O]}{MW}$$

These calculations assume that carbon is mineralised to CO_2, hydrogen to H_2O, and that sodium and potassium are oxidised to Na_2O and K_2O respectively.

4.4.2 Calculation of theoretical carbon dioxide production (TCO_2)

The theoretical amount of CO_2 that can be generated by PYRONIL 45 was calculated in the following way:

$$TCO_2 = mgCO_2/mg \text{ test material}$$

$$= \frac{\text{estimated carbon content (\%) x MW of } CO_2}{100 \text{ x MW of carbon}}$$

4.4.3. Calculation of chemical oxygen demand (COD)

The complete oxidation of potassium hydrogen phthalate under the conditions of the COD test, and the linearity of response (6), allows the direct determination of the COD of each sample.

4.4.4 Calculation of biochemical oxygen demand (BOD)

The amounts of oxygen consumed in test and control vessels in the bacterial inhibition test were calculated in the following way:

$BOD_n = (M_0 - M_{tn}) - (M_0 - M_{bn})$

where n = the number of days after the start of the test

M_0 = the mean oxygen level in vessels containing uninoculated mineral salts medium at the start of the test.

M_{tn} = the mean oxygen level in test vessels n-days after the start of the test.

M_{bn} = the mean oxygen level in inoculated mineral salts medium n-days after the start of the test.

The percentage degradation during the test was calculated from the mean weight of material added to BOD bottles in each group in the following way:

Degradation expressed in terms of ThOD

$$\% \text{ degradation } n = \frac{\text{mgBOD/l at n days}}{\text{mg Test Material/l x ThOD}} \times 100$$

In groups containing both sodium benzoate and PYRONIL 45 the apparent BOD of sodium benzoate has been calculated by subtracting the mean oxygen consumption in vessels containing test material alone at 2 and 10 mg/l from that of mixtures. The resulting BOD has been expressed in terms of the ThOD of sodium benzoate.

4.4.5 CO_2 production

Carbon dioxide production by control, reference and test mixtures was calculated in the following way.

Since 1 mmol of carbon dioxide is produced for every 2 mmol of hydrochloric acid titrated, the amount of CO_2 precipitated as $BaCO_3$ in a Drechsel bottle containing 100 ml $Ba(OH)_2$ is:

$$mgCO_2 = \frac{[N \times (Vs - Vt)]}{2} \times \frac{100}{V} \times MW(CO_2)$$

where:

N = normality of hydrochloric acid

Vs = volume of hydrochloric acid used to titrate the stock solution of barium hydroxide

Vt = volume of hydrochloric acid used to titrate test samples

V = volume of sample titrated

$MW(CO_2)$ = molecular weight of carbon dioxide

On each occasion when CO_2 levels were determined, the CO_2 level in the control group was subtracted from those in reference and test groups to give the blank-corrected value. The cumulative production of CO_2 in each group was calculated by adding successive estimates of blank-corrected CO_2 production.

Estimates of the percentage degradation of test and reference substances at intervals during the test were calculated from blank-corrected cumulative CO_2 production in the following way:

$$\% \text{ degradation} = \frac{\text{cumulative } CO_2(mg)}{TCO_2} \times 100$$

5. RESULTS

5.1 Calculations of ThOD and TCO_2 production

The ThODs of PYRONIL 45, potassium hydrogen phthalate and sodium benzoate were calculated to be:

PYRONIL 45 ($C_{24}H_{34}O_4Br_4$, MW = 706)	=	1.34 mgO_2/mg
Potassium hydrogen phthalate ($C_8H_5O_4K$, MW = 204)	=	1.18 mgO_2/mg.
Sodium benzoate ($C_7H_5O_2Na$, MW = 144)	=	1.67 mgO_2/mg.

The TCO_2 production of sodium benzoate was calculated to be 2.137 $mgCO_2/mg$ and that of PYRONIL 45, 1.50 $mgCO_2/mg$.

The total TCO_2 production of mixtures containing PYRONIL 45 in the Modified Sturm test was calculated to be 45.0 $mgCO_2$ (three litres, 10 mg/l), and 90.0 $mgCO_2$ (three litres, 20 mg/l).

The total TCO_2 production of the reference mixture was calculated to be 128.22 $mgCO_2$ (3 litres, 20 mg/l).

5.2 Determination of COD

The mean of six COD determinations, 0.92 mgO_2/mg, was 69% of its theoretical oxygen demand, which indicated that the material was not completely oxidised under the conditions of this test.

5.3 Bacterial inhibition assay

The mean PYRONIL 45 concentration in Day 5 and Day 10 BOD bottles containing nominally 2 mg/l, calculated from the weight of material added, was 3.8 and 3.3 mg/l respectively. At the nominal concentration of 10 mg/l, the mean calculated concentration was 10.5 mg/l.

The results of oxygen, pH and temperature measurements during the test are given in Table 1, and the computed values for oxygen consumption and the concentration of PYRONIL 45, calculated from the mean weights of material added to duplicate BOD bottles in each test group, in Table 2.

The BOD (mgO_2/mg) of PYRONIL 45 was calculated by dividing the observed BOD (mgO_2/l) by the mean weight of material added to the duplicate bottles in each group. The percentage degradation of PYRONIL 45 and sodium benzoate, calculated by dividing the BOD (mgO_2/mg) by the respective ThOD (mgO_2/mg), is given in Table 3.

Oxygen consumption had reached only 0.06 and 0.05 mgO_2/mg (4% of its ThOD) respectively at 2 and 10 mg/l after ten days. These results indicate that PYRONIL 45 was not readily degradable under the conditions of this preliminary test.

Estimates of the degradation of sodium benzoate after five and ten days (63% and 66%) indicate that the inoculum was viable and exerting normal biodegradative activity. In the presence of test material at 2 and 10 mg/l, estimates of the degradation of benzoate ranged from 54% to 66%, which indicates that the test material was not inhibitory to the bacterial inoculum.

Oxygen consumption at ten days in vessels containing mineral salts medium alone and inoculated mineral salts medium respectively was 0.4 and 0.3 mgO_2/l.

The temperature of the distilled water, measured before the preparation of batches of media for the test, was 20.5°C. The temperatures of the contents of bottles used for DO measurement ranged from 20.1°C to 20.5°C at the start of the test and 19.9°C to 20.0°C at the end of the test.

The pH of test and control mixtures ranged from 7.02 to 7.27 at the start of the test and 6.42 to 6.99 at the end of the test.

Temperatures in the test area ranged from 20.0°C to 20.5°C during the test.

5.4 Modified Sturm test

Estimates of blank-corrected CO_2 production and the percentage degradation at intervals during the test are given in Table 4.

Cumulative CO_2 production in the control group (20.1 mg) was less than the proposed maximum of 50 $mgCO_2$ at 28 days. The degradation of sodium benzoate was rapid and achieved 66% of its TCO_2 after six days and 91% after 28 days. These results confirm that the inoculum was viable and that the test was valid.

Degradation of PYRONIL 45 was first detected on Day six of the test when CO_2 production at 10 and 20 mg/l was equivalent to 2% of its TCO_2. At the nominal 10 mg/l, this level of degradation remained unchanged until the end of the test; the level of degradation at 20 mg/l increased from 2% to 3% of its TCO_2 following acidification on Day 27.

These results indicate that the test material was not readily biodegradable under the conditions of this test.

Mean values for duplicate, blank-corrected determinations of the DOC of test and reference mixtures measured at the start and end of the test are given in the following table:

Test group	DOC(mgC/l) Day 0	Day 28	Percent degradation
Reference (Sodium benzoate, 20 mg/l)	11.50	0.91	92
PYRONIL 45, 10 mg/l	0.43	0.06	NC
PYRONIL 45, 20 mg/l	0.00	0.00	0

NC - not calculated.

The nominal concentrations of carbon in test preparations at 10 and 20 mg/l, calculated from the empirical formula and molecular weight of the substance, were 4.1 and 8.2 mg/l respectively.

The results of DOC analysis shows that the test material was not sufficiently soluble to allow the calculation of reliable estimates of degradation based on the its removal.

The pH of test mixtures, measured at the start and end of the test, are given in the following table.

Test mixture	Day 0	Day 27
Control	7.22	7.31
Reference	7.27	6.55
PYRONIL 45, 10 mg/l	7.29	7.19
PYRONIL 45, 20 mg/l	7.22	7.15

Temperatures of the test area during the test ranged from 18.5 to 21°C.

Measured air flow-rates through test and control vessels, ranged from 43 to 67 ml/min during the test.

7. REFERENCES

1. OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES. (1984) Method C.6 Degradation, Biotic Degradation: Closed Bottle Test. L251, **27**, 19 September 1984, ISSN 0378-6987.

2. OECD GUIDELINES FOR TESTING OF CHEMICALS. (1981). "Ready Biodegradability: Closed bottle test", Procedure 301D, adopted 12 May 1981.

3. OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES. (1984) Method C.5. Degradation, Biotic Degradation: Modified Sturm Test, L251, **27**, 19 September 1984, ISSN 0378-6987.

4. OECD GUIDELINES FOR TESTING OF CHEMICALS. (1981). "Ready Biodegradability: Modified Sturm Test", Procedure 301B, adopted 12 May 1981, ISBN 92-64-12221-4.

5. AMERICAN PUBLIC HEALTH ASSOCIATION. (1971). "Standard methods for the examination of water and wastewater", 13th. ed., New York, p.489.

6. JIRKA, A.M. and CARTER, M.J. (1975). Analytical Chemistry, **47**, (8), 1397, (1975)

TABLE 1

Bacterial inhibition assay -
Recordings of temperature (°C), concentrations of dissolved oxygen (DO, mg/l) and pH in individual test and control vessels

Test group	Day 0			Day 5			Day 10		
	°C	DO	pH	°C	DO	pH	°C	DO	pH
Mineral salts alone	20.3	9.0	7.26	20.0	8.9	6.93	19.9	8.6	6.99
	20.3	9.0	7.20	20.0	8.9	7.10	19.9	8.6	6.97
Inoculated mineral salts	20.4	9.0	7.27	20.0	8.9	6.91	19.9	8.7	6.86
	20.3	9.0	7.23	20.0	8.9	6.82	19.9	8.7	6.82
Sodium benzoate (2 mg/l)	20.5	9.0	7.25	20.0	6.8	6.53	19.9	6.4	6.52
	20.3	9.1	7.18	20.0	6.8	6.54	19.9	6.5	6.51
PYRONIL 45 (2 mg/l)	20.1	9.0	7.22	19.8	8.7	7.02	19.9	8.5	6.78
	20.2	9.0	7.24	19.8	8.8	6.98	20.0	8.4	6.76
PYRONIL 45 (10 mg/l)	20.3	9.0	7.16	19.9	8.6	6.90	19.9	8.2	6.75
	20.3	9.0	7.03	19.9	8.7	7.00	19.9	8.2	6.75
Sodium benzoate (2 mg/l) + PYRONIL 45 (2 mg/l)	20.3	9.1	7.13	19.9	6.8	6.78	19.9	6.2	6.46
	20.3	9.0	7.10	19.9	6.7	6.73	19.9	6.3	6.47
Sodium benzoate (2 mg/l) + PYRONIL 45 (10 mg/l)	20.2	9.0	7.02	19.9	6.5	6.70	19.9	6.6	6.47
	20.3	9.0	7.03	19.9	6.7	6.74	19.9	6.1	6.42

Oxygen determinations are corrected to a standard pressure of 760 mm Hg.

TABLE 2

Bacterial inhibition assay - Oxygen consumption in test and control groups

Test group	Mean oxygen conc. (mgO_2/l)			Oxygen consumption (mg BOD/l)	
	Day 0	Day 5	Day 10	Day 5	Day 10
Mineral salts alone (M_o)	9.0	8.9	8.6	0.1	0.4
Inoculated mineral salts (M_{bn})	9.0	8.9	8.7	0.1	0.3
Sodium benzoate (2 mg/l)	9.1	6.8	6.5	2.1	2.2
PYRONIL 45 (2 mg/L)	9.0 *(2.6)*	8.8 *(3.8)*	8.5 *(3.3)*	0.1	0.2
PYRONIL 45 (10 mg/l)	9.0 *(9.2)*	8.7 *(10.5)*	8.2 *(10.5)*	0.2	0.5
Sodium benzoate (2 mg/l) + PYRONIL 45 (2 mg/l)	9.1 *(3.3)*	6.8 *(2.5)*	6.3 *(3.1)*	2.0	2.2
Sodium benzoate (2 mg/l) + PYRONIL 45 (10 mg/l)	9.0 *(10.7)*	6.6 *(9.9)*	6.4 *(11.4)*	2.1	1.8

Values in italics beneath mean oxygen levels give the concentration of PYRONIL 45 in mg/l calculated from the mean weight of material added to duplicate BOD bottles in the respective group.

TABLE 3

Bacterial inhibition assay - BOD (mgO_2/mg)
and percentage degradation in each test group

Test group	BOD(mgO_2/mg)		% Degradation (BOD/ThODx100)	
	Day 5	Day 10	Day 5	Day 10
sodium benzoate	1.05	1.10	63	66
PYRONIL 45 (2 mg/l)	0.03	0.06	2	4
PYRONIL 45 (10 mg/l)	0.02	0.05	1	4
sodium benzoate + PYRONIL 45 (2 mg/l)	1.00	1.10	60	66
sodium benzoate + PYRONIL 45 (10 mg/l)	1.05	0.90	63	54

TABLE 4

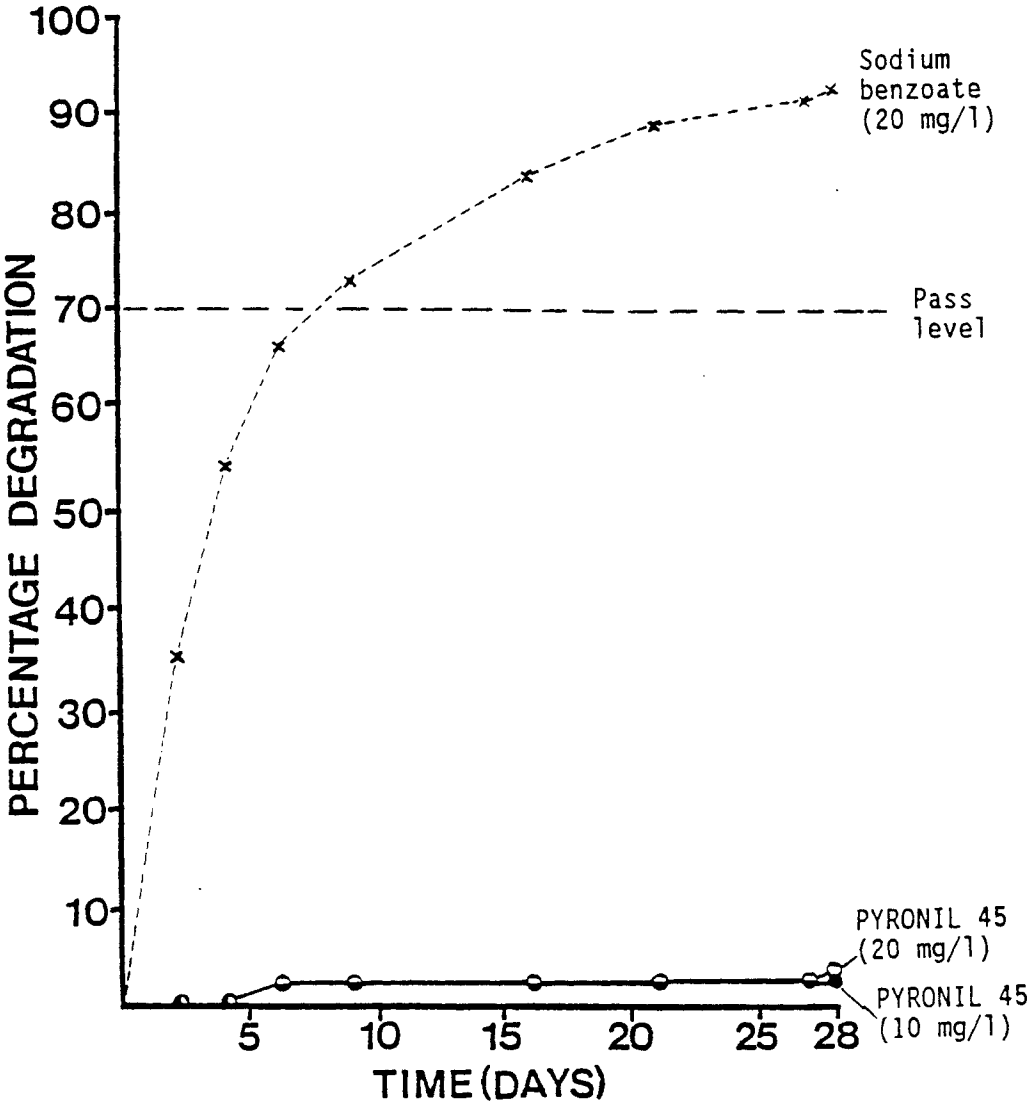
Modified Sturm test - Blank-corrected cumulative CO_2 production and degradation as a percentage of TCO_2 in reference and test mixtures

Day	Reference		PYRONIL 45 10 mg/l		PYRONIL 45 20 mg/l	
	CO_2(mg)	$\%TCO_2$	CO_2	$\%TCO_2$	CO_2	$\%TCO_2$
2	44.3	35	0.0	0	0.0	0
4	69.0	54	0.0	0	0.0	0
6	84.1	66	1.1	2	1.7	2
9	93.6	73	1.1	2	1.7	2
16	106.0	83	1.1	2	1.7	2
21	112.2	88	1.1	2	1.7	2
27	115.5	90	1.1	2	1.7	2
28	116.6	91	1.1	2	2.3	3

----- Denotes acidification on day 27.

FIGURE 1

Degradation of the test and reference substance, (CO_2/TCO_2).



APPENDIX 1

Preparation of mineral salts medium for the bacterial inhibition test

1. Dilution water

The dilution water used to prepare stock and test solutions of mineral salts was tap water that had been softened and treated by reverse osmosis before being distilled.

2. Mineral salts medium

The medium was prepared by adding 1 ml of each of the following stock solutions to each litre of distilled water:

	g/l
Stock 1	
Potassium dihydrogen phosphate	8.50
di-Potassium hydrogen phosphate	21.75
di-Sodium monohydrogen phosphate dihydrate	33.30
Ammonium chloride	1.70
Stock 2	
Magnesium sulphate heptahydrate	22.51
Stock 3	
Calcium chloride dihydrate	36.40
Stock 4	
Iron (III) chloride hexahydrate	0.25

Minor variations in the weights taken do occur but these are not considered to be significant.

APPENDIX 2

Preparation of mineral salts medium for the Modified Sturm Test

1. Dilution water

The dilution water used to prepare stock and test solutions of mineral salts was tap water that had been softened and treated by reverse osmosis before being distilled.

2. Mineral salts medium

The medium was prepared by adding the stated volumes of each stock solution to each litre of distilled water:

	concentration (g/l)	volume added (ml) per litre
Stock 1		
Iron (III) chloride hexahydrate	0.25	4.0
Stock 2		
Magnesium sulphate heptahydrate	22.50	1.0
Stock 3		
Calcium chloride dihydrate	36.40	1.0
Stock 4		
Potassium dihydrogen phosphate	8.50	
di-Potassium hydrogen phosphate	21.75	
di-Sodium monohydrogen phosphate dihydrate	33.40	2.0
Ammonium chloride	1.70	
Stock 5		
Ammonium sulphate	40.02	1.0

Minor variations in the weights taken do occur but these are not considered to be significant.