

# THE BIODEGRADABILITY OF ACETAMINOPHEN USING MULTIPLE OECD 301 GUIDELINES AND THE OECD 307 GUIDELINE

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## INTRODUCTION

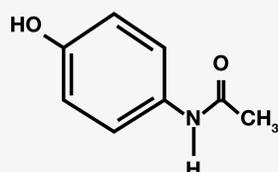
The ready biodegradability of Acetaminophen is assessed in this study based on several OECD 301 Guidelines and its biodegradability in the natural environment is determined using a simulation test based on the OECD 307 soil metabolism guideline.

Several datasets are publicly available that show Acetaminophen being biodegradable in activated sludge. However, most of these studies do not follow the strict requirements of the OECD 301 Ready Biodegradability Guidelines. This study aims to determine if Acetaminophen can pass a Ready Biodegradability Test and if the results are repeatable using several different test designs permitted by the OECD 301 Guidelines. The exact OECD guidelines followed were the OECD 301B, 301D, OECD 301F and OECD 310 Guidelines. Tests based on the OECD 209 and 302B were also performed.

The use of Acetaminophen as a potential reference compound is evaluated, and the reasons for the differences of the CO<sub>2</sub> evolution results between the different 301 studies and the OECD 307 soil simulation test are discussed in relationship to inoculum, contact, non-extractable and bound residues.

## MATERIALS AND METHODS

The test substance used in all of the studies following the OECD Ready Biodegradability Guidelines was Acetaminophen, which was obtained from Sigma Aldrich and its structure is presented below.



Acetaminophen  
C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub> MW = 151.16

Figure 1: Chemical Structure, molecular formula and molecular weight of Acetaminophen.

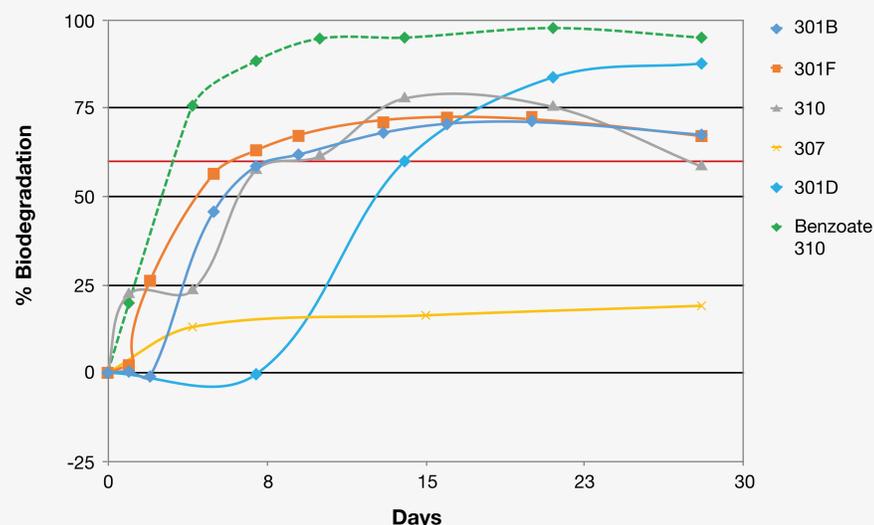
The test substance used in the soil metabolism study following the OECD 307 Guideline was also Acetaminophen as pictured above in Figure 1. Except it was uniformly radiolabeled with carbon-14 on the benzene ring. The radiolabeled [<sup>14</sup>C]Acetaminophen was received from Moravek Biochemicals, Inc.

Procedures followed in these experiments were based on the OECD 301B, 301D, 301F, 310, 209, 302B and 307 Guidelines. Activated sludge used as inoculum in the 'ready biodegradation' studies was obtained from a local Waste Water Treatment Facility in Wareham, Massachusetts USA. The soil metabolism study utilized two soils from the mid-western United States and they were characterized as a sandy loam and a loamy sand. Quantitation of CO<sub>2</sub> sequestered as carbonate in hydroxide solutions during the OECD 301B and 310 experiments was measured with a Shimadzu TOC analyzer with infrared detection, which also measured dissolved organic carbon (DOC) in the 302B test. An Oxi-Top Respirometer was used in the OECD 301F experiment to directly measure the amount of CO<sub>2</sub> evolution that occurred from this test. The OECD 301D test utilized a BOD Oxygen Probe made by YSI. The soil metabolism study utilized a Beckman Liquid Scintillation Counter (LSC) and an Agilent HPLC with radiometric detection. An oxidizer manufactured by R.J. Harvey was used to quantitate non-extractable residues. A StrathKelvin Respirometer was used in the experiment used to assess toxicity of Acetaminophen to activated sludge based on the OECD 209 Guideline.

## RESULTS AND DISCUSSION

### RESULTS OF THE READY BIODEGRADATION TESTS, THE OECD 209 AND THE OECD 307

The ready biodegradability results for Acetaminophen, based on CO<sub>2</sub> evolution, obtained in the OECD 301B, 301F and 310 portion of this investigation are presented in the graph below along with the sodium benzoate results from the 310 test. The ready biodegradation results for the OECD 301D test based on O<sub>2</sub> uptake and the mineralization results for Acetaminophen as percent of applied radioactivity (%AR) in the OECD 307 soil metabolism test are also presented in this graph.



In addition to the biodegradation testing, an OECD 209 Activated Sludge Respiration Inhibition Test was conducted in activated sludge from the same source as used for the ready biodegradation testing. This toxicity test produced percent inhibition values of 8.0,

23.1 and 31.5% for the test concentrations of 10, 100 and 1000 mg/L, respectively. Based on these results, Acetaminophen should not be toxic to the inoculum at the concentrations used for the biodegradation testing.

### RESULTS OF THE OECD 302B TEST

The inherent biodegradability results for Acetaminophen, based on DOC decline, obtained in the OECD 302B portion of the study showed that Acetaminophen biodegraded completely (100%) by Day 4, but only 3.6% at Day 1.

### RESULTS OF THE OECD 307 TEST

The average CO<sub>2</sub> evolution results obtained in the soil metabolism study using two soils are presented below:

Days	% Applied Radioactivity, AR					
	0	4	15	33	64	123
<sup>14</sup> CO <sub>2</sub>	0	13.0	16.3	19.8	23.5	26.5
%NER	77.4	79.0	74.6	70.3	68.5	57.0

Table: %CO<sub>2</sub> Evolution and Non-extractable residues (NER) based on 1 mg/Kg concentration of Acetaminophen

The parent [<sup>14</sup>C]Acetaminophen had completely degraded by day 4 of the experiment in both soils and as shown above the non-extractable residues remained above 50% in both soils as well, but they did decline as CO<sub>2</sub> was formed.

## CONCLUSIONS

The results of the OECD 209 experiment showed that the concentrations used in the biodegradation testing are not toxic to activated sludge microorganisms. However there was approximately 10% inhibition at 10 mg/L and this could be a reason for a slight lag phase in some of the biodegradation tests and possibly for the variation in replicates in the more dilute inoculum of the OECD 310 test.

The OECD 301B and OECD 301F experiments clearly show that Acetaminophen is 'ready biodegradable'. The OECD 310 experiment also determined that Acetaminophen produced >60% CO<sub>2</sub> evolution by day 10. However, some of the replicates were less than 60% at day 21 and 28, whereas the replicates of the reference compound were nearly identical to each other. It is possible that the more dilute inoculum in the OECD 310 test, i.e., secondary effluent, may not be as robust as using the 30 mg/L inoculum of activated sludge solids as used in the OECD 301B and 301F tests. Therefore, when possible, these ready biodegradation tests should be considered over tests that use weaker, more dilute inoculum. It is interesting to note that an OECD 302 B test with a thicker sludge inoculum, although showing complete biodegradability, there was a slight lag phase versus the sodium benzoate procedural control.

The use of Acetaminophen as a potential reference compound may be of interest to some, however, more work using dilute inoculums may be needed. To further this point, an OECD 301D test with Acetaminophen was conducted at 2 mg/L and it was shown to be 'ready biodegradable' but did show at least a one week lag phase.

The results of the OECD 307 soil metabolism tests show a drastic difference in CO<sub>2</sub> evolution versus the ready biodegradation tests. The soils maintained a very high level of non-extractable or bound residues throughout the tests. However, the NER does decline as CO<sub>2</sub> is formed. The fact that the amount of NER declines in and of itself, is indicative that the bound residues are not parent but metabolites on their way to CO<sub>2</sub>, albeit, slowly. Several reasons for this slower rate in the soils may be possible. One is that when Acetaminophen is in water, as it is in the ready biodegradation tests, there is better contact with the microorganisms and at the higher 10 ppm carbon concentration or more, Acetaminophen may be available for both cell maintenance and respiration, and therefore amenable to degradation. Another reason is, the lower concentration of Acetaminophen in soil, coupled with the high microbial biomass in soil allows for the rapid transfer of the test substance into the microbial cells where they are sequestered and used only for biomass rather than for biomass and respiration. Therefore, for some small molecules, ready biodegradation tests may provide faster ultimate biodegradation routes than the higher tiered soil or even water/sediment metabolism tests.

## REFERENCES

- OECD Guidelines. OECD Guidelines for Testing of Chemicals 209 and OECD 300 Series.