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English - Or. English

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

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Test Guidelines Programme

DRAFT UPDATED TEST GUIDELINE 451, 452 AND 453: COMMENTS/ RESPONSES

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

31st March-2nd April 2009, OECD Headquarters, Paris, France

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English - Or. English

This document presents the comments received by Canada, Denmark, Finland, Ireland, Japan, Sweden, the United Kingdom, the United States, BIAC and ICAPO on the last versions of the draft Test Guidelines 451, 452, and 453. Comments have been addressed by a consultant under contract, in consultation with the expert group on TG 450s.

The three final draft Test Guidelines, with track changes showing how comments have been addressed, and with a few comments added by the consultant, are available on the protected website for the Test Guidelines in "Draft Test Guidelines and Guidance Documents", under the title "WNT21".

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 three attached tables.***

TG 451

Paragraph	Country	Comment	Response
General	Canada	We support the intent to obtain additional information from the animals used in the study and providing further detail on dose selection. Please avoid broad generalizations. The word "normally" should be clarified in the context of the validity of the mouse model for carcinogenicity (see below for comment to paragraphs 12). It is recommended to qualify the validity of the mouse model. There are several references to other sources of information throughout the test guideline. While these references are definitely useful, providing a summary of the essential information contained in some of these references within the test guideline will make the guideline more of a stand-alone document and a more useful resource. Specific examples where additional information can be provided in the test guideline are indicated in some of the comments below.	Discussion of the validity of the mouse model will be included in the Guidance Document, as will details of essential information contained in some of these references and specific examples. It is beyond the scope of the TG to include the type of additional information sought by Canada
General	SW	Sweden welcomes the updated versions on the test guidelines for carcinogenicity and chronic toxicity. They are well structured and easy to follow. Please, see our comments as follows.	No response needed, thank you
General	UK	The UK considers that it is important that the mode of action is included in the initial considerations. The optimal design may differ depending on whether the substance is a known or suspected genotoxin. This may also affect the choice between a carcinogenicity study or a combined toxicity/carcinogenicity study. We agree that detailed discussion belongs in the Guidance Document but the Test Guidelines 451 and 453 should draw attention to this issue.	Introduced in paragraph 6

Initial considerations	UK	The Initial Considerations should include a paragraph on the statistical analysis of the results. It is important that this is considered before the study is initiated. However, most of the detail should be in the Guidance Document. We propose the text below is added as a separate paragraph: "The statistical methods most appropriate for the analysis of results, given the experimental design and objectives, should be established before commencing the study. Issues to consider include whether the statistics should include adjustment for survival, analysis of cumulative tumour risks relative to survival duration, analysis of the time to tumour and analysis in the event of premature termination of one or more groups. Guidance on the appropriate statistical analyses and key references to internationally accepted statistical methods are given in the Guidance Document."	Introduced as paragraph 8
General	US 1	Please acknowledge in the guideline that some details may differ for pharmaceuticals.	Addressed in paragraph 1
General	US 2	Agree with FDA CDER that it is important to acknowledge in the guidelines that some details may differ for pharmaceuticals, pesticides and other industrial chemicals. **For example, in the carcinogenicity guideline 451:** 30a. For pesticides, OPP requires an 18-month mouse study to differentiate tumorgenicity versus natural aging process alterations (i.e., age-related tumors). 30b. OPP criteria for pesticides states that at 15 months in mice and 18 months in rats, the number of animals in any group should not fall below 50%; And at study termination (18 months in mice and 24 months in rats), the survival in any group should not fall below 25%. 38. Historical control data are useful to determine	Addressed in paragraph 1 but only for pharmaceuticals?

		whether the results for the concurrent controls differ from the laboratory's typical results and how consistent the data are over time, from one study to another.	
4	Canada	Reference is made to TG 412 and 413 to assist in the design of longer term studies involving exposure via the inhalation route. Suggest making reference to guideline TG 410 for the dermal route	Added
5	US 3	We are looking for not only increased incidence, but also reduced time to appearance of tumors. Change the first bullet to say something like: "the identification of the carcinogenic properties of a chemical, resulting in an increased incidence of neoplasms INCREASED PROPORTION OF MALIGNANT NEOPLASMS, OR A REDUCTION IN THE TIME TO APPEARANCE OF NEOPLASMS, compared with concurrent control groups."	Changed
7	Canada	The value of the study is applicable but was not included, as it was in OECD guidelines 452 and 453. Consider adding: The carcinogenicity study provides information on the possible health hazards likely to arise from repeated exposure over the majority of the entire lifespan (in rodents). The study will provide information on the toxic effects of the substance including potential oncogenicity, and may indicate target organs and the possibility of accumulation. It can provide an estimate of the no-observed-adverse effect level, which can be used for establishing safety criteria for human exposure. The need for careful clinical observations of the animals, so as to obtain as much information as possible, is also stressed.	Added as para 5
7	DK	Wouldn't it be an idea to cite the particular paragraph 62 referred to	Done
9	Canada	Consider adding an explanation of why a combined chronic toxicity/carcinogenicity study is preferred, as phrased in OECD guideline 453: The combined test provides greater efficiency in	Added

		terms of time and cost compared to conducting two separate studies, without compromising the quality of the data in either the chronic phase or the carcinogenicity phase.	
11	DK	last line: delete "also"	Done
12	Canada	Recommend qualifying the use of the mice in carcinogenicity testing as follows: Although there are some questions regarding certain aspects of the mouse model for carcinogenicity testing e.g., some mouse liver tumours may have limited relevance to humans due to physiology differences (21, 22, 23), testing this species is considered valuable and necessary in some regulatory programmes. Consider adding in a reference for non-rodents: The design and conduct of carcinogenicity studies in non-rodent species, when required, should be based on the principles outlined in this Guideline together with those in OECD TG 409, Repeated Dose 90-day Oral Toxicity Study in Non-Rodents.	There has been a lot of discussion about this point, and earlier text has been deleted, this should not be expanded in the TG but addressed in guidance Added in para 2
13	Canada	Guidance of the age of the animal at study initiation is useful. Paragraph 15 can be referred to here.	Other comments said it was repetitious to have it in both paras, and it was deleted here
13	DK	first line: I have always been told that "strain" refers to inbreed animals while "stock" covers random breed animals. Is that correct?	I think you are right, but I think strain is used throughout the TGs
14	DK	line one: "small groups": could cause problems among others concerning "n" for food consumption etc. Maybe a range or an upper limit or a suggestion of two animals per cage (the rat is the preferred species).	Suggest this is discussed in guidance, rather than make an attempt to define it here
14	UK	Reference to cage placing sought but not yet received	Noted
15	Canada	Consider moving the phrase "...separately for each sex" earlier in the sentence i.e., "At the commencement of the study, the weight variation for each sex should be minimal..."	Have added your suggestion "for each sex" earlier in the sentence but retained "separately for each sex\2
15	DK	line 4: why have "we" changed from week 6 in the present version to 8 in the draft?	It was inconsistent and there was a preference for 8
17	ICAPO	Recognizing the variations in opinion, as reflected in comments,	"Where such information is already available

		on the issue of interim kills and/or satellite animals, ICAPO appreciates the requirement for scientific justification, and recommends that in addition, the following text is added: "Groups for interim kills should not be included if the results of subacute and subchronic studies are available," or similar.	from previous subacute and subchronic studies on the substance, interim kills may not be scientifically justified. " added to this paragraph (now 20)
18	Canada	Recommend defining "limit test" as a study using one dose level of 1000 mg/kg body weight as well as expanding on its conditions of use i.e., when is it appropriate or inappropriate. For instance, it is inappropriate for use if you expect a chemical to be carcinogenic at the limit test because a quantitative risk assessment is not possible with a single treatment group. This may be more appropriate in its own paragraph; perhaps cross-referencing to the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6) would be appropriate. The alternative may be supported if the limit dose is further elaborated upon as described.	Reference to limit test deleted
18	DK	last sentence: I will suggest deleting that sentence as it doesn't give any "useful" information here the first time limit test is mentioned	Reference to limit test deleted
20	DK	last line: insert "normally" between "should" and "not exceed..". I am not too happy with a limit test at all. In addition I have some difficulties reading the paragraph (probably personal problems more than editorial).	Last sentence deleted as agreed in conference call. Consultant still thinks the paragraph reads OK and is necessary, but has amalgamated it with previous para now that the last sentence has gone
20	SW	In the first sentence it says that a top dose lower than the dose which provides evidence of toxicity may be chosen. This is followed by an example which may need a clarification. An "adverse effect of concern that nonetheless has little impact on lifespan or body weight" which occurs below the dose which elicit evidence of toxicity – please clarify when a top dose other than the one that gives evidence of toxicity could be chosen.	SW point is noted, but as stated above to DK consultant thinks the paragraph reads OK and is necessary, I don't think it is possible to give examples here as these could be difficult to agree (e.g. liver enzyme induction, hormonal changes?). will be expanded in guidance
21	US 3	I am not comfortable with the emphasis on identifying a NOAEL, because this is not necessarily relevant for cancer studies. Can	Reflecting the discussion during the conference call, have changed to "Dose levels and dose

		this be re-worded in some way, such as: "…to establish a dose-response AND/OR NOAEL?"	level spacing may be selected to establish a dose:response and, depending on the mode of action of the test substance, a NOAEL or other intended outcome of the study, e.g. a BMD….".
24	Canada	It is recommended that the last sentence "....the additional pair-fed control group may be useful to allow for this." be modified to: "....the additional pair-fed control group may be useful to serve as a more suitable control."	Changed
24	IRL	Please explain the use of the term "pair-fed control group". Also it will need to be clarified if a 20% reduction in dietary intake is correct	Expert group considered there was no need to explain in TG 20% removed
24	UK	The UK supports the need for a qualification of reduced dietary intake and 20% is an appropriate value. Please delete 'of the order of' as you suggest. The room for case-by-case judgement is covered in the latter part of the sentence 'may be useful'. Replace "palatability" with "unpalatability". Not raised in this TG, but is in TG 452 and is equally applicable. The unpalatability of the diet would be expected to be known prior to commencement of the study and therefore an additional control group assigned from the outset.	There was a view that the whole of the last sentence should be removed, but having looked at it again I think it should stay in, reworded to remove reference to the 20% . Changed to "reduced palatability" Noted
25	Canada	It is recommended that the following, "For dietary or environmental chemicals including pesticides, administration should be via the diet or drinking water." be modified to: "For..., administration is typically via the diet or drinking water." It is recommended that the following sentence be added after the above-noted sentence: "However, for some pesticide uses (e.g., fumigants or insect repellents) or scenarios (e.g., occupational exposure), administration via other routes may be more suitable."	Changed Added. Acceptable to others?

26	UK	The wording on testing for stability and homogeneity requires revision **Homogeneity**. The Guidelines TG 451,452 and 453 indicate that the homogeneity of the substance in the diet containing the test article should be confirmed analytically before the start of the study. The wording about testing periodically throughout the study is so vague that it is not very helpful. A UK expert in assessing data for the authorization of veterinary medicinal products for administration via animal feed advised that homogeneity is unlikely to change in the absence of the triggers proposed below but periodic checks are normally carried out in most testing laboratories. I would suggest that revision of the text on testing for homogeneity as follows: "If the treated feed is transported from the site of preparation to a different site for administration, the homogeneity should be tested at the site of administration Additional testing for homogeneity during the study are required if/when: The method of preparation is altered The source or type of the feed is changed Routine checks for homogeneity may also be carried out at the study director's discretion and the results of these analyses should be included in the study report." **Stability** All the TGs should indicate that the stability of the substance in	Have changed this to ". Information should be available on the stability of the test substance and the homogeneity of dosing solutions or diets (as appropriate) under the conditions of administration (e.g., diet). Note TG 408 just contains the sentence "The stability of the test substance under the conditions of administration should be determined." and the only reference to homogeneity is in the results.

		the feed maintained at temperatures appropriate for the diet stored and during administration must be tested for the maximum time that may elapse between preparation and consumption. Some thought will be needed to draft a text to make clear the requirements for stability testing of solutions and suspensions used for gavage or following addition to the drinking water and for testing homogeneity of suspensions (solutions need to be tested for stability but not for homogeneity) but I think the consultant can do this.	
27	Canada	For clarity we recommend amending the last sentence, "... (mg/kg diet or ppm)... weekly basis (mg/kg body weight/day) may be used; the method should be specified."	Change made
28	Canada	Please modify the first sentence as follows: "In the case of oral or dermal administration, the animals are dosed with the test substance daily (seven days per week) up to 18 (for mice or hamsters) to 24 months (for rats) (see also paragraph 30)." Consider recommending a duration of exposure for the dermal route e.g., 6 hours per day, similar to OECD guideline 410. The paragraph does not specifically indicate the duration of dosing for inhalation exposure. Consider revising the last sentence: "Dosing by the inhalation route is carried out for 6 hours per day, 5 days per week, for a minimum of 12 months (chronic phase satellite groups) up to 18 or 24 months (carcinogenicity phase)."	Amended Added Duration added
28	DK	we should stick to every day i.e. 7 days a week irrespective route of administration (there could maybe be better arguments for changing dosing e.g. kinetics?). That's scientifically plausible. I don't think we should not deal with practical problems jeopardising our credibility.	Text revised in accordance with TG 412
28	ICAPO	Since specific guidance notes for mice have been inserted into this draft, it is recommended to consult TGs 412/413, as there was discussion during the revisions of those guidelines to recommend 4 hours per day for mice in repeated-dose inhalation	Text revised in accordance with TG 412 It was agreed at the start of the process that the TG would focus on the oral route and not get

		studies. In addition we would reiterate that a recommendation should be made for whole-body exposure rather than nose-only exposure for chronic inhalation exposure. Such recommendation could be made in the GD and reference to the GD made in this paragraph.	into detail on the other routes, but refer to guidance and other TGs on e.g. inhalation
29	US 1	The FDA only allows up to 10 ml/100g dose volume for gavage studies. Higher volumes may exceed the capacity of the stomach. Why is a larger volume given for aqueous mixtures?	Reference to 20 ml/kg has been removed
30	SW	The anticipated lifetime of the strain which is used in the test should determine the duration of the study. It means that for certain strains a duration of 24 months and 18 months, for rats and mice respectively, may be too short. Please, indicate that for certain strains a duration of 30 months and 24 months, respectively, may be more appropriate. See the current version of TG 451. In the current TG 451 there are considerations for a study to be accepted as negative. It is removed in the draft revision; what is the justification for that?	See new wording introduced "Shorter or longer study durations may be used, dependent on the lifespan of the strain of the animal species in the study, but should be justified. For specific strains of mice, e.g., AKR/J, C3H/J or C57BL/6J strains a duration of 18 months may be more appropriate." There was no agreement on this wording
30	ICAPO	Delete reference to hamsters	Not agreed by expert group, but replaced by "rodent"
30	US 1	For the FDA and for pharmaceuticals worldwide, the duration of the standard carc study for mice is 2 years and has been for about 20 years; an 18-month study is not considered acceptable. Survival of the mice has not been a problem. Also studies for pharmaceuticals are not terminated when survival reached 25% in controls. Studies for the FDA are terminated when there are 20 controls left; a particular dose group may be terminated when 15 animals are reached. Doses	Wording now "The duration of the study will normally be 24 months for rodents" as agreed in conference call Noted

		may be adjusted downward, if survival or body weight issues during a study, especially if early in the study.	
30	US 3	The normal duration should be 24 months for mice.	Wording now "The duration of the study will normally be 24 months for rodents" as agreed in conference call
31	Canada	Reference is made to paragraph 34 in TG 452 for a description of specific signs of toxicological relevance. Given the number of references identified in TG 451 (e.g., TG 412 and 413 for inhalation testing, the guidance document on the design and conduct of chronic toxicity and carcinogenicity studies), including the specific signs of toxicological relevance in TG 451 will assist the reader and minimize reliance on other references.	This reference now deleted
31	DK	third line: I would prefer to delete "working" and consequently the following sentence based on arguments like those presented just above.	Deleted, and the paragraph has been reworded, reflecting the discussions in the conference call on 21.1.09 and the comments of NL
32	Canada	Consider rephrasing the last sentence as follows to ensure that it is clear that water consumption should be measured at the stated intervals for studies in which the test substance is administered in drinking water and that these measurements should also be considered when drinking activity is altered: "Water consumption should be measured at least weekly for the first 13 weeks and at least monthly thereafter when the substance is administered in drinking water. Water consumption measurements should also be considered for studies in which drinking activity is altered."	Canadian suggestion accepted
33	Canada	Consider recommending that if appropriate, blood sampling for haematological and clinical chemistry determinations, and urinalysis be conducted mid-way during the study and study termination on a minimum of 10 per sex per group.	Canadian text added, with slight modification
33	DK	concerning blood samples wouldn't it be appropriate to	Covered by Canadian suggestion?

		mention/include interim kill (a reference to paragraph 17) when stating "at the end of the test period" (first line on page 8) or is that implicit?	
33	ICAPO	retro-orbital bleeding is well-known to be distressing for the animals, and ICAPO recommends that at the very least the animals are anaesthetized prior to the procedure, or another blood collection method is recommended.	Pont noted and agreed. Will be added in guidance. Reference to anaesthesia introduced
34	Canada	Although the development of tumours may confound the usefulness of organ weight data, please consider requiring organ weights from interim sacrifice animals as well as from at least 10 animals per sex per group at terminal sacrifice. This would contribute to the knowledge of the toxicological effects, particularly if there are no carcinogenic effects.	Consultant considers that to introduce this requirement at this stage of the process would cause too much debate, and the possibility of collecting organ weight data is covered by the latter part of the para
35	Canada	Rectum and nose preservation should be required, as in the EPA guideline guidelines (870.4200, 870.4300). Consider clarifying why the tissues in square brackets are optional. Consider moving the sentence defining the use of square brackets to the beginning of the paragraph i.e., The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (24) (tissues in square brackets are optional)...	Have changed rectum from optional to obligatory and added [nose] as optional Sentence moved, but consider that a clarification of the optional tissues is beyond the TG, will be explained in guidance
35	Japan	Not only preservation of female mammary gland but also preservation of male mammary gland would be useful to detect any important changes at mammary glands. Therefore, we recommend that the "male" mammary gland should also be preserved	Added
35	SW	For better readability, please arrange all the tissues in columns	Done
37	Canada	Consider adding that summary data tables <u>should also provide the means and standard deviations</u> (for continuous test data) of animals showing toxic effects or lesions, <u>in addition to the grading</u> of lesions.	This seems sensible, added
38	Canada	Consider adding that historical data should also be presented by	New text agreed on this para as a result of the

		individual study as well as an average of the data available. All data summary tables should include group sizes (N values) and means and standard deviations for continuous test data. Also add that the historical control, if evaluated, should be submitted from the same laboratory and animal supplier. Please delete "Historical control data should be used only if concurrent controls appear to be significantly different." It is not appropriate to restrict the use of historical data as described.	conference call
38	SW	It is important to note that if reference is made to historical control data they must originate from the same time point as data from the study conducted, in order to be relevant. For example if a study is teminated at 18 months and the historical control data originate from a termination at 24 months, such data are not relevant. Please, insert	New text agreed on this para as a result of the conference call
38	US 1	The sentences about historical and concurrent controls are confusing. Historical values can never take precedent over concurrent controls, especially when concurrent controls differ from historical controls. Control values may differ dramatically because the animals from the same source may differ widely from historicals, especially after animals are rederived. The pathologists may also be different. Historical controls are most useful for determining how uncommon a particular neoplasm may be.	New text agreed on this para as a result of the conference call
40	Canada	Under Test substance: · Recommend that a certified chemical analysis be included in the report. Under Test conditions: · For the test substance preparation, please recommend that analytical data be provided to confirm homogeneity, stability, and concentration. Under Results: · Consider providing more detailed guidance with regard to data supporting the Results texts. Supporting data should be in the form of raw individual animal data as	Changes made

		well as summary tabular data. · Consider adding Ophthalmoscopy (if available), Haematology (if available), Clinical biochemistry (if available), Urinalysis (if available). · For statistical treatment of results, the bullets are only a partial list of parameters that require statistical treatment. Consider deleting the subsequent bullets which are only a partial list of parameters that require statistical treatment, or adding the other parameters for which statistical treatment is appropriate e.g., clinical chemistry (if available). Under "Discussion of results including": Add "NOAEL and/or LOAEL determination"	

TG 452

Paragraph	Country	Comment	Response
General	Canada	We support the intent to obtain additional information from the animals used in the study and providing further detail on dose selection. Please avoid broad generalizations. The word "normally" should be clarified in the context of the validity of the mouse model for carcinogenicity (see below for comment to paragraphs 12). It is recommended to qualify the validity of the mouse model. There are several references to other sources of information throughout the test guideline. While these references are definitely useful, providing a summary of the essential information contained in some of these references within the test guideline will make the guideline more of a stand-alone document and a more useful resource. Specific examples where additional information can be provided in the test guideline are indicated in some of the comments below.	Discussion of the validity of the mouse model will be included in the Guidance Document, as will details of essential information contained in some of these references and specific examples. It is beyond the scope of the TG to include the type of additional information sought by Canada
General	SW	Sweden welcomes the updated versions on the test guidelines for carcinogenicity and chronic toxicity. They are well structured and easy to follow. Please, see our comments as follows.	No response needed, thank you
4	Canada	Reference is made to TG 412 and 413 to assist in the design of longer term studies involving exposure via the inhalation route. Suggest making reference to guideline TG 410 for the dermal route	Added
4	UK	We do not think it is necessary to expand this beyond the current wording.	Noted
6	Canada	Consider that the carcinogenicity "should" (rather than "may") be carried out after initial information on toxicity has been obtained from repeated dose 28-day and/or 90-day toxicity test 28/90 day study. This revised wording is consistent with OECD guideline 453.	I think Canada means "chronic toxicity" rather than "carcinogenicity". Have replaced "may" by "should only"

9	Canada	As this is not the guidance document for TG 453, consider deleting the last sentence, "This is particularly relevant in the situation where a combined chronic toxicity and carcinogenicity study (TG 453) is to be carried out (paragraph 10).".	Consultant considers it is preferable to retain this sentence as a reminder, even though it is not specifically relevant to this TG.
Initial considerations	UK	The Initial Considerations should include a paragraph on the statistical analysis of the results. It is important that this is considered before the study is initiated. However, most of the detail should be in the Guidance Document. We propose the text below is added as a separate paragraph: "The statistical methods most appropriate for the analysis of results, given the experimental design and objectives, should be established before commencing the study. Issues to consider include whether the statistics should include adjustment for survival and analysis in the event of premature termination of one or more groups. Guidance on the appropriate statistical analyses and key references to internationally accepted statistical methods are given in the Guidance Document."	Inserted as paragraph 8, thank you for the text
10	Canada	Consider adding an explanation of why a combined chronic toxicity/carcinogenicity study is preferred, as phrased in OECD guideline 453: The combined test provides greater efficiency in terms of time and cost compared to conducting two separate studies, without compromising the quality of the data in either the chronic phase or the carcinogenicity phase.	Added
12	Canada	Recommend modifying the first and third sentences, including deletion of the second sentence, as follows: "The test substance... of experimental animals for a period of <u>at least</u> 12 months, although longer or shorter durations may also be chosen (see paragraph 30). A duration longer than 12 months may be required depending on regulatory requirements but a period less than 12 months must be justified."	Have inserted the word "normally" rather than "at least" (I know Canada doesn't like it!) but have not deleted the second sentence
15	UK	Reference to cage placing sought but not yet received	Noted

16	Canada	Consider moving the phrase "...separately for each sex" earlier in the sentence i.e., "At the commencement of the study, the weight variation for each sex should be minimal..."	Have added your suggestion "for each sex" earlier in the sentence but retained "separately for each sex\2
17	Canada	Recommend removing the word "normally" in the third sentence as it is a broad generalization, and inaccurate, since the minimum of 20 animals/sex/group applies pertains only to rodents. Recommend including the recommended minimum group sizes for non-rodents and amending the sentence as follows, "For rodents, each dose group and concurrent control group should contain at least 20 animals per sex while for non-rodents a minimum of 4 per sex per group is recommended."	Canadian text used, but "normally" retained, given the controversy about 20 animals per group
18	Canada	Consider stating the minimum number (at least 10/sex/group) for interim kills.	Added
18	ICAPO	Recognizing the variations in opinion, as reflected in comments, on the issue of interim kills and/or satellite animals, ICAPO appreciates the requirement for scientific justification, and recommends that in addition, the following text is added: "Groups for interim kills should not be included if the results of subacute and subchronic studies are available," or similar.	"Where such information is already available from previous subacute and subchronic studies on the substance, interim kills may not be scientifically justified. " added to this paragraph (now 19)
20	Canada	The last sentence may be misinterpreted to mean that approximately 10% change in serum enzyme levels is considered evidence of toxicity and is an adequate demonstration of toxicity to justify the highest dose level. Consider revising to: "...the highest dose level should be chosen to elicit evidence of toxicity, as evidenced by, for example, depression of body weight gain (approximately 10%)." Recommend cross-referencing the example of toxicity e.g., the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (5).	Have deleted example of enzyme levels and made reference to the guidance
21	SW	In the first sentence it says that a top dose lower than the dose which provides evidence of toxicity may be chosen. This is followed by an example which may need a clarification. An "adverse effect of concern that nonetheless has little impact on	SW point is noted, but consultant does not think it is possible to give examples here as these could be difficult to agree (e.g. liver enzyme induction, hormonal changes?). will

		lifespan or body weight" which occurs below the dose which elicit evidence of toxicity – please clarify when a top dose other than the one that gives evidence of toxicity could be chosen.	be expanded in guidance
25	Canada	The last sentence requires revision - "....the additional pair-fed control group may be useful to allow for this." should read "....the additional pair-fed control group may be useful to serve as a more suitable control."	Changed
25	IRL	Please explain the use of the term "pair-fed control group". Also it will need to be clarified if a 20% reduction in dietary intake is correct	Expert group considered there was no need to explain in TG 20% removed
25	UK	The UK supports the need for a qualification of reduced dietary intake and 20% is an appropriate value. Please delete 'of the order of' as you suggest. The room for case-by-case judgement is covered in the latter part of the sentence 'may be useful'. Replace "palatability" with "unpalatability". **IP5** The unpalatability of the diet would be expected to be known prior to commencement of the study and therefore an additional control group assigned from the outset.	There was a view that the whole of the last sentence should be removed, but having looked at it again I think it should stay in, reworded to remove reference to the 20% . Changed to "reduced palatability" Noted
27	Canada	It is recommended that the following, "For dietary or environmental chemicals including pesticides, administration should be via the diet or drinking water." be modified to: "For..., administration is typically via the diet or drinking water. It is recommended that the following sentence be added after the above-noted sentence: "However, for some pesticide uses (e.g., fumigants or insect repellents) or scenarios (e.g., occupational exposure), administration via other routes may be more suitable."	Changed Added. Acceptable to others?
28	UK	The wording on testing for stability and homogeneity requires revision	Have changed this to ". Information should be available on the stability of the test substance and the homogeneity of dosing solutions or

		Homogeneity. The Guidelines TG 451,452 and 453 indicate that the homogeneity of the substance in the diet containing the test article should be confirmed analytically before the start of the study. The wording about testing periodically throughout the study is so vague that it is not very helpful. A UK expert in assessing data for the authorization of veterinary medicinal products for administration via animal feed advised that homogeneity is unlikely to change in the absence of the triggers proposed below but periodic checks are normally carried out in most testing laboratories. I would suggest that revision of the text on testing for homogeneity as follows: "If the treated feed is transported from the site of preparation to a different site for administration, the homogeneity should be tested at the site of administration Additional testing for homogeneity during the study are required if/when: The method of preparation is altered The source or type of the feed is changed Routine checks for homogeneity may also be carried out at the study director's discretion and the results of these analyses should be included in the study report." **Stability** All the TGs should indicate that the stability of the substance in the feed maintained at temperatures appropriate for the diet stored and during administration must be tested for the maximum time that may elapse between preparation and consumption.	diets (as appropriate) under the conditions of administration (e.g., diet). Note TG 408 just contains the sentence "The stability of the test substance under the conditions of administration should be determined." and the only reference to homogeneity is in the results.

		Some thought will be needed to draft a text to make clear the requirements for stability testing of solutions and suspensions used for gavage or following addition to the drinking water and for testing homogeneity of suspensions (solutions need to be tested for stability but not for homogeneity) but I think the consultant can do this.	
29	Canada	For clarity we recommend amending the last sentence, "... (mg/kg diet or ppm)... weekly basis (mg/kg body weight/day) may be used; the method should be specified."	Change made
30	Canada	Please modify the first sentence as follows: "In the case of oral or dermal administration, the animals are dosed with the test substance daily (seven days per week) for a minimum of 12 months, although a longer duration may be required depending on regulatory requirements." There is no retrospective analysis available to confirm the statement that 12 months is the "normal" duration in rodents. A retrospective analysis would be required to determine whether 12 months is a sufficient duration for cumulative effects to manifest. Consider recommending a duration of exposure for the dermal route e.g., 6 hours per day, similar to OECD guideline 410. The paragraph does not specifically indicate the duration of dosing for inhalation exposure. Consider revising the last sentence to the following: "Dosing by the inhalation route is carried out for 6 hours per day, 5 days per week, for a minimum of 12 months (chronic phase satellite groups) up to 18 or 24 months (carcinogenicity phase)."	Modified Added Duration added
30	ICAPO	Since specific guidance notes for mice have been inserted into this draft, it is recommended to consult TGs 412/413, as there was discussion during the revisions of those guidelines to recommend 4 hours per day for mice in repeated-dose inhalation studies. In	Text revised in accordance with TG 412 It was agreed at the start of the process that the TG would focus on the oral route and not get

		addition we would reiterate that a recommendation should be made for whole-body exposure rather than nose-only exposure for chronic inhalation exposure. Such recommendation could be made in the GD and reference to the GD made in this paragraph.	into detail on the other routes, but refer to guidance and other TGs on e.g. inhalation
33	Canada	Reference is made to IPCS Document No. 40 for observations of neurofunctional and neurobehavioural signs. Consider including examples of the recommended daily observations from the reference	These are covered in the following paragraph, cross reference added
33	UK	Suggest that the text after "retro-orbital sinus" to the end of the sentence be deleted as it does not anything useful.	Is this a reference to para 39? Have deleted in para 39
36	Canada	Consider rephrasing the testing schedule for neurotoxicity assessments e.g., ... may optionally be conducted before commencement of the study and at 3 month periods after study initiation up to and including 12 months, as well as at study termination (if longer than 12 months).	Changed
37	Canada	Consider rephrasing the testing schedule for immunotoxicity e.g., ... may optionally be conducted at study termination.	Changed
37	SW	When there are indications of immunotoxic effects further investigations of this effect could be conducted. It would be valuable with examples of appropriate tests to conduct with references (in analogy with para 36 on neurotoxicity).	Although I'd be happy to include this, I think at this stage it could be difficult to get general agreement from all countries and organisations as to what these tests should be. If Canada can come up with a proposal, circulate it to the expert WG and get agreement on it by the end of February, it could be considered for insertion at the WNT meeting
38	Canada	Consider rephrasing the last sentence as follows to ensure that it is clear that water consumption should be measured at the stated intervals for studies in which the test substance is administered in drinking water and that these measurements should also be considered when drinking activity is altered: "Water consumption should be measured at least weekly for the first 13 weeks and at least monthly thereafter when the substance is administered in drinking water. Water consumption measurements should also be	Canadian suggestion accepted

		considered for studies in which drinking activity is altered."	
39	Canada	Consider rephrasing the schedule for haematological examinations e.g., ... should be carried out....at 3, 6, and 12 months, as well as at study termination (if longer than 12 months), using the same animals throughout." It is unclear when samples for non-rodent animals should be collected, as the phrase "at the end of the designated test period" is ambiguous. Recommend rephrasing and rearranging the sentence as follows: "Samples should be collected at interim sampling times and at termination as described for rodents. Measurements at 3 months need not be conducted..." Please add a fasting recommendation for haematological examinations, as in paragraph 40, "Overnight fasting of the animals (with the exception of mice) prior to blood sampling is recommended."	Canadian suggestion accepted Canadian suggestion accepted Reluctant to add this at this stage, as it may cause further discussion
39	BIAC	Hematology: Suggest considering revising the statement: "In addition, Heinz bodies or other atypical erythrocyte morphology and methhemoglobin should be investigated if there is any indication of anemia or other hematopoietic diseases." Based on our experience, this statement appears to be too forceful. Not all hematopoietic diseases results in Heinz bodies, or atypical erythrocyte morphology and methhemoglobin formation. We believe there should be a flexible approach (similar to what is stated in the last 4 lines of para 40). Therefore, suggest re-wording along the lines of: "*Other hematology parameters such as Heinz bodies or other atypical erythrocyte morphology or methhemoglobin may be measured as appropriate depending on the toxicity of the substance. Overall, there is a need for a flexible approach depending on the observed and/or expected effect from a given substance*	Text revised. Briefly discussed in conference call and no objections
39	ICAPO	ICAPO does not recommend the blood collection method of retro-	Point noted and agreed. ICAPO information

		orbital bleeding (ROB). As stated in the US Public Health Service Guide for the Care and Use of Laboratory Animals, ROB has "the potential to cause pain or distress that cannot be reliably controlled." Researchers with the U.S. Department of Agriculture Agricultural Research Service (USDA ARS) have noted that while ROB "is a rapid and efficient bleeding method, it is extremely inhumane to the mouse. In fact, many countries have banned the procedure." Several papers authored by European governmental agencies, industry associations, and nongovernmental associations have reported serious potential adverse effects of ROB. An initiative between the European Federation of Pharmaceutical Industries Association (EFPIA) and the European Center for the Validation of Alternative Methods (ECVAM) resulted in a paper that identifies ROB as the only method of blood collection in mice and rats for which "the potential for tissue damage" is moderate to high.[1] Some of the potential adverse effects include "retrobulbar haemorrhage resulting in haemotoma and excessive pressure on the eye which is almost certainly painful for the animal," "corneal ulceration, keratitis, pannus formation, rupture of the globe and micro-ophthalmia," "damage to the optic nerve … which can lead to deficits in vision and even blindness," and "fracture of the fragile bones of the orbit and neural damage by the micro-pipette." The authors cite "histological changes, abnormal clinical signs and evidence of discomfort" as having "led to animals having to be killed on humane grounds and so lost from the study." The report concludes that "retrobulbar sampling with recovery should only be used when other routes are not practical." The paper emphasizes, "Bleeding from this plexus always should be carried out under general anaesthesia in all species and anaesthesia is a requirement in some national regulations."	will be added in guidance. Reference to anaesthesia introduced

[1] Diehl K-H, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal J-M, van de Vorstenbosch C. 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology, 21:15-23. Available at: http://www.ff.up.pt/farmacologia/pdf/good_practice_lab_animals.pdf.

		There much guidance available on blood collection methods, and perhaps this can be discussed in more detail in the planned guidance document. However, clearly, the test guideline should not recommend the ROB method without anaesthesia, and especially ROB should not be recommended for blood collection at intermediate intervals during the study. [2]	
41	Canada	Consider deleting the term "recent," since it is a relative term.	Deleted
42	Canada	Recommend amending the first sentence for clarity: "...baseline haematological and clinical biochemistry variables <u>are required for dog studies</u>, but need not be determined <u>in rodent studies</u> before treatment."	Changed
44	Canada	The sentence "Organ weights should be collected from all animals, other than those excluded by the latter part of paragraph 42." should read "....paragraph 43."	Noted
44	IRL	*Organ weights should be collected from all animals*, <u>*other than those excluded by the latter part of paragraph 42*</u>" There is no reference to animal exclusions in paragraph 42 , either in this or the previous version. Please clarify what the latter part of this sentence refers to.	Note from consultant: should have been para. 43
44	BIAC	A new requirement appears to have been added: " In the case of paired organs e.g. kidney, adrenal, both organs should be weighed separately." Unfortunately, there is no rationale provided for this new requirement. Collecting separate weight data of left and right organs would increase the chances of statistical false positive and negatives and presents difficulties in data interpretation. A test compound with potential systemic toxicity is expected to affect both paired organs simultaneously.	Requirement deleted

[2] Federal Labs Consortium (FLC). 2007. 2007 FLC Awards. Available at: http://www.federallabs.org/pdf/2007_FLC_Awards_Program.pdf.

		Furthermore, a recent Society of Toxicologic Pathology Position paper recommends " Paired organs routinely should be weighed together" Reference: Sellers, R.S, et al (2007) : Society of Toxcologic Pathology Position Paper: Organ weight recommendations for Toxicology studies: Toxicologic Pathology, 35: 751-755 Therefore, suggest removing the need for weighing paired organs separately	
45	Canada	Rectum and nose preservation should be required, as in the EPA guideline guidelines (870.4200, 870.4300). Consider clarifying why the tissues in square brackets are optional. Consider moving the sentence defining the use of square brackets to the beginning of the paragraph i.e., The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (27) (tissues in square brackets are optional)...	Have changed rectum from optional to obligatory and added [nose] as optional Sentence moved, but consider that a clarification of the optional tissues is beyond the TG, will be explained in guidance
45	Japan	Not only preservation of female mammary gland but also preservation of male mammary gland would be useful to detect any important changes at mammary glands. Therefore, we recommend that the "male" mammary gland should also be preserved	Added
45	SW	For better readability, please arrange all the tissues in columns	Done
47	Canada	Consider adding that summary data tables should also provide the means and standard deviations (for continuous test data) of animals showing toxic effects or lesions, in addition to the grading of lesions.	Added
48	Canada	Consider adding that historical data should also be presented by individual study as well as an average of the data available. All data summary tables should include group sizes (N values) and	New text agreed on this para as a result of the conference call

		means and standard deviations for continuous test data. Also add that the historical control, if evaluated, should be submitted from the same laboratory and animal supplier. Please delete "Historical control data should be used only if concurrent controls appear to be significantly different." It is not appropriate to restrict the use of historical data as described	
48	SW	It is important to note that if reference is made to historical control data they must originate from the same time point as data from the study conducted, in order to be relevant. For example if a study is terminated at 18 months and the historical control data originate from a termination at 24 months, such data are not relevant. Please, insert	New text agreed on this para as a result of the conference call
49	Canada	Under Test substance: · Recommend that a certified chemical analysis be included in the report. Under Test conditions: · For the test substance preparation, please recommend that analytical data be provided to confirm homogeneity, stability, and concentration. Under Results: · Consider providing more detailed guidance with regard to data supporting the Results texts. Supporting data should be in the form of raw individual animal data as well as summary tabular data. · For statistical treatment of results, the bullets are only a partial list of parameters that require statistical treatment. Consider deleting the subsequent bullets which are only a partial list of parameters that require statistical treatment, or adding the other parameters for which statistical treatment is appropriate e.g., clinical chemistry. Under "Discussion of results including": Add "NOAEL and/or LOAEL determination"	Changes made
52	Canada	The reference for pathology studies is more likely reference 28,	

		rather than 27. 27. EMEA (draft) document „Non-clinical guideline on drug-induced hepatotoxicity. (Doc. Ref. EMEA/CHMP/SWP/a50115/2006) 28. Crissman JW, Goodman DG, Hildebrandt PK et al. (2004). Best Practices Guideline: Toxicological Histopathology. Toxicologic Pathology 32, 126-131.	

TG 453

Paragraph	Country	Comment	Response
General	**Canada**	Comments relate to: · Clarifications and suggestions, and · Objections to the proposed alternative study designs with regard to dose groups. We support the objective of the combining the chronic toxicity and carcinogenicity studies to improve the efficiency in terms of time and cost, in comparison to conducting two separate studies. However, the alternative proposed study designs which may lessen the amount of work or resources in conducting a combined chronic toxicity and carcinogenicity study, the proposed alternatives could potentially complicate the assessment of toxicity potential and the determination of a NOAEL. For instance, the proposed alternatives with respect to fewer animal numbers and/or dose groups are considered to diminish the ability to detect toxicological effects and determine a dose-response curve. We therefore do not support nor recommend the proposed alternatives. Please ensure that the objective of determining the carcinogenic potential is achieved, by ensuring that the following criteria are required: 4 doses including a control dose, a dose that can be considered a NOAEL, and a dose to elicit evidence of toxicity. It should be emphasized that other objectives e.g., determination of a BMD, should be satisfied by additional doses groups meeting a minimum of 20 animals/sex/dose. Please qualify the avoid broad generalizations. The word "normally" should be clarified in the context of chronic or carcinogenicity study duration, or study objective (see below for	Views noted The design now agreed will meet these objectives, and will be further addressed in the guidance Other countries have generally supported the use of "normally", as providing an indication that alternative approaches can be used. Discussion of the validity of the mouse model will be included in the Guidance Document, as will details of essential information contained in some of these references and specific examples. It is beyond the scope of the TG to include the type of additional information

		comments to paragraphs 11, 17, and 31, or 11, or 22, respectively). There is no retrospective analysis available to confirm the statement that 12 months is the "normal" duration in rodents. A retrospective analysis would be required to determine whether 12 months is a sufficient duration for cumulative effects to manifest. In addition, utility of the mouse model for carcinogenicity testing was generalized as limited and it is recommended to qualify the validity of the mouse model (see comments below to paragraph 12). There are several references to other sources of information throughout the test guideline. While these references are definitely useful, providing a summary of the essential information in some of these references within the test guideline will make the guideline more of a stand-alone document and a more useful resource. Specific examples where additional information can be provided in the test guideline are indicated in some of the comments below.	sought by Canada
General	**Finland**	We fully support the option that the full dose-response should be evaluated for chronic toxicity (i.e.ALTERNATIVE (1) in the draft). If only a control group and the high dose group are included, no dose-response is received. However, the dose-response is crucial for evaluation of the results, understanding the toxicity and for further use of the data for risk assessment.	Noted, this approach will be adopted
General (and paras 16, 19 and others)	**Japan**	In response to the call for comments on OECD-TG453, we recommend "Option 1B" or "Option 1C" due to the following reasons: **** Consultant note: Option 1B is the design involving at least 3 dose groups + control in the chronic phase of the study, with 20 animals per sex per group in the top dose group and 10 animals per sex per group in other groups, while Option 1C is the design involving at least 3 dose groups + control , with 10 animals per sex per group** 1. One of the purposes of the TG 453, which is combined chronic toxicity and carcinogenicity tests, is to achieve maximum	Japan's views and information provided are noted

		efficacy of the study in terms of possibilities for reduction in numbers of animals. However, NOAEL/BMD estimation is another main purpose of the TG 453 as is in the TG 452. Therefore, we believe that in the chronic toxicity phase at least three dose groups would be essential for the NOAEL/BMD estimation with dose-response relationship which cannot be derived from Option 2. (Only in the case that hematological and serum biochemical exams which are not obligatory in the carcinogenicity phase of Draft TG (Option 2) are mandatory for at least 10 animals per group, the lack of data from middle and low dose groups might be partially complemented in the chronic toxicity phase.) 2. From the view point of animal protection, the draft TG (Option 1A) that requires "at least 20 animals per sex in all groups" which is the same as separate execution on the TG 452could not be accepted. Otherwise, draft TG 453 may be practically useless for almost all chemical manufacturers. Thus, "Option 1B" or "Option 1C" is recommended. 3. Mortality is rather one of the important toxicological signs. Even though high mortality in high dose group in Option 1C (*i.e.* 10 animals) may prevent statistical evaluation, it may not affect NOAEL estimation. (This logic could be also applied for the TG 452. Thus we also suppose the number of animals in the TG452 could be "at least 10" instead of "at least 20" per group). 4. It may be possible to detect most of degenerative and/or necrotic alterations due to testing compounds by single dose toxicity test through 90 days repeated dose toxicity tests. Although spontaneous and/or similar toxicity changes due to testing compound may appear in early phase of the test, for example, early appearance of focal myocarditis due to beta-blocker or high dose of androgenic hormonal treatment, or an early development of chronic nephrosclerosis after pesticide treatment, etc., however these morphological alterations generally	

		develop more than a year after the treatment. Simultaneously, longer period of treatment with testing compounds makes differences equivocal between spontaneous and treatment-derived pathological findings. 5. So called precancerous lesions in hepatic alterative lesions in the liver may be detectable, if applicable, when more than a year of treatment would be given, which may be useful information for carcinogenicity evaluation. 6. More than five cases of typically induced pathological alterations, at least, make pathological evaluation possible generally in the histopathological examination. 7. Except recent trend of relatively high incidence of spontaneous leukemias and/or spontaneous breast tumors observed in SD/IGS rats, the incidence of spontaneous neoplasms/diseases develops minimum within a year of observation in most of strains, thus, the protocol may provide more than seven cases for statistically robust evaluation.	
General	SW	Sweden welcomes the updated versions on the test guidelines for carcinogenicity and chronic toxicity. They are well structured and easy to follow.	No response needed, thank you
General	SW	<u>Design of the TG</u> We have doubts regarding the appropriateness of conducting a combined carcinogenicity/chronic toxicity study due to the complex design of such a study; separate studies may in individual cases be preferable. If the combined study still is an option it should be carefully considered whether there are advantages of such a study which outweigh the advantages with separate studies. Where a combined study is found to be the most appropriate, we believe provisions should be made for the generation of dose-response data for chronic toxicity which could be used for risk assessment. It means for example that by only using a high dose group and a control group the generated data will be a point	Sweden's views are noted

		estimate for frank chronic toxicity which would not be possible to use as a basis for risk assessment. Furthermore, if the generated data are not useful it means an unnecessary use and a waste of animals, which is unethical. Therefore, when chronic toxicity should be investigated provisions should be made for dose-response assessment.	
General	UK	The UK firmly supports the use of the combined study to maximise the information that can be obtained from these long term studies. We consider the advantages of running the toxicity test alongside the carcinogenicity test greatly enhance the interpretation of the latter without the need for additional animals for an interim kill. The combined study is not just two studies tacked together. Perhaps the use of the phraseology "phase" is misleading here. The toxicity endpoints may be important in deciding on the critical endpoint and dose in the risk assessment for carcinogenicity.We strongly support adoption of Option 1C** proposed by the consultant. This decision will affect the text. For detailed comments, see comments on TG 451 and TG 452 **** Consultant note: Option 1C is the design involving at least 3 dose groups + control in the chronic phase of the study, with 10 animals per sex per group**	UK views noted – can UK think of an alternative to phase
General	UK	The UK considers that it is important that the mode of action is included in the initial considerations. The optimal design may differ depending on whether the substance is a known or suspected genotoxin. This may also affect the choice between a carcinogenicity study or a combined toxicity/carcinogenicity study. We agree that detailed discussion belongs in the Guidance Document but the Test Guidelines 451 and 453 should draw attention to this issue.	Introduced in paragraph 7
General	US	**U.S. Position:** **Agree that Option 1, sub-option 1A is the most comprehensive design proposed -provided statistical validation. The**	The design now agreed will meet these objectives, and will be further addressed in the guidance

		advantages of this study design are greater than the disadvantages. **(Option 1: Chronic toxicity phase of TG453 should contain "at least 3 dose groups and a control" (at least 20 males and 20 females per group, as in the chronic toxicity TG452.)** There are 3 possible sub-options in this design: 1A: At least 20 animals per sex in all groups as per TG 452 1B: At least 20 animals per sex in the top dose group and at least 10 animals per group in all other groups 1C: At least 10 animals per sex in all groups These 3 options obviously differ in the reduction in animal numbers they offer. A reduction to 10 animals per sex per group may require statistical validation, but may be justified on the basis of further information being available from animals on the carcinogenicity phase of the study **Option 2: Chronic toxicity phase of TG453 should contain "2 satellite groups (a treated group and a control"** There are 3 possible sub-options in this design: 2A: At least 20 animals per sex in the treated group and 10 animals per sex in the control group, as in the original TG452.) 2B: At least 20 animals per sex in both the treated group and the control group. 2C: At least 10 animals per sex in both the treated group and the control group. These 3 options obviously differ in the reduction in animal numbers they offer. A reduction to 10 animals per group may require statistical validation, but may be justified on the basis of further information being available from animals on the carcinogenicity phase of the study.)	
General	US 1	I agree with Option 1, sub-option A, which is the most comprehensive design proposed -provided statistical validation. The advantages of this study design are greater than the disadvantages. Contrarily, Option 2 raises concerns about compromise of data quality reliability (e.g., dose reliability) in the study.	The design now agreed will meet these objectives, and will be further addressed in the guidance

General	US 2	Agree with Option 1, sub-option A.	The design now agreed will meet these objectives, and will be further addressed in the guidance
General	ICAPO	While we look forward to a forthcoming guidance document that will address recent advances in scientific thinking with regard to mode of action, intelligent testing, testing strategies, animal welfare, and related topics within chronic and carcinogenicity testing, ICAPO considers this version of TG 453 to be a step backwards. The elimination of a savings of animal numbers by conducting the combined protocol, an option in place since 1981, is discouraging and unadvisable. As we are reminded in some alternative text in paragraph 22, this combined guideline is intended to be focused on carcinogenicity, and screen for chronic toxicity, which explains the study design in the original protocol. If the intent is to eliminate this option, such a discussion is for the WNT and not the expert consultation group. ICAPO notes that several commenters offered the suggestion to assign 10 animals to each treatment and/or control group, for the chronic portion of the study. The current US EPA test guideline, OPPT TG 870.4300 Combined Chronic Toxicity/Carcinogenicity (http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-4300.pdf), recommends10 animals/sex/dose. ICAPO urges the WNT to consider options that would involve the same number of animals or fewer than currently prescribed.	ICAPO views noted. The comments received show a preference for the "at least 3 dose groups and control" design for chronic toxicity, with 10 males and 10 females per dose group
4	Canada	Consider inserting a reference to guideline TG 410 for the dermal route of exposure.	Done
5	US 3	We are looking for not only increased incidence, but also reduced time to appearance of tumors. Change the first bullet to say something like: "the identification of the carcinogenic properties of a chemical,	CPSC suggestion included

		resulting in an increased incidence of neoplasms INCREASED PROPORTION OF MALIGNANT NEOPLASMS, OR A REDUCTION IN THE TIME TO APPEARANCE OF NEOPLASMS, compared with concurrent control groups."	
6	Canada	Consider that the carcinogenicity "should" (rather than "may") be carried out after initial information on toxicity has been obtained from repeated dose 28-day and/or 90-day toxicity test 28/90 day study. This revised wording is consistent with OECD guideline 453.	
Initial considerations	UK	The Initial Considerations should include a paragraph on the statistical analysis of the results. It is important that this is considered before the study is initiated. However, most of the detail should be in the Guidance Document. We propose the text below is added as a separate paragraph: "The statistical methods most appropriate for the analysis of results, given the experimental design and objectives, should be established before commencing the study. Issues to consider include whether the statistics should include adjustment for survival, analysis of cumulative tumour risks relative to survival duration, analysis of the time to tumour and analysis in the event of premature termination of one or more groups. Guidance on the appropriate statistical analyses and key references to internationally accepted statistical methods are given in the Guidance Document." See	Introduced as paragraph 9
9	ICAPO	reference to reducing in animal use should be reinstated. As discussed above and below, a reduction in the number of animals used should be a consideration in choosing the combined study over separate studies.	Have introduced ", and some reduction in animal use,"
11	Canada	We do not support the use of "normally" in this paragraph and elsewhere in the document (paragraphs 17 and 31), as it is considered a broad generalization that may be inaccurate e.g., the duration of a chronic toxicity phase of the study is <u>not</u> normally of	Changes suggested have been incorporated, although "normally" has been retained (other countries have favoured use of this word in various places)

		1 year, and a carcinogenicity study in mice is not normally 2 years. A retrospective analysis is required to confirm the statement that 12 months is sufficient for cumulative effects to manifest. · The study duration is In the first sentence consider deleting the duration for both phases that are considered "normal" and referencing the appropriate paragraph i.e., "The study design consists of two parallel phases, a chronic phase and a carcinogenicity phase (for duration see paragraphs 31 and 32, respectively)." Recommend amending the following: For the chronic phase,... one dose level per group for a period of a minimum of 12 months, although a longer duration may be required depending on regulatory requirementsthe dosing regime.	
12	Canada	Recommend qualifying the use of the mice in carcinogenicity testing as follows: Although there are some questions regarding certain aspects of the mouse model for carcinogenicity testing e.g., some mouse liver tumours may have limited relevance to humans due to physiology differences (21, 22, 23), testing this species is considered valuable and necessary in some regulatory programmes.	There has been a lot of discussion about this point, and earlier text has been deleted, this should not be expanded in the TG but addressed in guidance
15	Canada	Consider moving the phrase "...separately for each sex" earlier in the sentence i.e., "At the commencement of the study, the weight variation for each sex should be minimal..."	Have added your suggestion "for each sex" earlier in the sentence but retained "separately for each sex\2
16	Canada	The proposed number of animals assigned for each group for terminal and/or interim sacrifices is very unclear for both paragraphs 16 and 17. Recommend inserting a table or a figure that would outline the proposed numbers per sex per group per sacrificial time point. We do not support either of the proposed alternatives. Both suggested alternatives would reduce the ability to determine the toxicological significance of effects observed, making it difficult to assess the dose response of treatment-related effects, and	I would prefer not to have to introduce a table at this late stage of the revisions of the TGs, but if the expert group think strongly that it would help I'll consider it The first option, rather than the options, is put forward

		consequently reduce the effectiveness of the study. · Our preference is to maintain the current wording, such that the minimum requirement for an acceptable chronic toxicity/carcinogenicity study is 50 animals of each sex for each of three dose groups and one control group for the carcinogenicity phase and 20 animals of each sex for each of three dose groups and one control group for the chronic phase. · We do not support alternative 1 as it is currently proposed, however, the following amended version may be considered: A sufficient number of animals must be tested such that for the chronic phase at study termination there is a minimum of 10 animals of each sex for each of the three 20 animals per sex per high dose group and 10 animals per sex for each of the other two dose groups and the control group. Additional animals are required for interim sacrifice, if required (minimum 10 animals per sex per group). · We do not support alternative 2 because no dose may be available to establish a NOAEL, which is required for our risk assessment purposes. Consider adding that additional mice may be required for haematological examinations due to their smaller blood volume.	The reference to additional mice is already included
16	IRL	Alternative 2 appears to place too many restrictions on the design of the study, some investigations may require the use of more than two dose levels in the chronic phase of the study.	Noted
16	BIAC	What is the need for 20 animals/sex/dose for the chronic phase of the study, particularly when only 10 animals/sex/dose are suggested (as one of the alternatives) for hematology (para 39), clinical chemistry (para 40) and urine parameters (para 41). 13-week sub chronic studies are typically conducted with 10 animals/sex/dose and has been well accepted. Is there sufficient scientific evidence to argue that 20 animals/sex/dose is necessary	Reduced to 10 in this TG

		in the high-dose group (or any other group) to detect treatment-related differences? It appears that this number (20 animals/dose/sex) has been carried over from the 1981 guidelines. We believe, from animal welfare, time and costs points of view, 20 is excessive.	
16	ICAPO	The current wording offers no reduction in the use of animals over doing the two studies separately (and presumably this extends to resources as well). The original TG 453 recommends a single high dose plus control, and if the original intent of the combined study is maintained (as described in the alternate version of paragraph 22), the updated TG 453 should maintain this recommendation. Regarding alternative 1, this option appears arbitrary without explaining the rational for choosing 20 animals/sex for only the high dose group. Alternative 2 using 20 animals per sex per group is the same as the original TG 453; using 10 animals per sex for the control would represent a reduction in animal use over the original TG 453 and would be our preference.	There seemed to be reasonable support in the conference call for the design now put forward
17	Canada	We do not support the use of "normally" in this paragraph and elsewhere in the document, as it is considered a broad generalization that may be inaccurate (see comments for paragraph 11). A retrospective analysis is required to support the statement that the duration of a chronic toxicity phase of the study is "normally" of 1 year duration. Highly recommend: · Deleting "normally of 12 months duration" that occurs twice in this paragraph. · Rephrasing from "Satellite groups... will <u>normally</u> be restricted to the highest dose level of the study plus control" to "Satellite groups... <u>may</u> be restricted to the highest dose level of the study plus control." We do not support the proposed alternative. Restricting the animals examined for chronic toxicity parameters to high and control doses will make it difficult to assess a dose response of	other countries have favoured use of this word in various places. Retained in the selected option Accepted Alternative deleted

		treatment-related effects and reduce the effectiveness of the study. As these values may be pivotal for hazard characterization and subsequent risk assessment, it is necessary to assess animals at other doses to determine a more accurate point of departure. While it is agreeable to have a minimum of 10 animals per sex for the interim kills, it is highly recommended that there be three dose groups and one control group. When assigning satellite groups to monitor the reversibility of toxicological changes, it is agreeable to examine only high and control doses, but it is recommended that the minimum number of animals required be specified (10 animals per sex per group). For sentinel animals it is agreeable to recommend 5 animals per sex .	
17	ICAPO	Recognizing the variations in opinion, as reflected in comments, on the issue of interim kills and/or satellite animals, ICAPO appreciates the requirement for scientific justification, and recommends that in addition, the following text is added: "Groups for interim kills should not be included if the results of subacute and subchronic studies are available," or similar.	"Where such information is already available from previous subacute and subchronic studies on the substance, interim kills may not be scientifically justified. " added to this paragraph (now 20)
19	IRL	In relation to the limit dose, the first alternative should be used: i.e. " …but may be appropriate in the chronic toxicity phase of this combined chronic toxicity/carcinogenicity study"	First alternative is used, new wording regarding limit test in chronic phase
19	Canada	We do not support the proposed alternative of using only a top dose and a control group for the chronic phase of the study is not acceptable. It is highly recommended that both the chronic and carcinogenicity phases should at least consist of three dose levels and a concurrent control. Minimizing the number of groups to high dose and control groups would limit the usefulness of the data (e.g., benchmark dose modelling should not be based on two values). Recommend defining "limit test" as a study using one dose level of 1000 mg/kg body weight as well as expanding on its conditions of use i.e., when is it appropriate or inappropriate. For instance, it is inappropriate for use if a chemical is expected to be carcinogenic at the limit test because a quantitative risk	At least three doses plus control is recommended This will be done in guidance, at this stage in the process I am reluctant to start drafting a new paragraph on limit tests

		assessment is not possible with a single treatment group. This may be more appropriate in its own paragraph; perhaps cross-referencing to the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6) would be appropriate. The alternative may be supported if the limit dose is further elaborated upon as described.	
19	ICAPO	The alternative language should be used for the first sentence, but not for the last.	At least three doses plus control is recommended, new wording regarding limit test in chronic phase
20	Canada	Recommend cross-referencing the example of what is considered a sign of toxicity or a toxic effect e.g., the OECD Guidance Document on the design and conduct of chronic toxicity and carcinogenicity studies (6).	I think this is covered under Observations, later
21	SW	In the first sentence it says that a top dose lower than the dose which provides evidence of toxicity may be chosen. This is followed by an example which may need a clarification. An "adverse effect of concern that nonetheless has little impact on lifespan or body weight" which occurs below the dose which elicit evidence of toxicity – please clarify when a top dose other than the one that gives evidence of toxicity could be chosen.	SW point is noted, but I don't think it is possible to give examples here as these could be difficult to agree (e.g. liver enzyme induction, hormonal changes?). will be expanded in guidance
22	US 3	I am not comfortable with the emphasis on identifying a NOAEL, because this is not necessarily relevant for cancer studies. Can this be re-worded in some way, such as: "…to establish a dose-response AND/OR NOAEL?"	Reflecting the discussion during the conference call, have changed to "Dose levels and dose level spacing may be selected to establish a dose:response and, depending on the mode of action of the test substance, a NOAEL or other intended outcome of the study, e.g. a BMD
22	Canada	Recommend rephrasing "the primary objective will be to obtain information for carcinogenicity risk assessment purposes, and information on chronic toxicity will normally be a subsidiary objective." This statement is contradictory to paragraph 5, in which the objectives appear equal. We do not agree that chronic	There have been no objections to this statement so far, and I am reluctant to change at this stage

		toxicity is ancillary to cancer risk assessment. We do not support the proposed alternative. The alternative concludes by saying that the study design only includes a top dose and a control group; 3 dose groups and a control group are what we consider minimally acceptable, as discussed in the comments for paragraph 19. We recommend that objectives other than carcinogenicity risk assessment should be reached by adding additional dose groups, ensuring that there are doses to establish a NOAEL and that elicit evidence of toxicity.	Alternative is deleted
22	IRL	Dose level spacing is appropriate for both phases of the study and not just for carcinogenicity	Alternative is deleted
22	ICAPO	The alternative language should be used	Alternative is deleted
23	Canada	Consider deleting the proposed alternative, for inclusion of an available fourth test group would assist in the interpretation of the dose-response curve in the chronic phase of the study, as well as the carcinogenicity phase of the study.	Alternative is deleted
23	IRL	Similar to paragraph 22, the additional group should not be limited to the carcinogenicity phase of the study.	Alternative is deleted
25	Canada	It is recommended that the last sentence "....the additional pair-fed control group may be useful to allow for this." be modified to: "....the additional pair-fed control group may be useful to serve as a more suitable control."	Changed
26	Canada	It is recommended that the statement: "For dietary or environmental chemicals including pesticides, administration should be via the diet or drinking water." be modified to: "For..., administration is typically via the diet or drinking water." It is recommended that the following sentence be added after the above-noted sentence: "However, for some pesticide uses (e.g., fumigants or insect repellents) or scenarios (e.g., occupational exposure), administration via other routes may be more suitable."	Changed Added.
28	Canada	For clarity we recommend amending the last sentence, "... (mg/kg diet or ppm)... weekly basis (mg/kg body weight/day) may be	Changed

		used; the method should be specified."	
29	Canada	Please modify the first sentence as follows: "In the case of oral or dermal administration, the animals are dosed with the test substance daily (seven days per week) for a minimum of 12 months (chronic phase satellite groups) up to 18 or 24 months (carcinogenicity phase), see also paragraphs 31 and 32." Consider recommending a duration of exposure for the dermal route e.g., 6 hours per day, similar to OECD guideline 410. The paragraph does not specifically indicate the duration of dosing for inhalation exposure. Consider revising the last sentence: "Dosing by the inhalation route is carried out for 6 hours per day, 5 days per week, for a minimum of 12 months (chronic phase satellite groups) up to 18 or 24 months (carcinogenicity phase)."	I think current wording is OK Added Duration added
29	ICAPO	Since specific guidance notes for mice have been inserted into this draft, it is recommended to consult TGs 412/413, as there was discussion during the revisions of those guidelines to recommend 4 hours per day for mice in repeated-dose inhalation studies. In addition we would reiterate that a recommendation should be made for whole-body exposure rather than nose-only exposure for chronic inhalation exposure. Such recommendation could be made in the GD and reference to the GD made in this paragraph.	Text revised in accordance with TG 412
31	Canada	We do not support the proposed alternative, for limiting the interim sacrifice to only high dose and control groups would be considered an unacceptable for the assessment of chronic toxicity. Please refer to comments in paragraph 16. We do not support the use of "normally" in this paragraph and elsewhere in the document. Please refer to comments for paragraph 11.	Alternative text deleted "normally" has been retained (other countries have favoured use of this word in various places)
31	IRL	The alternative text should be used here to reflect the use of satellite animals	Alternative text deleted

31	US 3	Can the duration be changed from "normally" to "at least" 12 months? Compare TG 452, paragraph 32.	normally" has been retained. Paragraph makes it clear that other durations are possible
32	SW	The anticipated lifetime of the strain which is used in the test should determine the duration of the study. It means that for certain strains a duration of 24 months and 18 months, for rats and mice respectively, may be too short. Please, indicate that for certain strains a duration of 30 months and 24 months, respectively, may be more appropriate. See the current version of TG 451. In the current TG 451 there are considerations for a study to be accepted as negative. It is removed in the draft revision; what is the justification for that	See new wording introduced "Shorter or longer study durations may be used, dependent on the lifespan of the strain of the animal species in the study, but should be justified. For specific strains of mice, e.g., AKR/J, C3H/J or C57BL/6J strains a duration of 18 months may be more appropriate." There was no agreement on this wording
32	ICAPO	Delete reference to hamsters	Not agreed by expert group, but replaced by "rodent"
32	US 3	The normal duration should be 24 months for mice	Wording now "The duration of the study will normally be 24 months for rodents" as agreed in conference call
33	Canada	Reference is made to IPCS Document No. 40 for observations of neurofunctional and neurobehavioural signs. Consider including examples of the recommended daily observations from the reference	These are covered in the following paragraph, cross reference added
36	Canada	Consider rephrasing the testing schedule for neurotoxicity assessments e.g., ... may optionally be conducted before commencement of the study and at 3 month periods after study initiation up to and including 12 months, as well as at study termination (if longer than 12 months).	Changed
37	SW	When there are indications of immunotoxic effects further investigations of this effect could be conducted. It would be valuable with examples of appropriate tests to conduct with	Although I'd be happy to include this, I think at this stage it could be difficult to get general agreement from all countries and organisations

		references (in analogy with para 36 on neurotoxicity).	as to what these tests should be. If Canada can come up with a proposal, circulate it to the expert WG and get agreement on it by the end of February, it could be considered for insertion at the WNT meeting
38	Canada	Consider rephrasing the last sentence as follows to ensure that it is clear that water consumption should be measured at the stated intervals for studies in which the test substance is administered in drinking water and that these measurements should also be considered when drinking activity is altered: "Water consumption should be measured at least weekly for the first 13 weeks and at least monthly thereafter when the substance is administered in drinking water. Water consumption measurements should also be considered for studies in which drinking activity is altered."	Canadian suggestion accepted
39	Canada	We do not support the proposed alternative. Refer to the comments for paragraph 16. In studies involving rodents, heamatological examinations should be conducted at 3, 6, and 12 months as well as at study termination (if longer than 12 months), using the same animals throughout. It is unclear when samples for non-rodent animals should be collected, as the phrase "at the end of the designated test period" is ambiguous. Recommend rephrasing and rearranging the sentence as follows: "Samples should be collected at interim sampling times and at termination as described for rodents. Measurements at 3 months need not be conducted..." Please add a fasting recommendation for haematological examinations, as in paragraph 40, "Overnight fasting of the animals (with the exception of mice) prior to blood sampling is recommended."	Alternative deleted Canadian suggestion accepted Canadian suggestion accepted Reluctant to add this at this stage, as it may cause further discussion
39, 40, 41	IRL	The alternative text emphasises that haematological, clinical chemistry and urin-analyses are only made on the animals from	Other countries did not agree with this at conference call

		the chronic phase of the study. The sampling should not be restricted and should include all the animals from the group.	
39	BIAC	Hematology: Suggest considering revising the statement: "In addition, Heinz bodies or other atypical erythrocyte morphology and methhemoglobin should be investigated if there is any indication of anemia or other hematopoietic diseases." Based on our experience, this statement appears to be too forceful. Not all hematopoietic diseases results in Heinz bodies, or atypical erythrocyte morphology and methhemoglobin formation. We believe there should be a flexible approach (similar to what is stated in the last 4 lines of para 40). Therefore, suggest re-wording along the lines of: "*Other hematology parameters such as Heinz bodies or other atypical erythrocyte morphology or methhemoglobin may be measured as appropriate depending on the toxicity of the substance. Overall, there is a need for a flexible approach depending on the observed and/or expected effect from a given substance*	Text revised. Briefly discussed in conference call and no objections
39	ICAPO	ICAPO does not recommend the blood collection method of retro-orbital bleeding (ROB). As stated in the US Public Health Service Guide for the Care and Use of Laboratory Animals, ROB has "the potential to cause pain or distress that cannot be reliably controlled." Researchers with the U.S. Department of Agriculture Agricultural Research Service (USDA ARS) have noted that while ROB "is a rapid and efficient bleeding method, it is extremely inhumane to the mouse. In fact, many countries have banned the procedure." See further detailed comments in our comments on this issue on TG 452	Pont noted and agreed. ICAPO information will be added in guidance. Reference to anaesthesia introduced
40	Canada	We do not support the proposed alternative. Refer to the comments for paragraph 16.	Alternative deleted
41	Canada	We do not support the proposed alternative. Refer to the comments for paragraph 16. Consider deleting the term "recent," since it is a relative term.	Alternative deleted Deleted

42	Canada	Recommend amending the first sentence for clarity: "...baseline haematological and clinical biochemistry variables are required for dog studies, but need not be determined in rodent studies before treatment."	Changed
44	BIAC	A new requirement appears to have been added: " In the case of paired organs e.g. kidney, adrenal, both organs should be weighed separately." Unfortunately, there is no rationale provided for this new requirement. Collecting separate weight data of left and right organs would increase the chances of statistical false positive and negatives and presents difficulties in data interpretation. A test compound with potential systemic toxicity is expected to affect both paired organs simultaneously. Furthermore, a recent Society of Toxicologic Pathology Position paper recommends " Paired organs routinely should be weighed together" Reference: Sellers, R.S, et al (2007) : Society of Toxcologic Pathology Position Paper: Organ weight recommendations for Toxicology studies: Toxicologic Pathology, 35: 751-755 Therefore, suggest removing the need for weighing paired organs separately	Requirement deleted
45	Canada	Rectum and nose preservation should be required, as in the EPA guideline guidelines (870.4200, 870.4300). Consider clarifying why the tissues in square brackets are optional. Consider moving the sentence defining the use of square brackets to the beginning of the paragraph i.e., The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination (36) (tissues in square brackets are	Have changed rectum from optional to obligatory and added [nose] as optional Sentence moved, but consider that a clarification of the optional tissues is beyond the TG, will be explained in guidance

		optional)...	
45 & 51	Japan	Not only preservation of female mammary gland but also preservation of male mammary gland would be useful to detect any important changes at mammary glands. Therefore, we recommend that the "male" mammary gland should also be preserved	Added
45 & 51	SW	For better readability, please arrange all the tissues in columns	Done
48	Canada	We do not support the proposed alternative. Refer to the comments for paragraph 16.	Alternative deleted
48	Canada	Please refer to comments for paragraph 38.	Canadian suggestion accepted
49	IRL	In relation to taking blood samples from animals at the termination of the carcinogenicity phase, these should be taken from animals in a fasting state to allow comparison with other samples taken during the study.	Noted, covered by footnote
49	ICAPO	retro-orbital bleeding is well-known to be distressing for the animals, and ICAPO recommends that at the very least the animals are anaesthetized prior to the procedure, or another blood collection method is recommended.	Pont noted and agreed. Will be added in guidance. Reference to anaesthesia introduced
50	Canada	Although the development of tumours may confound the usefulness of organ weight data, please consider requiring organ weights from interim sacrifice animals as well as from at least 10 animals per sex per group at terminal sacrifice, in agreement with the EPA guidelines. This would contribute to the knowledge of the toxicological effects, particularly if there are no carcinogenic effects.	Consultant considers that to introduce this requirement at this stage of the process would cause too much debate, and the possibility of collecting organ weight data is covered by the latter part of the para
51	Canada	Please refer to paragraph 45.	Have changed rectum from optional to obligatory and added [nose] as optional Sentence moved, but consider that a clarification of the optional tissues is beyond the TG, will be explained in guidance
52	Canada	The reference for pathology studies is more likely reference 36, rather than 31. 31. Moser, V.C., McDaniel, K.M., Phillips, P.M. (1991). Rat	Correct

		Strain and Stock Comparisons Using a Functional Observational Battery: Baseline Values and Effects of Amitraz. Toxicol. Appl. Pharmacol., 108, 267-283. 36. Crissman JW, Goodman DG, Hildebrandt PK et al. (2004). Best Practices Guideline: Toxicological Histopathology. Toxicologic Pathology 32, 126-131.	
53	Canada	Consider adding that summary data tables should also provide the means and standard deviations (for continuous test data) of animals showing toxic effects or lesions, in addition to the grading of lesions.	Added
54	Canada	Consider adding that historical data should also be presented by individual study as well as an average of the data available. All data summary tables should include group sizes (N values) and means and standard deviations for continuous test data. Also add that the historical control, if evaluated, should be submitted from the same laboratory and animal supplier. It is highly recommended to truncate the sentence pertaining to historical data, because the use of historical data should not be restricted as described. Please delete "Historical control data should be used only if concurrent controls appear to be significantly different" because the use of historical data should not be restricted as described	Done
54	SW	It is important to note that if reference is made to historical control data they must originate from the same time point as data from the study conducted, in order to be relevant. For example if a study is teminated at 18 months and the historical control data originate from a termination at 24 months, such data are not relevant. Please, insert	New text agreed on this para as a result of the conference call
56	Canada	Under Test substance: · Recommend that a certified chemical analysis be included in the report. Under Test conditions: · For the test substance preparation, please recommend that	Changes made

		analytical data should be provided to confirm homogeneity, stability, and concentration. Under Results: · Consider providing more detailed guidance with regard to data supporting the Results texts. Supporting data should be in the form of raw individual animal data as well as summary tabular data. · Consider removing "(chronic toxicity phase animals only)" following ophthalmoloscopy, haematology, and clinical biochemistry, and urinalysis measurements. The data should be required to be provided for all time points that they were measured, they should be summarized in tables, as well as be provided for individual animals. · For statistical treatment of results, the bullets are only a partial list of parameters that require statistical treatment. Consider deleting the subsequent bullets which are only a partial list of parameters that require statistical treatment, or adding the other parameters for which statistical treatment is appropriate e.g., clinical chemistry. Under "Discussion of results including": · Add "NOAEL and/or LOAEL determination"	

US 1: FDA
US 2: OPP
US 3: Consumer Product Safety Commission