

**Simulation Test to Assess the Primary and Ultimate biodegradability of Chemicals**  
**COMMENTS RECEIVED ON THE JULY 2006 DRAFT AND RESPONSE TO THESE COMMENTS**

Country / Organisation	Comments	Expert Response ( <i>red italics</i> ) Secretariat Response (blue)
Denmark	<p><b>General comments</b></p> <p>The proposal is overall very good at the technical level. The experimental procedures are described very well and appear reasonable simulation tools.</p> <p>As in the guideline for surface water, OECD TG 309, a kinetic or process approach is taken aiming at producing realistic rate constants for use in chemical fate model calculations. By contrast to the thorough experimental part, however, the kinetics part is insufficient as is. <b>The simple “two-compartment models” should be further documented (what assumptions are made?) and explained (when should the different equations be used?).</b></p> <p>Furthermore when such methods are used it also raises questions on how the results should be used in relation to regulatory frameworks/decisions/ criteria.</p> <p>The proposed tests also suffer from <b>a lack of guidance as to the practical use of the obtained biodegradation results for risk assessment or other regulatory purpose.</b> A few examples:</p> <ul style="list-style-type: none"> <li>-</li> <li>- Biodegradation in a Sewer System Test. It is not clear how biodegradation results shall be used when obtained during a test period of 4 d, which is longer than the residence time in many sewer</li> </ul>	<p><i>The section on kinetics has been expanded for clarity and completeness. Since these analyses may not always be appropriate, this section is now labelled as optional.</i></p> <p><i>No other guideline in the OECD family of fate methods provides guidance on how the data should be used in a regulatory context. Since governments differ in how they might use the data, it is inappropriate to be overly prescriptive. We are sympathetic to comments and previously have provided some guidance in the FAQ document; however, we don't feel this should be a formal part of the guideline.</i></p> <p><i>The length of a test may be extended beyond normal residence time to help establish whether ultimate degradation occurs in this compartment and to provide sufficient data for estimating degradation rates. The test duration and the sampling schedule sections for this method have been</i></p>

	<p>systems.</p> <ul style="list-style-type: none"> <li>- Biodegradation in Treated Effluent-Surface Water Mixing Zone. It is also not clear how the results of this test shall be used. How large is the mixing zone recipient, where the results should be applied? If someone takes the test duration of up to 28 days as an indication for the size of the mixing zone (where a dilution factor of 10 applies as a default), it gives a totally misleading picture: E.g., in theory, the distance an effluent may pass during 28 days is 2419 km with a river flow of 1 m/s ! This indicates that the definition of the mixing zone / duration of test is not reasonable. Anyway a better guidance as to defining the boundaries of the mixing zone seems to be needed. Furthermore the whole issue of biodegradation in treated effluent- surface water mixing zone could be questioned in relation to regulatory use in environmental risk &amp; exposure assessment. Currently no account is for example taken of biodegradation when PEC local is estimated according to the <b>TGD of the EU. In relation to PEC regional and regional scenarios</b>, it is important to know the size of the mixing zone relative to the total surface water area. Only if the mixing zone is large, the biodegradation here is really influencing the overall biodegradation in the total water body, which is used for the PEC regional.</li> <li>- Biodegradation in Untreated Wastewater-surface water Mixing Zone. The comments above apply.</li> </ul>	<p><i>edited to make this point more clear The appropriate residence time would be incorporated into the risk assessment, where the data are used.</i></p> <p><i>There is no way to perfectly simulate what happens to a chemical moving downstream in a packet of water , which is continually receiving new inputs of chemicals and nutrients as well as being diluted by these inputs. The current EU-TGD addresses this issue by treating the water in a region as a fully mixed reactor within a fugacity context, which isn't particularly realistic either.</i></p> <p><i>This test tries to assess the potential for ultimate biodegradation under conditions in a generic or specific mixing zone scenario as well as determine a rate for this degradation. The length of the test is based upon the time necessary to establish whether ultimate degradation is occurring and the rate it occurs rather than t how long such a condition would be maintained through space in the real world. The relevant hydraulic residence time of the mixing zone would be that relevant within the specific risk assessment.</i></p> <p><i>While we would agree that this is not the most satisfactory situation, it is no worse than what is currently assumed within the EU-TGD and the resulting data may have more relevance than a ready test. As consolation, it should be recognized that a chemical and the microbes degrading it move downstream together and are subject to the same dilution. Furthermore, the rates from this type of study have been slower than those reported for in-stream die-away studies conducted in the field in the few cases where corresponding data exist (referenced in the FAQ document).</i></p> <p><i>The current EU-TGD paradigm was written without the prospect of having the type of data that these tests provide. It may be time to debate how these data could be used to revise and improve the TGD.</i></p>
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**All tests** – Suggest to include the procedure for extrapolation from tests conducted at standard laboratory temperature at 20-25°C to the field, where the temperature is usually lower (or higher for anaerobic digester). The default of the TGD for generic environmental risk assessment is currently by performing a temperature correction to convert degradation rates obtained at lab temperature to a default temperature for the environment (12 degrees Celsius for surface water).

#### Specific comments

**Page 15, lines 26-28:** The statement “when this standard for simulation is not achieved (i.e., trace level of test chemical; *added by authors*), observed biodegradation rates will be slower and not representative of those under steady state conditions, which should be considered in the interpretation of the results” is only true if related to the *initial* degradation rate. Initial degradation rates are often impossible to measure (not sufficient data points), and observed degradation rate may be grossly overestimated due to growth of degrading micro-organisms. Therefore, the wording “slower and not representative” in the above sentence should be **changed** to “slower **or higher** and not representative”.

**Page 25:** Section “Preparation of the Test Treatments”. It should be stated somewhere in this section **how maintenance of anaerobic conditions is monitored.**

**Page 25, lines 24-25:** Delete sentence “The sludge can be diluted ... too high” as it is repeated in lines 28-29.

*This is manipulation of the test results and is based upon assumptions that might differ as a function of the regulatory agency and country involved. No other test method guideline contains a similar manipulation of the data. Such a manipulation is currently described in the EU-TGD and is not appropriate for a guideline whose purpose is to describe the development of the data that could be manipulated in a variety of contexts.*

**Done.**

*We prefer to just modify this sentence to read “When this standard for simulation is not achieved, observed biodegradation rates may not be fully representatives, which should be considered in the interpretation of the results. This factor is particularly important for continuously released chemicals, which often reach steady state conditions in wastewater systems.” Page 17, line 31-34*

*This is done by ensuring proper operation of the anaerobic chamber and will differ from system to system. The level of guidance provided is consistent with that in the existing OECD 311 guideline*

	<p><b>Page 27, line 19:</b> Suggest <b>addition of new sentence:</b> “Direct measurement of <math>^{14}\text{CO}_2</math> and <math>^{14}\text{CH}_4</math> is only possible in the sealed flow-through batch system with connected base traps”.</p> <p><b>Page 31, lines 32-34:</b> Same comment as to page 15, lines 26-28.</p>	<p><b>Done</b></p> <p><b>Done</b></p> <p><b>Done</b></p> <p><i>We prefer to just modify this section to read “In most circumstances due to analytical considerations, it will be impossible to test at actual surface water concentrations. Consequently, observed biodegradation rates may not be fully representative of those under actual environmental conditions and should be considered in the interpretation of the results.”</i>  <b>Page 33, lines 29-31</b></p>
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Country / Organisation	Comments	Expert Response ( <i>red italics</i> ) Secretariat Response (blue)
Germany	<p>We appreciate the effort made developing 5 new Test Guidelines to assess the primary and ultimate biodegradability of chemicals discharged to wastewater. In general we think the proposed guidelines as well as the reduced test program are felicitous.</p> <p>Using radio labeled test substances in the tests is very useful in order to determine metabolite pathways which are often necessary for chemical assessment. We consider it therefore expedient to adjust the present proposal more on the detection of the metabolites especially in case of radio labeling by means of formulation (e.g. <b>instead of "optional" "preferred"</b> see also special comments).</p> <p>Because of two aspects <b>we would not like to recommend open test systems</b>. First misinterpretation of data can easily occur and second well working closed systems do already exist (see OECD TG308). The open systems would not work for volatile test compounds and compounds, which form volatile metabolites during degradation. Especially if the metabolic pathway of a compound is unknown, open systems should not be used. Volatile metabolites would be interpreted as mineralization. The second also important aspect is that the use of radio labelled test compounds is recommended.</p>	<p><b>Optional is kept.</b> An OECD TG is an instrument that includes the elements for RA and regulatory requirements, but it does not dictate them. <i>Concur with the Secretariat</i></p> <p><i>We prefer the closed system, when there is a need to definitively establish that a <sup>14</sup>C-labelled test chemical is mineralized. Furthermore, it is mandatory for volatile test chemicals. We also see the usefulness of open systems for tritiated chemicals and those whose ability to be mineralized is well established, where the goal is to refine the kinetics for risk assessment purposes. The following has been added in the General Principles sections (Page 3, lines 1-4) to provide guidance on this matter.</i></p> <p><i>“Tests can be performed using an open batch system or a closed gas flow-through batch system where traps are used to capture evolved <sup>14</sup>CO<sub>2</sub> or <sup>14</sup>CH<sub>4</sub>. The closed flow-through system is absolutely mandatory for volatile test materials. It is also usually preferred for <sup>14</sup>C-labeled test chemicals, Open systems are appropriate for non-volatile <sup>3</sup>H test chemicals and for refining the biodegradation kinetics of non-volatile <sup>14</sup>C test materials, whose ability to be mineralized has previously been established”</i></p>

<p>We would also like to make suggestions in order to simplify application of the Test Guidelines. Since The TG3xx consists of basically 5 independent tests [Biodegradation in A) Sewer System, B) Activated Sludge, C) Anaerobic Digester Sludge, D) Treated Effluent-Surface water Mixing Zone, E) Untreated Wastewater-Surface water Mixing Zone] where the phrases/wording in the separate tests are basically identical it would be helpful to have an <b>appendix table, where the differences between the tests are systematically listed</b> (e.g. oxygen content, etc.). It may also be helpful to generally take some aspects / details from OECD TG308 (e.g. test design, sampling intervals, analytical methodology), or a reference could be given to OECD TG 308.</p> <p>It is not quite clear to us what will happen to the document "Frequently asked Questions" but we think it should be published.</p> <p><b>Specific comments:</b>  page 2, line 5 Industry experts who performed comparable tests in their laboratories in the past IND <b>suggested to allow static test systems for test B</b> (Activated Sludge Test), C Anaerobic Digester Sludge), see OECD TG308. For test B (Activated Sludge test) biometer flasks with soda lime traps could be used for trapping of 14CO2.  For test C (Anaerobic Digester Sludge), also the gas trapping technique using gas sampling bags should be allowed. This avoids the necessity to continuously run the oxidation oven and allows the set-up of multiple</p>	<p><i>We liked this idea in the beginning, but as we began to put it together, there were so many possible variables dependent upon what is being simulated and the type of test chemical, that the table became more confusing than helpful and unlike the table in the 301 guideline, we found it to have little real value.</i></p> <p>The FAQ document is not for publication. <i>Some of the more useful aspects within the FAQ have been incorporated into the GL per United States Comments (see below)</i></p> <p>It is written: open or flow through. <i>No change</i></p> <p><i>We are concerned about using a closed static system for activated sludge given its high oxygen demand. The use of static closed systems has caused problems for us in the past so we migrated to open and flow-through systems. While we believe valid data can be generated using static systems, they would not be our first choice and including them in the guideline would require different apparatus and sampling techniques than those currently described.</i></p>
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	<p>replicates with minimal effort.</p> <p>page 4 line 27-38 'Validity of the Tests' <b>We understand that recovery will only affect validity of the test if it is not maintained for the parent. If this is the case we disagree.</b> To our understanding the emphasis that is put on the recovery of the parent in this case is not justified (e.g. ' Under such circumstances, the primary biodegradation data from the biotic treatment would likely be accurate as parent recovery should be optimized and the losses attributed to poor recovery of metabolic products.'). Important to us is the recovery of the total radioactivity since it is an important parameter for the quality of the study. For that reason we prefer the alternative of <sup>14</sup>CO<sub>2</sub> determination in a closed system compared to determining the difference between the biotic and abiotic treatments in an open system. From our experience better recoveries are possible that way.</p> <p>page 4, line 49 'Sensitivity of analytical methods' <b>The LOD for transformation products should be raised to 2 % of the initial amount</b> (rather than 1%). A LOD of 1 % would be a hard criteria for a study in which environmental relevant concentrations are tested because</p>	<p><i>We agree with the importance of determining mass balances as part of the validity criteria and have added a paragraph on this subject to this section. Page 5, lines 44-48.</i></p> <p><i>1% is kept.</i> It is in line with other TGs (307 and 308). <i>Concur with the Secretariat</i></p>
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	<p>overdosing will have impacts on the outcome of the study.</p> <p>page 5 Section Data and Reporting: Reference should be made to the latest revision of the FOCUS (2006) document .<b>Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.</b></p>	<p>Reference added in the bibliography list. <i>The reader is directed to this document in the revised kinetics section, reference 20. page 7, line 31</i></p>
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Country / Organisation	Comments	Expert Response ( <i>red italics</i> ) Secretariat Response (blue)
The Netherlands	<p><b>General comments</b></p> <ul style="list-style-type: none"> <li>• The proposals offer a very useful set of guidelines to assess the biodegradability of chemicals in various wastewater discharges that will help to assess and predict as realistically as possible the half-life and fate of chemicals in the aquatic environment.</li> <li>• The guidelines are generally clearly written and can be understood by experienced biodegradations experimentalists.</li> <li>• One major emission pathway for volatile and lesser volatile chemicals is emission to air. Volatile chemical may be emitted to air quite easily and less volatile chemicals may be emitted to air as well in well-aerated parts of wastewater treatment. Although some of the guidelines mention that they can deal with volatile chemicals, probably since abiotic controls are involved, interpretation of tests conducted with (less) volatile chemicals will probably be difficult. <b>Each individual guideline should mention the suitability of testing <u>and</u> of interpreting the tests conducted with (less) volatile chemicals.</b> Furthermore, the scheme 'Pathways for</li> </ul>	<p><i>Modifications for volatile materials were clarified in General Principles in response to the comments from Germany. All tests, but not all test systems are suitable for volatile testing. This has hopefully been clarified by the revisions. Page 3, lines 1-4</i></p> <p><i>Volatilization can occur from any compartment and the tests are not designed to measure this process. They are only designed to allow the biodegradation of volatile materials to be evaluated.</i></p>

	<p>Chemicals Discharged to Wastewater’ in the FAQs document should at least mention this important pathway to the environment.</p> <ul style="list-style-type: none"> <li>• In several tests, interpretation of the results in terms of determining half-lives heavily depends on which test concentration is used. <b>More guidance is needed to find out whether the tested concentration is sufficient or whether more test concentrations are needed to derive those half-lives.</b> This comment is particularly relevant for 3xxA, 3xxB, 3xxD, and 3xxE (in this case also relevant for the 1<sup>st</sup> and 2<sup>nd</sup> phase).</li> <li>• Many abbreviations need to be explained, whether obvious (DO=dissolved oxygen) or not (HACH=?).</li> </ul> <p><b>Proposed OECD 3xx Guideline</b></p> <ul style="list-style-type: none"> <li>• Page 1: please mention that the tests are <b>not designed for marine waters</b>, but for surface waters only, to avoid possible misunderstandings.</li> <li>• Page 2: <b>a summary table listing the key characteristics of the different tests</b> and showing their applicability would help the user to select the most appropriate test(s) and to help understand which environment and which route of entry to the environment the individual test</li> </ul>	<p><i>The statement regarding observed half-life and test concentration is true. The expanded kinetics section should help clarify this fact. Testing various concentrations is relevant if one is trying to assess half-life under different concentration scenarios or determine if the observed kinetics are true first-order. In most circumstances, the observed rates will be quasi first-order and the question of true first-order is academic. Testing multiple concentrations is always an option but depends upon the objective of the test.</i></p> <p><b>HACH is a commercial name: removed.</b>  <i>We have tried to explain abbreviations at their first mention, some of which occur in the Test Report section, which may have been overlooked. Page 8</i></p> <p><i>Replaced with ‘surface freshwaters’.</i>  <i>We disagree. There is no scientific reason why the mixing zone tests could not be used to simulate where treated or untreated wastewater enters estuarine or marine environments. While the test would most commonly involve the use of fresh water, estuarine or marine water should not be ruled out a priori without a good scientific reason. The freshwater designation was removed.</i></p> <p><i>We liked this idea in the beginning, but as we began to put it together, there were so many possible variables dependent upon what is being simulated and the type of test chemical, that the table became more confusing than helpful and unlike the table in the 301 guideline, we found it to have little</i></p>
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	<p>is simulating.</p> <ul style="list-style-type: none"> <li>• Page 3, line 1””It’s’ should read ‘It is’.</li> <li>• Page 5, please <b>add equations to derive half-lives</b> for each individual phase when two- or multiple compartment models are involved.</li> <li>• Page 6, lines 8-17: please also list the <b>geographical location</b> (latitude etc) as a requirement under “Environmental Samples”.</li> </ul> <p><b>3xxA Biodegradation in a sewer system</b></p> <ul style="list-style-type: none"> <li>• Page 7, line 20: <b>is studying one test concentration sufficient</b>, or are more test concentrations needed to estimate kinetics under environmental realistic conditions?</li> </ul> <p><b>FAQs document</b></p> <ul style="list-style-type: none"> <li>• Page 1, scheme: please add emission route to air.</li> <li>• Page 2, line 7: Typo ‘form’ should read ‘from’.</li> </ul>	<p><i>real value.</i></p> <p><i>Done</i></p> <p><i>Added to extended kinetic section pages 6-7</i></p> <p><i>Done</i></p> <p><i>See previous comment on this point</i></p> <p><i>The FAQ document is not for publication with the TG.</i></p>
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Country / Organisation	Comments	Expert Response ( <i>red italics</i> ) Secretariat Response (blue)
<p><b>United Kingdom</b></p>	<p>This is a useful gathering together of tests applicable to environmental situations, four of which are aerobic and one is anaerobic. Each of the 5 parts is self-contained so that continual reference to different parts of the guideline is minimised, or even unnecessary. The kinetics of degradation of chemicals in wastewaters are followed from within the sewer to final discharge to a receiving body of water. The kinetics of mineralization and transformation in anaerobic digester sludge is also described.</p> <p>A serious drawback is that a radioactive form of the test chemical is required, unless a very sensitive chemical method is available to be able to measure the chemical at the normally very low concentrations at which they occur in the environment. But even then only primary biodegradation could be investigated.</p> <p>Also, for the uninitiated, it would have been convenient and enlightening to have examples shown of the two conditions described in "Data and Reporting" p.5 lines 11 to 35. Examples of percentage remaining - time curves, as well as T<sub>1/2</sub> values, should be given.</p> <p><b>NOTE</b> The paragraphs are not yet numbered, so page and line numbers are given.</p> <p><b>1.p.1, line 17:</b> Simulation tests employ a continuous flow of sewage/synthetic sewage and are <u>not batch</u> tests.</p> <p><b>2.p.1, line 18:</b> Wrong word - "principle" should be "principal".</p> <p><b>3.p.1, line 20:</b> It is logical to put "characterization" before "quantification".</p> <p><b>4.p.1, line 20:</b> Word order "to assess accurately".</p> <p><b>5.p.2, lines 18-23: Would the Authority (EU) require this detailed analysis in every case?</b></p>	<p><i>The kinetics section has been expanded, hopefully making an example unnecessary.</i></p> <p><b>Amended:</b> "open batch system or closed flow-through batch system"</p> <p>Done</p> <p>Done</p> <p>Done</p> <p>No change</p>

	<p><b>6.p.3, line 1:</b> Preferably to say “It is ...”</p> <p><b>7.p.3, line 35:</b> Word order. Write as "to monitor comprehensively".</p> <p><b>8.p.3, line 36:</b> Delete comma after “molecule”- redundant</p> <p><b>9.p.4, line 12:</b> recall that OECD 209 originally also gave the French version of the toxicity test (using the BOD test) with a considerably lower density of micro-organisms, i.e. simulating the 301 series of tests. Unfortunately, it has been omitted on revision. It is likely that with some chemicals, at least, the difference in cell densities, about <math>10^3</math> cells/ml up to about <math>10^7</math> cells/ml would lead to differences in toxicity.</p> <p><b>10.p.4, line 36:</b> Better to put "efficiently" just before or just after <math>^{14}\text{CO}_2</math></p> <p><b>11.p.6, lines 11, 33, 36:</b> End of each statement should have a semi-colon, except the last statement, which has a full stop.</p> <p><b>12.p.7, line 13:</b> UK experience is that flowing sewage in most sewers investigated contained <math>&gt;2\text{mgDO/l}</math>; it was never anaerobic. In lab experiments the DO - time curves in un-aerated batches of raw sewage were linear down to about <math>0.5\text{mg DO/l}</math>. The "saturation" constant was around <math>0.1\text{mg DO/l}</math> - but if nitrification occurred it was at least double.</p> <p><b>13.p.7, line 32:</b> Insertion of a comma after “continuous” is desirable.</p> <p><b>14.p.8, line 10:</b> Insert hyphens between "water" and "soluble" (twice). Insert a comma after "non-volatile".</p> <p><b>15.p.8, line 20:</b> OECD usually denotes litres by "L". If not, we prefer the French spelling "litre".</p> <p><b>16.p.8, line 49:</b> "dry-ice" is "spoken" in the UK but we write "solid <math>\text{CO}_2</math>"</p> <p><b>17.p.9, lines 32,33:</b> In the UK the SS and BOD concentrations of typical sewages are much the same as in the US but tend towards the higher end of the ranges.</p> <p><b>18.p.10, line 9:</b> Insert a hyphen – “water-miscible”.</p> <p><b>19.p.10, line 11:</b> "neat". If this phrase "added in a neat form" means, "added without</p>	<p>Done</p> <p>Done</p> <p>Done</p> <p>No change</p> <p>Done</p> <p>Done</p> <p>No change</p> <p>Done</p> <p>Done</p> <p>Done</p> <p>Done <i>Changed to “solid <math>\text{CO}_2</math> (dry ice)”</i></p> <p>No change</p> <p>Done</p>
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	<p>water", then it is acceptable.</p> <p><b>20.p.10, line 26:</b> “non-toxic”- a hyphen.</p> <p><b>21.p.10, line 26:</b> Is it necessary to state "20.1°C" and "15.6°C" to the nearest 1st place of decimals?</p> <p><b>22.p.10, line 26:</b> In the UK we would say - "allowed to stand overnight" as a matter of course; but "sit" seems to jar! But if they sat all night could the loss of CO<sub>2</sub> correctly be described as "stripping"? Stripping seems to require some obvious action.</p> <p><b>23.p.16, line 20:</b> Hyphens after "water", twice</p> <p><b>24.p.16, line 30:</b> sp. “litre” or “L .</p> <p><b>26.p.17, line 4:</b> "solid CO<sub>2</sub>" for "dry ice".</p> <p><b>27.p.17, lines 25-27:</b> If release is episodic, should the collection of activated sludge be made only during a dosing period; or should two collections/tests be made with sludge collected during dosing and also with sludge collected when the chemical is not being discharged?</p>	<p>Done</p> <p>Done</p> <p>No Change</p> <p>Done</p> <p>Done</p> <p>Done</p> <p>Done <i>Changed to “solid CO<sub>2</sub> (dry ice)”</i></p> <p><i>This is good perspective. However, without an understanding of the complex hydraulics associated with an episodic release, it is not practical to sample at the peak release. From a biological perspective, we feel that total concentration is the most important variable, which can be set as a test condition in the laboratory.</i></p>
	<b>28. p.18, line 3</b>	
	"too low" not "to low".	
	<b>29. p.18, line 17</b>	Done
	Hyphen after "Water-".	
	<b>30. p.18, line 18</b>	Done
	Hyphen "non-toxic".	
	<b>31. p.18, line 19</b>	Done

"neat" take any necessary action - see 19 above.	Done	
<b>32. p.19, line 39</b>		
Word order "to measure accurately".		
<b>33.p.23, line 12</b>	Done	
"or can be further fractionated using to determine uptake into various components of biomass". This seems to need word(s) between "using" and "to".	'using' deleted	
<b>34. p.23, line 15</b>		
Insert: - Hyphen after "water" - twice.		
<b>35. p.23, line 25</b>		
sp. "litre".	Done	
<b>36. p.23, line 26</b>		
sp. "litre".	Done	
<b>37. p.23, line 46</b>		
"is" for "are".	Done	
<b>38. p.24, line 6</b>		
"solid CO <sub>2</sub> " for "dry ice".	Done	
<b>39. p.24, line 40:</b> <b>For usually no more than 30 days". 30 days is only around 1 "retention time" - should the experiment not last for say, 3 x retention times?</b>	Done <i>Changed to "solid CO<sub>2</sub> (dry ice)"</i>  <i>Changed to 60 days to simulate 1<sup>st</sup> and 2<sup>nd</sup> stage digestion</i>	
<b>41. p.25, line 23:</b> With anaerobic sludge, is it usual to determine total solids, not suspended solids?		
<b>42. p.25, line 29</b>		
"medium" for "media".	<i>Changed to total solids</i>	
<b>43. p.25, line 51</b>		
Hyphens after "Water" and "non".	Done	
<b>43p.26, line 14:</b> "to simulate the static conditions which occur within a digester, the test vessels are not usually continuously mixed.	Done	
<b>44 p.26, line 48</b>		
Add "s" to "chemical".	Done	
<b>45 p.27, line 4</b>	Done	

" <sup>14</sup> Gases" - is this meant or should it be " <sup>14</sup> C Gases"?		
<b>46 p.27, line 9</b>		
"1ml" - is this not too small an amount of sludge to be able to pipette accurately in replicate?	Done	
<b>47 p.27, line 14</b>		
Should " <sup>14</sup> Gas" be "14C Gas"?	No change	
<b>48 p.27, line 36</b>		
Delete "a" before "centrifuge"?	Done	
<b>49 p. 43</b>		
Should "with" be inserted after "combined"?	Done	
<b>50 p.29, line 2</b>		
Ref.7 is not Sutherland et al in my Ref. list.	Done	
<b>52. p.31, line 19</b>		
"pseudo" describes "first-order" but <u>not</u> "second-order" - is this correct?	Done	
<b>53. p.31, line 29</b>		
"saturation" This is ambiguous; does it refer to solubility or kinetics?	<i>Sentence removed per earlier comment</i>	
<b>54. p.31, line 33</b>		
It is better to use " <b>lower</b> ", rather than "slower".	<i>Sentence removed per earlier comment</i>	
<b>55. p.32, line 10</b>		
Insert hyphen - "flow-through".	Done	
<b>56. p.32, line 15</b>		
" <sup>3</sup> H" for "3H".	Done	
<b>57. p.32, line 18</b>		
Insert commas after "and" and "appropriate".	Done	
<b>58. p.32, line 25</b>		
Insert hyphens after "water", twice.	Done	
<b>59. p.32, line 35</b>		
sp. "litres".	Done	
<b>60. p.33, line 9</b>		
"solid CO <sub>2</sub> " for "dry ice".	Done	
<b>61. p.38, line 29</b>		
Insert hyphen after "site".	Done <i>Changed to "solid CO<sub>2</sub> (dry ice)"</i>	
<b>62. p.33, line 46</b>		
Sp. "litre".		
<b>63. p.34, line 5</b>	Done	

Hyphen "site-specific".		
<b>64. p.34, line 39</b>	Done	
Hyphen after "non".		
<b>65. p.36, line 15</b>	Done	
Word order "to measure accurately".		
<b>66. p.37, line 43</b>	Done	
As earlier, should "with" follow "combined"?		
<b>67. p.39, line 12</b>	Done	
"biochemical" for "biological".		
<b>68. p.39, line 26</b>	Done	
As earlier - "pseudo-" does not describe "second-order"?		
<b>69. p.40, line 30</b>	Done	
Insert hyphens after "water", twice.	<i>Pseudo has been replaced with quasi</i>	
<b>70. p.40, lines 40, 41</b>	Done	
sp. "litre(s)".		
<b>71. p.40, line 41</b>	Done	
Insert hyphens - "2- or 4- litre".		
<b>72. p.40, line 46</b>	Done	
"values" for "levels" (the first time only!).		
<b>73. p.41, line 22</b>	Done	
"solid CO <sub>2</sub> " for "dry ice".		
<b>74. p.42, lines 7-11</b>		
It is generally accepted that sewage in US is somewhat weaker than in Europe partly because of the higher daily/higher daily use/person of water in the US.	<del>Change to "higher CO<sub>2</sub> higher"</del>	
<b>75. p.42, line 28</b>		
TOC determinations (i.e. with sample containing suspended solids are not very reproducible)	No change	
<b>76. p.42, line 33</b>		
Presumably the final concentration of HgCl <sub>2</sub> in the mixture is to be 0.1 g/l.	No change	
<b>77. p.42, line 42</b>		
Insert hyphens after "Water" and "non".	Clarified	
<b>78. p.43, line 44</b>		
Add "s" to "chemical".	Done	
<b>79. p.45, line 11</b>		
	Done	

	Word order. "to extract directly".		
	<b>80. p.45, line 35</b>	Done	
	"This information combined its sequence of formation and disappearance....". Should "combined"?	"with" be inserted after <i>This sentence has been rewritten to read "This information combined with known biochemical reactions along with when a metabolite appears and disappears in the sequence of biodegradation can form an additional basis for inferring its identity. "</i>	
	<b>81. p.46</b>		
	Contains a list of 17 References.	24 references <i>References updated, there are now 25</i>	

31 October 2007

<b>Country / Organisation</b>	<b>Comments</b>	<b>Expert Response (<i>red italics</i>) Secretariat Response (blue)</b>

Country / Organisation	Comments	Expert Response ( <i>red italics</i> ) Secretariat Response (blue)
<p><b>United States</b></p>	<p>The needs for this test guideline may have particular significance for pharmaceuticals when compared to the testing needs for other consumer products and industrial chemicals.</p> <p>Need: Ready biodegradation test as required in certain regulatory test guidance do not provide applicable data for most pharmaceuticals, which typically do not mineralize during STP, but do biotransform. There is a gap in current methods when one considers the specific conditions and desired endpoints needed for the environmental assessment. The proposed sludge die-away provides a method to assess primary degradation as well as mineralization at realistic levels; enables an assessment of metabolite profiles resulting from STP; provides a means for developing a <math>K_e</math> for primary &amp; ultimate degradation. STP is a significant environmental “compartment” for pharmaceuticals, and may provide significant depletion prior to release into the aquatic compartment. <math>K_e</math> for primary degradation is very useful in refining PEC surface water for pharmaceuticals. Data obtained are also amenable to current designations around “readily biodegradable”.</p> <p>Application: Pfizer is currently using this test for product registrations in lieu of ready biodegradation protocols. Data have been shared through poster presentations at SETAC 2005 (Baltimore) &amp; DIA (Stockholm) 2006; and will be presented at SETAC 2006 (Montreal). To ensure the most efficient allocation of resources, Pfizer continues to assess where unnecessary testing may be eliminated and/or replaced with tests that provide more relevant information.</p> <p>What is the definition of “combined sludge”</p> <p>Page 22, line 32-34.....the statement “ultimate biodegradation cannot be determined unless the biodegradation pathway is well understood.....”; why</p>	<p><i>No Change</i></p> <p><i>Reworded; changed to digester sludge. Combined sludge referred to the composition of digester solids, i.e. primary and secondary sludge solids.</i></p>

	<p>can't one just measure total CO<sub>2</sub> and CH<sub>4</sub> production and compare to the abiotic control? Why does one need to understand the pathway?</p> <p>Page 23, line 30: Suggested wording change: "...and are incubated <i>inside</i> an anaerobic chamber containing an oxygen-free atmosphere.</p> <p>Page 26, line 28-29: "These chambers are maintained....." <b>does this sentence belong here??</b> If so why use the term "chambers", since we were talking about vessels' previously??</p> <p>Page 1, lines 10-31: The Introduction sets forth the scope of the TG and how it relates to other types of tests, but this discussion is only at a superficial level. The first of the FAQs, in the separate FAQs document, has more detail. More detail from that discussion of scope should be included in the TG.</p> <p>Pages 1-6: There is no meaningful discussion of how to determine test substance concentration. The TG refers to "environmentally relevant concentrations" but that's about it. It is suggested that the material on this topic in the FAQs document be inserted into the TG in the first few pages, as a new subsection.</p> <p>Page 3, line 1: it is, not it's.</p> <p>Page 4, line 50: Where it says "at least 1%", is "<math>\leq 1\%</math> meant?</p> <p>Page 17, lines 33 and 36: neither HRT nor SRT is explained at first use. In line 36,</p>	<p><i>Edited statement to include "non-radiolabelled test substance. Page 2, line 38</i></p> <p><i>Change made</i></p> <p><i>Changed "Chambers" to "Replicates".</i></p> <p><i>Included wastewater pathway diagram from FAQ. Included where these tests fit into a tiered test approach. Page 2 line 24-25.</i></p> <p><i>Added guidance for setting test concentrations to the introduction section of each method as well as a reference to Holman (1981), "Estimating the environmental concentrations of consumer product components" and the EU-TGD.</i></p> <p><i>Changed</i></p> <p><i>Changed to <math>\leq 1\%</math></i></p>
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<p>'2 to3 SRTs is awkward</p> <p>Page 18, line 18: non-toxic, not non toxic.</p> <p>p 20, line 22 : 'benchmark' is jargon and in actuality its meaning is not all that clear.</p> <p>Page 21, line 17: Should be "combined <u>with</u> its sequence"</p> <p>The proposed guidelines are very thorough and well-written. Our compliments to the authors! The proposed guidelines will provide very useful guidance when such simulation tests are deemed necessary. It should be noted throughout the guideline that these are to be included amount the tests which are defined by OECD as "Simulation Tests" - which are intended to simulate the fate processes which occur within specific environmental compartments. Considering the almost infinite diversity of these "environmental compartments" along with the diversity of synthetic chemistries in commerce, it could be said that no two such "simulation tests" will be alike. Therefore, it cannot be overemphasized that the proposed guideline should be regarded as general guidance for conduct and interpretation of such simulation tests for wastewater environments; and that strict adherence to these recommendations should not be regarded as grounds for acceptance/rejection of the test by regulatory authorities. In other words, there needs to be suitable flexibility in the proposed guidelines to account for differences in the chemicals and environments to be studied. The authors have done an excellent job of allowing this flexibility within the proposed guideline.</p> <p>In the Introduction section, it is noted that the proposed guideline is intended to determine the rate/extent of degradation for substances whose route of entry to the</p>		<p><i>Explained the acronyms. Changed line 36 to "2 to 3 times the SRT".</i></p> <p><i>Changed</i></p> <p><i>Removed "benchmark" and clarified intended meaning</i></p> <p><i>This sentence has been rewritten to read "This information combined with known biochemical reactions along with when a metabolite appears and disappears in the sequence of biodegradation can form an additional basis for inferring its identity.</i></p> <p><i>No Change</i></p>
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	<p>environment begins with discharge to wastewater. However, considering the complexity and cost of these simulation tests, it should be further emphasized that such tests should not be required for all such substances. A number of standardized screening tests are available which, together with suitably conservative interpretation and extrapolation, can demonstrate adequate margins of safety in screening-level environmental risk/exposure assessments. For example, conservative default biodegradation half-lives are currently assigned to wastewater, surface water, soil, and sediment compartments based on results of the OECD Ready Biodegradability Tests. In cases where these screening level exposure assessments indicate an unacceptable margin of safety (or potential risk of harm), the further refinement of degradation kinetics which is afforded by these simulation tests may be deemed necessary to better define potential risk.</p> <p>Page 5, line 14: "...percent of the dosed radioactivity..." Change to "...percentage of the dosed radioactivity..."</p> <p>Page 5, line 19: "...the percent parent as a function of time..." Change to "...percentage of parent substance remaining as a function of time..."</p> <p>Page 5, line 26: <math>k_1</math> and <math>k_2</math> should be described as first-order rate <i>constants</i> (with unites of time<sup>-1</sup>), and not rates (unites of concentration/time). Change to " <math>A</math> equals the initial percentage of parent substance which is degraded according to first-order rate constant <math>k_1</math> and <math>B</math> equals the initial percentage of parent substance which is degraded according to first-order rate constant <math>k_2</math>."</p> <p>Page 5, lines 28-29: Provide similar clarification of <math>k_1</math> and <math>k_2</math>, <math>A</math> and <math>B</math>, with respect to the mineralization models. Are no <math>A</math> and <math>B</math> representing the upper asymptotes of CO<sub>2</sub> evolution for each fraction of the added parent substance?</p>	<p><i>Added the following sentence to the Introduction section: "These tests are intended as higher tier tests for assessing the biodegradation of chemicals, which do not biodegrade in OECD screening tests, or for refining biodegradation rates used for exposure assessment". Page 2 line 24-25</i></p> <p><i>Noted in revised kinetics section</i></p> <p><i>Noted in revised kinetics section</i></p> <p><i>Noted in revised kinetics section</i></p>
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	<p>Page 5, line 33: “Half –lives (<math>T_{1/2}</math>) can be calculated from the estimated first-order rates (<math>k</math>) using ...” Change to “Half-lives (<math>T_{1/2}</math>) can be calculated from the estimated first-order rate constant (<math>k</math>) using....”</p> <p>Page 6, line 36: “...correlation coefficients (<math>R^2</math>) and F statistics for the selected models...” Change to “ ...coefficients of determination (<math>R^2</math>) and F statistics for the selected models...”</p> <p>Page 7, line 4: Delete redundant use of “rates “ in ...designed to provide rates of primary and ultimate biodegradation rates for a ...”</p> <p>Page 7, lines 11-13: While it is agreed that low D.O. may occur in sewers, it may be difficult o achieve and maintain a level of &lt; 1 mg/L. Should a lower threshold of DO also be stated, such that anoxic conditions are avoided? A D.O. control system is mentioned elsewhere, and is described on Pg. 38. However, laboratories might not have this capability.</p> <p>Page 7, line 19: Suggest the use of an alternative to the “equilibrium” in describing the relationship between emitted chemical and its degrading population. For example “... the chemical and its degrader population will not be in equilibrium and the ....”See preferable text used in Page 15 lines 14/15. This comment may also apply to (page #/line?): 7/19, 15/12, 22/13, 31/19, 39/25.</p> <p>Page 9, line 49: The concentration of mercuric chloride (1 g/L) seems excessive, and will likely cause significant precipitation of minerals in the abiotic controls. Our experience has shown that lower concentrations (e.g. 250 mg/L) are often sufficient. Further, autoclaving for 90 minutes seems excessive (depending on bulk volumes</p>	<p><i>Noted in revised kinetics section</i></p> <p><i>Noted in revised kinetics section</i></p> <p><i>Changed</i></p> <p><i>Changed</i></p> <p><i>A lower D.O. limit was added for the test as well as a statement that the oxygen concentration in test vessel headspace, should be monitored periodically if a D.O control system is not available. Page 10, line 31-33.</i></p> <p><i>Replaced “equilibrium” with “steady state”</i></p>
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	<p>treated). More so than chemical sterilization, autoclaving results in denaturing of sludge solids, and thus, a different (lessened) potential for adsorption. Also, there may be waste removal costs associated with mercuric chloride wastes that are generated for the abiotic controls.</p> <p>Page 10, line 9: Recommend inserting a comment on use of solid carriers/dispersants for poorly soluble/oil substances. This comment also applies to (page#/line#): 25/51, 34/39, 42/42. Change to "...in question. For example, the use of inert solid carriers (e.g. silica gel) may be used to introduce and disperse insoluble/oily substances. Water-miscible, non-toxic solvents....."</p> <p>Page 10, line 14: Insert the word "solution" as shown: "The volume of added stock solution should be of sufficient..."</p> <p>Page 11, lines 21 and 47: Use of 0.5% HCl is mentioned here and elsewhere in the document. Recommend stating an equivalent (and more commonly used) volumetric concentration (e.g. 0.2 N). This suggestion may also apply to (page#/line#): 13/12, 19/29, 20/9, 27/49, 36/6, 36/36, 37/51, 44/6, 44/32. For example at page 11 line 21, change to "...sufficient acid (e.g. 1 ml of 0.5% or 0.2 N HCL ) to lower sample pH to &lt;2..."</p> <p>Page 17, line 19: "High pressure liquid chromatography" is used here, but "high performance liquid chromatography (HPLC)" is used in lines 15/16. The is latter is preferred. Also, "mass spectrometry" should be "mass spectrometer" in this context.</p> <p>Page 21, line 17: Insert the word 'with' into this sentence. Viz., "This information, combined with its sequence of formation and disappearance and known biochemical</p>	<p><i>No Change. This combination of high mercuric chloride concentration and heat sterilization has been necessary to prevent microbial mediated degradation of labile materials. The procedure is written to allow for other methods of chemical/heat sterilization. HgCl<sub>2</sub> is disposed of as hazardous waste.</i></p> <p><i>Added the following statement to the <b>Test Substance Preparation</b> sections, "For materials which are poorly soluble and typically associated with suspended solids in wastewater, it may be appropriate to adsorb the test material onto an inert solid carrier, which is then dosed to the test system" ..</i></p> <p><i>Changed</i></p> <p><i>Changed unit from % to normality</i></p>
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	<p>reactions can form.....”</p> <p>Reference Substance – suggest the guideline provide substances for benchmarking QC of the conduct of these tests over time</p> <p>Solubility vs. concentration - There is some concern with taking subsamples, which may not be homogeneous. Although more labor intensive, may want to have some flexibility in test design, to allow sacrificing whole test systems at each sampling interval by using smaller test systems.</p>	<p><i>Changes made</i></p> <p><i>This sentence has been rewritten to read “This information combined with known biochemical reactions along with when a metabolite appears and disappears in the sequence of biodegradation can form an additional basis for inferring its identity.</i></p> <p><i>In a simulation test, the reference compound would serve as a viability control unless the purpose of the test was to compare the result of the reference material to the test compound. Viability can be monitored by other means such as plate counts, etc. Also, from an added cost standpoint, a reference substance is not recommended.</i></p> <p><i>Amended the <b>Test Substance Preparation</b> sections to include the following statement,” If the test material can not be evenly distributed within the test system prior to the initial sampling point, individual test systems can be prepared that are destructively sampled at each sampling interval”.</i></p>
	<p>Isomeric/complex mixtures- no guidance is provided for testing these types of substances – e.g. (a) the tests are not currently designed for them; (b) they can be</p>	<p><i>Knowledge of the test material, including the presence of stereoisomers, is critical to</i></p>

	modified to evaluate dual or multiple radiolabelled substrates (how to interpret kinetics?) (c) methodology can be used for individual testing of representative structures of the mixtures; or (d) other.	<i>the interpretation of any test. Complex mixtures can lead to complex results and we are not sure how to address this fundamental issue for any test for any purpose.</i>
	Page 5, line 19: Results – data plotting & half-life calculations – clarify data treatment with lag phase, since the guidelines states “in the absence of a lag phase.....”	<i>The kinetics section was expanded and now includes clarification on how to deal with degradation rates that do not follow first order or quasi-first order kinetics.</i>
	Page 39, lines 12-22 (and elsewhere): In the open batch tests, $^{14}\text{CO}_2$ and potentially $^{14}\text{CH}_4$ are not trapped and allowed to enter the atmosphere. i.e. the lab environment. This may need some clarification in regard to possible violations of radio-licensing & safety criteria	<i>Modified statement to include .....and located in a fume hood.</i>
	Page 39, lines 27034: Untreated wastewater-surface water mixing zone test – it is not clear how to report phase 1 versus phase 2 data when calculating half-lives.	<i>Kinetics section has been revised for clarity and is now optional. Would recommend that if DT values are reported for phase 1 of study that they are also reported for phase 2 regardless if the results are first order..</i>
	Also, there may be waste removal costs associated with mercuric chloride wastes that are generated for the abiotic controls. EMBSI switched to another chemical because the costs were prohibitive for the $\text{HgCl}_2$ removal.	<i>The procedure is written to allow for other methods of chemical/heat sterilization. If using <math>\text{HgCl}_2</math> it is disposed of as a hazardous waste.</i>