INFLUENCE OF INOCULUM SOURCE ON THE BIODEGRADABILITY OF PROPRANOLOL AND ATENOLOL

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Introduction

Active pharmaceutical ingredients (APIs) and most other classes of chemical are assessed for their biodegradability using a ready biodegradability test (OECD 301 or OECD 310 test). The result of this test is used to set the rate of degradation used in environmental risk assessment and persistence classification.

Many chemicals fail to fulfil the criteria for ready biodegradability. This may be because of the test conditions (very high test substance concentration and low biomass concentration), or that the molecules are too complex for the microorganisms to degrade them within the allotted time (28 days).

The aim of this research project was to test the hypothesis that the source of the inoculum (activated sludge) could affect the result of the ready biodegradability test.

Materials & Methods

Activated sludge from 3 different sewage treatment plants were tested at intervals over a period of 3 years.

Each sludge sample was used in an OECD 301 ready biodegradability test:

Test substances – atenolol and propranolol (along with standard reference substances: sodium benzoate and aniline)

Test substance concentration – 1.0 & 0.1 mg/L (not 10 - 100 mg/L); reference substance concentration – 100 mg/L

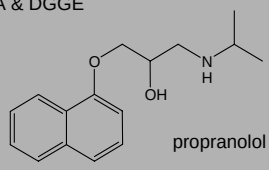
Atenolol & propranolol analysis – LCMS (not mineralisation); reference substance analysis – mineralisation

Inoculum – 30 mg/L biosolids (activated sludge)

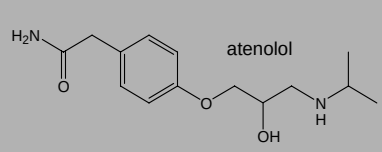
Microbiology – Biolog™, PLFA & DGGE

Sewage treatment plant operating conditions

	Population equivalents	Total daily flow (m^3/day)		
		July 07	October 07	March 08
Newton Abbot	82 000	24 200	15 600	42 000
Exeter	140 000	40 300	30 900	62 200
Totnes	19 000	7 200	3 200	8 100



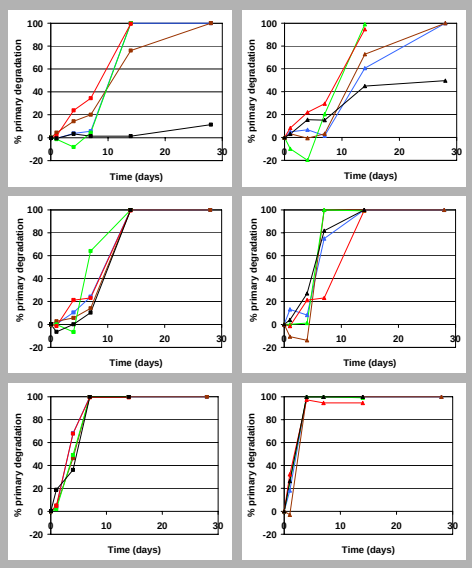
propranolol



atenolol

Results

1.0 mg/L atenolol 0.1 mg/L

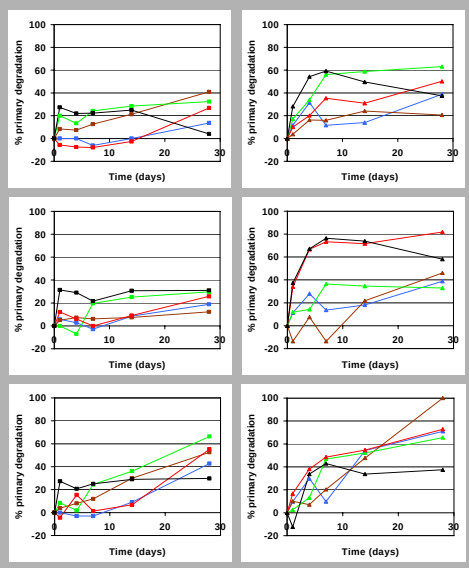


Newton Abbot

Exeter

Totnes

1.0 mg/L propranolol 0.1 mg/L



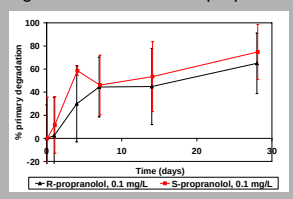
July 2007
October 2007
March 2008
October 2008
January 2009

Similar results were observed for the reference substances (data not presented), but not so pronounced as for propranolol and atenolol. Aniline did not "pass" the ready biodegradability criteria, but sodium benzoate did.

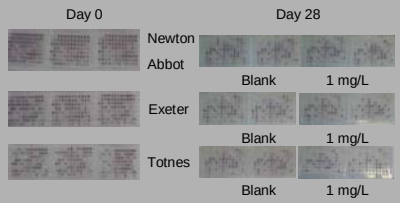
Preliminary investigation into the inhibitory effect of propranolol

Concentration of propranolol	Lag time (time to produce 10% mineralisation, in days)
100 mg/L	>56
100 µg/L	13
10 µg/L	9

Degradation of R- and S-propranolol



Biolog™ analysis of the microbial population



PLFA – Not sensitive enough for dilute inoculum (30 mg/L activated sludge)

DGGE – See SETAC poster WE180

Conclusions

The data suggests that:

- **The source of the inoculum may be an important factor in determining the result of the ready biodegradability test for some substances. It is therefore recommended that a blend of inocula be used in order to increase the probability of inoculating the test with the appropriate microorganisms.**
- **The use of low test substance concentrations should be considered where this is feasible, and if ^{14}C-labelled test substance is available this should be used to increase sensitivity and avoid potentially inhibitory test substance concentrations.**
- **There were no obvious seasonal variations, so this seems not to be an influencing factor.**

References

OECD (1992). Organisation for Economic Co-operation and Development (OECD) guideline for testing of chemicals, No. 301. Ready Biodegradability

OECD (2006). Organisation for Economic Co-operation and Development (OECD) guideline for testing of chemicals, No. 310. Ready Biodegradability - CO_2 in Sealed Vessels (Headspace Test)

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