

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

Test Guidelines Programme

**DRAFT PROPOSAL FOR A NEW TEST GUIDELINE 436: ACUTE INHALATION ATC METHOD:
COMMENTS/RESPONSES**

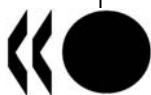
21st Meeting of the Working Group of National Coordinators of the Test Guidelines Programme

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This document presents the comments received after the last circulation of the new draft Test Guideline 436 on “*Acute Toxicity Testing-Acute Toxic Class (ATC)*”. The 2nd version of the draft TG was circulated on 28 November 2008 with a deadline of 9 January 2009. Comments were received from USA, the UK, the Netherlands and ICAPO. Ireland and Denmark stated they had no comments.

The final draft TG 436 (Version 3) with mark-up changes is available on the protected website for the Test Guidelines Programme in the section “*Draft Test guidelines and Guidance Documents*”, under the title “*WNT 21*”.

ACTION REQUIRED: *The Working Group of National Coordinators of the Test Guidelines Programme is invited to take note of the comments and responses included in the attached table.*

Compilation of comments and the Secretariats responses on the draft TG 436 on “Acute Inhalation ATC method” after the 1st circulation round with a deadline of 23 Jan-09. Comments were received from USA, UK, NL and ICAPO. IRL and DNK stated they had no comments. 11 February 2009.

MEMBER COUNTRY	GENERAL COMMENTS	SECRETARIATS RESPONSE
UK	<p>Generally, our opinion is that the documents provide for improved acute inhalation testing and updated guidance, as compared to the current situation. Opposition to the draft TG 433 led us to suspend inclusion of this proposal in the acute inhalation package as the UK was concerned that its inclusion would delay or prevent any progress in the area. However, UK remains convinced that the approach to acute inhalation toxicity in the UK proposed draft TG 433 is preferable to either the revised TG 403 or TG 436. The UK is still committed to the development of this approach as we consider the use of evident toxicity as an endpoint is justified on the basis of the use of the results to protect human health and on animal welfare grounds. The UK hopes to be able to re-submit a revised proposal in the near future. The work that has gone into the development of TG 403, TG 436 and GD 39 provides excellent basis for the development of a revised TG 433. Some amendments to the text are requested to avoid creating obstacles to future advances in refinement.</p>	<p>The Secretariat welcomes the UK initiative to continue work on the draft TG 433 and look forward to receive the submission in due time.</p>
UK	<p>The advice on testing corrosive and irritant materials is not strong enough. TG 403 says "When exposing animals to these materials, the targeted concentrations should be low enough to not cause marked pain and distress, yet sufficient to extend the concentration-response curve to levels that reach the regulatory and scientific objective of the test. These concentrations should be selected on a case-by-case basis and justification for concentration selection should be provided (see GD 39).", which rather defeats the object of these acute tests as it means that only non-lethal concentrations can be used.</p> <p>The UK considers that an LC50 test should not be performed with substances classified as corrosive, because the results are predictable. As both TG 403 and TG 436 depend on lethality, the purpose of such testing is unclear and will inevitably involve considerable suffering in the exposed animals. On the other hand, a repeated dose toxicity study using non-corrosive concentrations should be</p>	<p>Paragraph 6 in TG 403 has been amended to read as follows:</p> <p>“5. Testing corrosive and/or irritating test articles at concentrations that are expected to cause severe pain and/or distress should be avoided to the extent possible. The corrosive/irritating potential should be evaluated by expert judgment using such evidence as human and animal experience (e.g., from repeat dose studies performed at non-corrosive/irritant concentrations), existing in vitro data, pH values, information from similar substances or any other pertinent data, for the purpose of investigating whether further testing can be waived. For specific regulatory needs (e.g., for emergency planning purposes), the TG 403 may be used for exposing animals to these</p>

MEMBER COUNTRY	GENERAL COMMENTS	SECRETARIATS RESPONSE
	considered as this would provide information of value in human health protection.	materials because it provides the study director or principal investigator with control over the selection of target concentrations. However, the targeted concentrations should not induce severe irritation/corrosive effects, yet sufficient to extend the concentration-response curve to levels that reach the regulatory and scientific objective of the test. These concentrations should be selected on a case-by-case basis and justification for concentration selection should be provided (see GD 39) (2).
UK	All the texts (e.g. TG 403 paras 5 & 34) should avoid the suggestion of intentional sequential testing by first TG 436 then TG 403. Wherever possible, a decision should be taken on the appropriate single text.	The text in paragraphs 5 and 34 of TG403 have been changed.
UK	<p>Generally, performing a C x t study under the new 403 guidance would be the preferred option for a general acute inhalation study as this tends to sit more with the concepts of the 3R's than the existing 403.</p> <p>Where simple labeling is the only requirement for the study, the 436 would seem appropriate, but TG 436 may require several more exposures per study and therefore become notably more expensive.</p> <p>NC: The 3Rs element will depend on the information needs. The cost argument is noted but should not outweigh other considerations, such as animal welfare.</p>	No comment necessary.
US 2	<p>Unless otherwise constrained by formatting conventions, I would suggest using cut lines to delineate footnotes from main text, have all footnotes at bottom of the page, and have continuing footnotes shown at the bottom of the next page. This is part of the automatic formatting options available in MS-Word.</p> <p>NOTE: All suggested changes shown below have insertions shown in <u>blue underline</u>, and deletions shown in red strikeout modes.</p>	Formatting done.

MEMBER COUNTRY	GENERAL COMMENTS	SECRETARIATS RESPONSE
US 6	<p>General comments: Overall, TG 436 is clear, concise, and complete. It is recommended that the following minor changes be made throughout the TG to be consistent with GD 39, TG 403, TG 412, and TG 413:</p> <ul style="list-style-type: none"> • Change “dose” to “concentration” • Change “dusts and mists” to “aerosols” • Change “test substance” to “test article” • Change “more sensitive sex” to “more susceptible sex” • Change “most susceptible sex” to “more susceptible sex” 	Changes done.
ICAPO	<p>OVERARCHING ISSUES</p> <p>Corrosive articles: While the animal welfare language in the TG so far urges study directors to consider animal welfare when testing potentially corrosive articles, ICAPO questions the scientific utility of testing dilutions of such articles to determine acute toxicity point estimates. Since regulatory authorities differ in recommendations for testing of corrosives, ICAPO suggests adding this sentence or similar to paragraphs containing discussions of testing corrosive articles: “Before conducting a study with a corrosive or irritating test article, it is advisable to check with the appropriate regulatory authorities, as some countries allow the testing of such articles to be waived, and some do not allow the testing of such articles at all.” These instances occur in Paragraphs 6 of TG 403, and in multiple places in the draft GD 39.</p>	See comment above from the UK.
ICAPO	<p>OVERARCHING ISSUES</p> <p>Nose-only exposure method: The extreme confinement associated with this method is troubling on ethical and scientific grounds. Even in acute exposure situations, the animals experience significant distress. This distress could affect</p>	Some additional text on nose-only exposure has been added to GD 39 in paragraph 56 and text have been revised for paragraph 11 in TG 403 and 436.

MEMBER COUNTRY	GENERAL COMMENTS	SECRETARIATS RESPONSE
	<p>the integrity of the study: Fechter et al found marked depletions in liver glutathione (GSH) levels in rats exposed to different types of jet fuel by the nose-only, but not whole-body, exposure scenario. For these reasons, the TGs and GD should at minimum refer to the potential for such distress and confounding factors, in sections that discuss exposure methods, of which there are many. Additionally, in GD 39, paragraph 58, text stating that acclimation to the exposure tubes before the test may be unnecessary should be changed to recommend acclimation.</p> <p>¹ Fechter LD, Nelson-Miller A, Gearhart C. 2008. Depletion of liver glutathione levels in rats: a potential confound of nose-only inhalation. Inhal Toxicol 20(9):885-90.</p>	

Specific Comments		
Paragraph 1		
US 2	<p>Sentence 6: "Lethality is used as <u>the</u> key endpoint."</p> <p>Sentence 7: "... in severe pain or distress, suffering or impending death..." First instance becomes redundant to use at the end of the sentence.</p>	Changes made.
US 3	<p>Page 1, item 1, 4th sentence: The readers need to understand that the analyses did not involve animal experimentation but statistical simulations. Thus, it is important that TG 436 defines "retrospective validation" in Appendix I.</p> <p>Also the 4th sentence conveys a subtle tone of conclusiveness about the validity of TG 436 for classification and labeling that is not present in the PAG report. For example, gases and vapors were not part of the biometrical evaluation and although it is expected that the classification properties would be similar to those found in dusts, the analysis did not explicitly show the results for gases and vapors. An alternative sentence would change the verb "showed" to "suggested".</p>	<p>The term retrospective validation has been exchanged with retrospective performance assessment.</p> <p>The PAG report and the presentations by the PAG at OECD Expert Meetings have resulted in recommendations that the TG436 can be used for CL for aerosols, gases and vapors. No change.</p>
Paragraph 2		
US 3	Page 1, item 2: reference 8 needs to be updated on page 11. The reference should cite GD39 not GD19.	Change made.
Paragraph 4		
US 3	Page 1, item 4: GD39 covers other conditions that would make an investigator to change from 436 to a 403 test. The readers should be aware that these conditions exist and one way to do it is to invite them to read item 33 in GD 39 (Page 25). Another way to address it is to explicitly talk about these conditions in TG 436.	Reference to GD39 has been inserted.
Paragraph 5		
US 5	Paragraph 5: wording could be enhanced to note that the "all available information... should be considered" should include identification of any existing studies whose data would support not doing additional testing, or assist in the	Changes made in accordance with comment.

	<p>choice of the most appropriate species, strains, gender, and mode of exposure for the study as judged for highest usefulness and relevance for human risk assessment purposes.</p> <p>This would also help for:</p> <ul style="list-style-type: none"> • Paragraph 9: "The preferred species is the rat. Justification should be provided if other species are used" and • Paragraph 12: "The use of non-default modes of exposure should be based on the focus of the study and should be justified in the study report." <p>Further, the "all available information" could be noted as identifying any potential for chemical interactions that:</p> <ul style="list-style-type: none"> • would form new substances in the test chambers or in human exposure environments (e.g., terpenes with ozone forming particulates) or • could otherwise impact the response and use in risk assessment (e.g., mitigation, or synergism or other exacerbation of response) or • could impact the potential for any chemical and physical hazard interactions (e.g., ototoxicity from solvents plus noise) and/or • could highlight the seeking of information on sensory irritation and delayed toxicity for any target organ. <p>It may be worth noting a goal of identifying and considering information for the article and related substances that could indicate that the Concentration X Time studies might provide unexpected (unusual) results.</p>	<p>Too much details for the TGs.</p>
<p>US 6</p>	<p>Consider adding the following sentence at the end of this paragraph: "When testing corrosive and/or irritating test articles (e.g., for emergency planning purposes) the use of TG 403 is recommended."</p>	<p>A reference to GD39 and TG403 has been inserted into paragraph 7.</p>
<p>Paragraph 6</p>		
<p>US 2</p>	<p>Sentence 4: "Depending on the mortality and/or the moribund status of the animals, more than 2 steps may not be necessary <u>sufficient</u> to allow judgement on the acute toxicity of the test substance."</p>	<p>Changes made.</p>

	<p>Sentence 5: Add “only” to the end after “... the more susceptible sex.”</p> <p>Sentence 6: Replace “i.e.,” with “such that:” and for option c), revise as follows: “...sex only (i.e., for the lower boundary estimates of the toxic class should be based on 6 animals per test concentration group, <u>regardless of gender</u>).</p>	
US 4	The single instance of “gender” should be changed to “sex” to match the rest of the paragraph. The word “or” is necessary at the end of step (b), else every step may be expected.	Change made.
US 6	This sentence is unclear. Consider changing “i.e., for the lower boundary...” to “i.e., the lower boundary...”	Change made.
Paragraph 6, 27		
UK	The guideline is all about Fixed Doses using groups of 3M and 3F, as indicated in para 6 but paragraph 27 refers to 5M and 5F.	Paragraph 27 has been amended, it contained text from TG403 that has been taken out.
Paragraph 8		
US 2	<p>Sentence 1: “Testing in GHS Category 5 should only be considered in this context, i.e., when there is a strong likelihood that results from such a test <u>will</u> have a direct relevance for <u>to</u> protecting human or animal health.”</p> <p>Sentence 2: Replace “rationalized” with “provided.”</p>	Paragraph 8 has been deleted and the suggested text inserted into paragraph 29, on Limit Test.
US 4	This paragraph is not nearly so well written as Par. 50 of GD 39, which has been used to fashion Par. 29 of this draft TG 436. Serious thought should be given to adopting some of the Par. 50 language instead. This is especially important since reference to Category 5 in this paragraph appears to come out of nowhere!	See comment above.
US 6	Consider changing the last word from “rationalized” to “provided” or “reported.”	See comment above.
ICAPO	<u>Para 8:</u> The second sentence in this paragraph is unclear; if the intention is to suggest that inhalation testing should not be performed if inhalation exposure is not likely, “this route” should be replaced by “the relevant route of exposure.”	See comment above.

Paragraph 10		
US 2	End of Sentence 2: "... at the same time point <u>age</u> ."	Change made.
Paragraph 11		
US 3	Page 2, item 11: Delete sentence that reads "Animals should be housed individually in whole-body inhalation chambers during exposure to aerosols to prevent ingestion of test article due to grooming of cage mates". This sentence is already present in 13.	The paragraph has been reworded.
US 6	The sentence, "Animals should be housed individually..." is duplicated in paragraph 13. Consider removing this sentence from paragraph 11.	See change above.
Paragraph 19		
US 2	Comment on Second Sentence: Should humidity be monitored and recorded three times, regardless of exposure duration? I would suggest adding a phrase to indicate that relative humidity should be measured at least three times <u>for durations of up to 4-hours, and hourly for shorter durations</u> .	Change made.
Paragraph 22		
UK	Last sentence - correct "sample's identify" to "sample's identity"	Change made.
US 3	Page 5, item 22, last sentence: "...sample's identify..." should be "...sample's identity". The typo also appears in TG 403.	Change made.
Paragraph 23		
US 3	There are several sentences throughout the document that direct the reader to GD39 to get additional information but the reference citation is missing. For example, the last sentence of item 23 should read: "Guidance for estimating t95 can be found in GD 39 (8)".	Agree, reference to GD39 has been inserted at several places.
US 3	Page 5, item 23, 6th sentence: Fix punctuation at the end of the sentence.	Change made.
US 5	Add reference (8) after GD 39 at the end of last sentence.	Change made.
Paragraphs 24		

US 3	Page 5, item 24: Modify sentence to say “...each phase may behave differently in an inhalation chamber so at least one indicator substance (analyte),...”. This is the way the sentence looks like in TG 403.	Change made.
Paragraphs 25		
US 3	Page 5, item 25, last sentence: Add “(see paragraph 14)” at the end of the sentence. This is the way the sentence looks like in TG 403.	Change made.
Paragraphs 26-27		
NL	It seems that paragraph 26 and 27 are in contradiction to each other. The main study as described in paragraph 27 seems not to fit in the study design and seems to come from the TG 403.	The paragraphs have been amended since it contained text from 403 that has been taken out.
Paragraph 26		
US 3	Page 6, item 26: Testing schemes are missing in Annex 2-4. Add testing schemes to the TG.	The testing schemes for annexes 2-4 was attached to the draft TG436 on submission.
US 4	This is currently (and has been) a very awkward sentence: “The testing schemes for gases and vapours included in Annexes 2-4 represent the testing with the cut-off values of the GHS categories for vapours (defined as Categories 1-4) for gases (100, 500, 2500, 20.000 ppm) (Annex 2), for vapours (0.5, 2, 10, 20 mg/L/4 h) (Annex 3) and for dusts and mists (0.05, 0.5, 1, 5 mg/L/4 h) (Annex 4).” It’s not at all clear why “vapours” appears twice. Is the following what is meant: “The testing schemes for gases and vapours [and dusts and mists?] included in Annexes 2-4 represent the testing with the cut-off values equivalent to those defining the GHS vapour Categories 1-4 for gases (100, 500, 2500, 20.000 ppm) (Annex 2), for vapours (0.5, 2, 10, 20 mg/L/4 h) (Annex 3) and for dusts and mists (0.05, 0.5, 1, 5 mg/L/4 h) (Annex 4).”?	The section contains some duplicative text that has been removed.
US 6	On line 4, place parentheses () around “included in Annex 2-4.”	Change done.
Paragraph 26, 29		
UK	Amend “20.000” to “20000”	Done

Paragraph 27		
UK	Paragraph 27 should not be in this guideline (e.g. wrong numbers of animals/test group, pre-determined number of concentration levels) but has slipped in from the TG 403. Some of the text will need to be retained but a thorough overhaul is required to render it appropriate for this TG 436.	The paragraph has been amended since it contained text from 403 that has now been taken out.
US 3	Page 6, item 27: The first sentence has the incorrect number of animals. It should be 3 males and 3 females or 6 animals of the susceptible sex.	See comment above.
US 4	I think the first two sentences of this paragraph should be deleted from here and only appear in Par. 31 of TG 403 (which is otherwise identical to this paragraph). They do not have meaning with this design, and contradict Par. 26.	See comment above.
US 6	Most of the text in this paragraph applies only to TG 403. Remove: “A main study is ... robust statistical analysis.” “This allows the study director ... scientific judgement.”	See comment above.
ICAPO	<u>Para 27:</u> This paragraph appears to be copied in error from TG403, since this TG uses 3 animals per sex or 6 of the same sex, and not 5/sex.	See comment above.
Paragraph 28		
US 3	Page 6, item 28, last sentence: If the material is expected to be highly toxic, TG 403 but not 436 should be conducted. GD39 covers when to switch to 403 and I noted that this information is not covered in the TG. As I noted above, the readers should be aware that these conditions exist and one way to do it is to invite them to read item 33 in GD 39 (Page 25). Another way to address it is to explicitly talk about these conditions in TG 436....”	A reference to GD 39 has been inserted.
US 6	In the first line, change “shown” to “known.”	Change made.
Paragraph 29		
US 2	Final sentence: “To avoid an unnecessary use of animals, a test run <u>without animals</u> should be ...”	Change made.
US 3	13. Page 6, item 29, 1st sentence: The last part of the sentence “which serves as a limit test for this Guideline” implies that other TGs may not use the same limit concentration cut-offs. Delete last part of the sentence or revise language to avoid confusion.	An “a” has been changed to “the”.

Paragraph 34		
US 4	Lest there be misunderstanding, additional examinations can extend the study's interpretive value only if they are carried out by design, for all animals or for a suitable sample of animals randomly selected across the entire experiment. <i>Ad hoc</i> examinations of individuals do not enlarge the domain of inference.	The pathology section of TG436 is now the same as the one in 403, with the adding "...included a priori by design...".
Data and Reporting		
US 3	<p>Page 8-9, Data and Reporting section, Inhalation chamber and Test Conditions subsection: Information is duplicated for the equipment used to monitor temperature, humidity and particle size distribution. My recommendation is to have the information in one subsection instead of having it in two subsections.</p> <p>Page 8-9, Data and Reporting section: There are circumstances when an investigator may choose to measure oxygen and carbon dioxide concentrations in the chamber (see item 18). When applicable, information about the equipment used to measure oxygen and carbon dioxide should be added to the study report, but this is not included in the current Data and Reporting sections.</p> <p>Page 8-9, Data and Reporting section: In some circumstances, investigators may switch from 436 to 403. If this occurs, the study report should note such a change and provide justification for the switch. This is not currently covered in the Data and Reporting section.</p>	Changes made
Literature		
US 5	<p>Last bullet; Reference for OECD Guidance Document on Humane Endpoints should be (7) and not (5)</p> <p>Change guidance document number from 19 to 39</p> <p>TG 430 title is In vitro skin corrosion – transcutaneous electrical resistance test (TER)</p> <p>Hyperlinks don't appear to link to the proper documents.</p>	Changes made.
US 4	Item 8 (which was item 7 in June 2007) should be changed to refer to Monograph 39, not to Monograph 19 (Humane Endpoints), and should give the appropriate URL (the current reference is to the draft guideline website).	Change made.

Annex 1		
US 3	<p>Page 13, Annex I, LC50 definition: Superscript is missing in mg/m³ units. Please change mg/m³ to mg/m3. Change may be needed in other parts of the document.</p> <p>Page 13, Annex I, limit concentration: The cut-off value for gases is incorrect. The value is 20,000 ppm based on the 2007 version of the GHS document,</p> <p>Page 13, Annex I, MMAD definition: The last sentence of the definition on page 14 is incomplete.</p> <p>Page 13, Annex I: add definitions for “moribund status” and “vapour”.</p>	Annex 1 deleted, all definitions are found in the GD39.
US 6	<p>Observations – Use all capitals: OBSERVATIONS</p> <p>Annexes – It is not clear what the second row of boxes are (e.g., 3-6, 0-2). Consider adding to the left side “Dead/Moribund.” I found the information in the bottom box to be confusing. Consider modifying the text or eliminate it altogether. In Annex 4, change “dusts and mists” to “aerosols.”</p>	Annex 1 deleted, all definitions are found in the GD39.
US 5	<p>Humane endpoint, Impending death, and Moribund status should refer to reference (7) and not (6)</p> <p>For consistency with Guidance Document 39, add definitions for fume, haze, smog, and smoke. Likewise, add the following verbiage to the end of the definition for aerosol: <i>See also dust, fog, fume, haze, mist, smog, and smoke.</i></p>	Annex 1 deleted, all definitions are found in the GD39.
Annex 2		
US 3	Annexes 2-4 are missing. Add testing schemes.	The annexes were submitted together with the draft TG436.
US 5	Annex 2 d: Title – replace 5000 ppm with 20000 ppm	Changes made
ICAPO	<u>ANNEX 2d</u> : There appears to be an error in this figure: the subtitle and the concentration in the “Start” box do not agree.	Changes made
Annex 3		
NL	Furthermore a typo on pg 21, Annex 4a should be annex 3a.	Changes made
US 4	Annex 3: Annex 3a currently is mislabelled Annex 4a.	Changes made

US 5	Title – replace ANNEX 4 a with ANNEX 3 a	Changes made
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