EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

23 September 2010
EMA/402716/2008 Corr*
Committee for Human Medicinal Products (CHMP)

Overview of comments received on Guideline on repeated dose toxicity (CPMP/SWP/1042/99 Rev 1)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	Merck Sharp & Dome (MSD)
2	Committee of the European Society of Toxicologic Pathology (GC-ESTP)
3	Scientific and Regulatory Policy Committee of the Society of Toxicologic Pathology (STP)
4	EFPIA
5	Schering-Plough Drug Safety
6	International Council on Animal Protection in Pharmaceutical Programmes (ICAPPP)
7	Association of the European Self-Medication Industry (AESGP)

* Comments no. 6 and 7 have been added.

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1. General comments – overview

Stakeholder no.	General comment	Outcome
2	The GC-ESTP is worried that this guideline, although comparable in scope to similar guidelines already issued by OECD and FDA, is far less detailed. For example, of concern to the pathologists, the tissue list (which, by the way, is not properly sorted and contains factual errors) is shorter that can be expected by other regulatory bodies. Following this guideline could thus compromises the acceptance of the study by another regulatory body. As a consequence the usefulness of this guideline, in view of the guidelines currently in use, is very low.	It is by purpose that the EU Guidance documents contain not too much detail. A lot of details are just the responsibility of the investigator (see General Principles). The example of the tissue list is remarkable, as the list from the STP (see link by EFPIA) contains two more and two less tissues, and the sorting is not alphabetically as is the STP list.
4	This draft guideline under review aims at providing guidance on characterizing the toxicological profile of a test compound by the use of repeated dose toxicity studies in laboratory animals. The document constitutes a revision of the 2000 "Note for guidance" that takes into account, notably, changes in the EU legal basis and available ICH and CHMP guidance documents. Changes regarding immunotoxicity testing have been introduced, however, these do not appear to be in line with the ICH S8 guidance. This revised guideline should also adhere to generally acceptable practices, in particular with regard to the list of tissues to be examined histologically (http://www.toxpath.org/Position_Papers/Tissues.pdf) and to the list for organ weights that was issued by a working group from the Society of Toxicology Pathology (http://www.toxpath.org/Organweightbestpractices.pdf).	The guidance is meant to be an update and should be in line with the ICH Guidance documents. The text on immunotoxicity has been revised, as apparently it can be read in different ways, see below. For comments on the list of tissues, see above.
6	ICAPPP welcomes the inclusion of overarching animal welfare considerations in the new "Legal Basis" section at the beginning of the guideline. We also urge the CHMP to include specific reference to OECD Guidance Document 19 on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals	A reference to the OECD Guidance Document 19 is now included in Section 8.2 and in the reference list.

Stakeholder no.	General comment	Outcome
	Used in Safety Evaluations (http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/NT00002E46/$FILE/00087372.PDF) where appropriate in the guideline.	
	One of the key differences between the current draft and the 2000 CMPM Note of Guidance on Repeated Dose Toxicity (CPMP/SWP/1042/99 corr.) is the deletion of content regarding immunotoxicity testing – presumably because this now exists as ICH S8. It is unclear, however, whether this change is intended to encourage applicants to undertake such studies separately, or whether they may be combined – at least for initial screening purposes – which is standard practice in other sectors. ICAPPP strongly favours the latter scenario, and invites CHMP to include additional guidance on this point. We also note the addition of guidance regarding triggers for functional neurotoxicity testing, but are not aware of the existence of validated test methods or guidelines for such evaluations in the pharmaceutical sector. Further guidance and technical references would be welcome on this point as well.	It is not appropriate to change this in the guideline, as in the ICH S8 Guideline defines the immune endpoints in Standard Toxicity Studies and mentions additional Immune Function Studies, where appropriate if there is a cause for concern.
	ICAPPP also urges CHMP to re-examine the value of routine testing in a second species in light of the findings of several recent retrospective reviews in other sectors, which have concluded, for both chronic (general) toxicity and carcinogenicity, that separate studies in dogs and mice contribute little or no "value added" relative to a single chronic toxicity/carcinogenicity test in rats (Doe et al., Regulatory Toxicology and Pharmacology 2006; 36: 37-68; Box & Spielmann, Archives of Toxicology 2005; 79, 615-26; Baetcke et al., US EPA 2005: http://www.epa.gov/scipoly/sap/meetings/2005/may2/dogstudymay05.pdf; Gerbacht & Spielmann. Archives of Toxicology 1998; 72, 319-29). Similarly, regulators in the agrochemicals sector have taken steps	In the present document it is stated: The use of one species is acceptable when clearly justified. This is better usually acceptable in ICH Guideline S6 Preclinical testing of Biotechnology Derived proteins.

Stakeholder no.	General comment	Outcome
	to abandon requirements for redundant subchronic testing in both rodents and dogs in favour of a single subacute (28-day) study in rodents and a single subchronic (90-day) study in dogs. Similar efforts to eliminate redundancies in the pharmaceutical sector would be welcomed.	

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
Paragraph 2	4	Comments: It should be clearly stated in the body of the text, in the introduction or (preferably) in section 7.1 that the duration of repeat dose studies in animals should be consistent with ICH recommendations and reference made to ICH M3.	This has been incorporated, although the ICH M3 (R2) Guideline is referred to already.
Paragraph 4	6	Specific reference to animal reduction and refinement measures would be welcome here. Proposed change: Add: "The investigator should justify the selected study design***, including efforts to refine and reduce animal use.***"	This is already included under "legal basis" and a repetition is not needed.
Scope	4	Comments: The previous guideline suggested that this guideline should be read together with other guidelines; this is useful to incorporate. Proposed change: Include information re: CPMP/ICH/300/95, CPMP/ICH/384/95.	Both guidelines are already included in the list of references. In the section on Duration of Administration they are now referred to.
P 2, lines 1-2	4	Comments: This sentence indicates that repeat dose toxicity studies should be carried out under GLP conditions. There is no differentiation between range-finding repeat dose studies and definitive repeat dose studies. This sentence, if taken literally, would mean that range-finding repeat dose studies would need to be performed	Not accepted. The Guideline is meant to describe the studies that are pivotal. Range-finding studies are just supportive, and are the responsibility of the investigator. It is obvious that GLP is applicable to pivotal studies. No change.

		under GLPs. Proposed change: Modify sentence to read: *"**Definitive/pivotal** repeated dose toxicity studies should be carried out in conformity with the provisions relating to …"*	
Section 5.1	5	Comments: "The substance used in the repeated dose toxicity studies should present a similar pattern of impurities as the product intended for use in human (clinical trials and marketing), as much as possible." Since repeated dose toxicity studies are often used to qualify impurities as well as to support clinical studies and on occasion the API may be spiked with higher levels of individual impurities to cover those situations were levels might increase as manufacturing batch size increases. This scenario should be accommodated within this repeat dose toxicity guideline. Proposed change: Suggest the wording be changed to: "The substance used in the repeated dose toxicity studies should present at least a similar pattern or levels of impurities as the product intended for use in human (clinical trials and marketing), as much as possible."	Accepted.
5.2 Excipients	4	Comments: Clarification is needed regarding for which situations studies of the active substance together with excipient(s) would be needed.	Not accepted. During the SWP meetings in September/December we have had a long discussion, with the conclusion that the second part of this paragraph is out of the scope of the document. Other

			members indicated that this statement is an old recommendation, and there is no need to change. This recommendation is already included in the previous document. After again long discussion no agreement could be reached on an example, such as a change in bioavailability, pharmacokinetics or biodistribution. Therefore the wording is not changed.
5.2 Excipients	5	Comments: Does this imply that repeated dose toxicity studies with the excipient alone need to be done in parallel with the studies conducted with the active ingredient alone, or is there an opportunity to evaluate the safety of the excipient as well as the active ingredient by conducting studies where both the API and excipient are co-administered - perhaps with an excipient alone arm as a comparator? Proposed change: Please clarify	Not accepted. Please see above.
5.2 Excipients	5	Comments: This section also states "In certain cases, studies with the active substance together with the excipient(s) used in the final product may be needed." Does this mean that this type of study can be used to ascertain the safety of both the API and the excipients in lieu of conducting studies with the API alone and/or with the	Not accepted. Please see above.

		excipients alone? Proposed change : Please clarify	
6.1 Animal species, 1st paragraph	4	Comments: Main human metabolite(s) should be ensured- Proposed change: It is suggested to include definition for main human metabolite – to make sure that it is understood the same way by all.	Not accepted. There is the MIST document from PhRMA. The SWP opinion is that this guidance document should not go into that discussion.
6.1	6	Comments: The reliability and relevance to humans of studies in artificially induced disease models in other species is considered highly dubious. More specific guidance and technical references are required in order to substantiate this point.	The sentence referred to allows a very flexible approach. More specific guidance would reduce flexibility and is not recommended. The use of a disease model should always be justified.
Section 6.2	7	Comments: It is stated that "normally, equal numbers of male and female animals should be used." Does this also hold true for drug developed for typical male or female indications? Should the number of animals per group be increased if only one gender is included in a study?	The study design should be justified by the sponsor. In case of gender-specific indications this can be justified easily. No need to change the text.
6.2 Sexes	4	Comments: It would be helpful having guidance on when only one sex would be appropriate. Does this also hold true for drug developed for typical male or female indications?	Not accepted. For rodents gender differences might occur which are not relevant to human situations. On the other hand, due to a different physiology even male animals might give indicators of

		Should the number of animals per group be increased if only one sex is included in a study? Proposed change: Add the following sentence: "**However, if the medicinal product is indicated only for male or female patients, it is acceptable to reduce the testing to the corresponding sex in the animal studies."**	toxicity which are relevant for women. Gender differences are mainly important for hormonal products related to reproduction, and in such cases one-gender studies are accepted. Generalization to all products that might be developed for one gender, is not appropriate.
6.3, Size of treatment groups P 3	4	Comments: This section needs to be expanded upon, with consideration given to adequate numbers for statistical analysis. The minimum numbers of animals in treatment groups should be given for usual circumstances, and in the case of recovery studies, recommendations of the number of animals to be retained relative to the initial group size (e.g., 25% of the original group). In addition, discussion of circumstances under which it would be acceptable to combine sexes in a dose group for statistical analyses would be helpful Proposed change : Recommend add bullet as follows: **▪ Unless specific circumstances drive greater or fewer numbers, standard group sizes are 10-15/sex/group for rodents and 3-4/sex/group for nonrodents, with the larger groups appropriate for the chronic studies of 6 and 9 months for**	During the discussion it has been decided that the EU guideline will not specify these numbers, as we can refer to OECD guidelines, which are more specific in this respect (see also general comments). It is to be expected that by giving more statistical support to the numbers per group will raise. The main difficulty is that there is no scientific agreement on the sensitivity that is needed to detect a difference. The observations in toxicity studies should be used to generate a signal for monitoring in clinical studies.

		rodents and nonrodents, respectively. Recovery and interim groups are added to these numbers.	
6.3	6	Comments: More specific guidance is needed here – especially in relation to upper limits. Proposed change:	Revise: "The size of the treatment group should be ***justified statistically, but in general should not exceed 5-10 rodents or 4 non-rodents per sex per group.*** ~~sufficient to allow meaningful scientific interpretation of the data generated~~." The reader is referred to the comment on the line above.
6.4. Number of species	4	Comments: "*The use of one species is acceptable if it has been unequivocally demonstrated that other available species are irrelevant as models for human safety assessment*." The sentence may be too restrictive. There are also circumstances where repeat dose toxicity testing in one species is considered acceptable, e.g. in qualifying impurities (rat), consideration should be given to mentioning such cases. Proposed change : "*The use of one species is acceptable* **with a clear scientific rationale."**	The example is so specific that it is irrelevant as an argument. The proposed text is always true and does not provide more guidance. On the other hand, as also the next comment indicates there more examples. The text can be changed: *The use of one species is acceptable when clearly justified.* The microdose-example by MSD, indicates that it is not only scientific, but also regulatory.
6.4. Number of species	1	Comments: "The use of one species is acceptable if it has been unequivocally demonstrated that other available species are irrelevant as models for human safety assessment" The sentence might be too restrictive; for example, one	See above.

		species is required to support microdose clinical studies. Proposed change: Replace the sentence by: "The use of one species is acceptable with clear scientific rationale"	
Section 6.4	6	Comments: As previously stated, the value of routine testing in two species is often dubious. We therefore recommend that a single (most relevant) species paradigm be accepted as a new default, with the potential to trigger a second-species study only in limited cases (e.g., equivocal results).	The default position is defined in Directive 2001/83 Annex 1. The sponsor has the possibility to defend another approach (see above).
Section 6.1	5	Comments: "Exposure to the main human metabolite(s) should be ensured. If this can not be achieved in toxicity studies with the parent compound, specific studies with the metabolite(s) should be considered." A quantitative definition of "main human metabolite(s)" would be useful. Proposed change : Suggest harmonization with US FDA level of 10% of parent levels for a metabolite to be considered for additional safety testing.	See above on metabolites.
Section 6.3	5	Comments: If a compound is developed for one gender only, the total number of animals per group can be reduced. This should be elucidated in this section.	See above for one gender. As the guideline does not specify numbers, a remark on numbers is not needed.
6.5 Animal	4	Comments:	Accepted.

Husbandry		It is suggested to store the records / certificates in relation to animal husbandry with the raw data, rather than in the report.	
6.5	6	Comments: The reference to a "high standard" of animal husbandry, while welcome, should be elaborated upon with specific examples. Proposed change: Add: "Care should be taken to provide group housing, solid flooring, bedding, daily health monitoring, and environmental enrichment in addition to appropriate food and water."	The company is free to add environmental enrichment when appropriate. This can be stimulated also by revising the European legislation on animal experiments. There is no need to suggest this in this guideline only.
7.1 Duration of administration	1	Comments: "When toxicity studies of three months duration or longer are needed, it is recommended that a repeated dose toxicity study of two or four weeks duration is carried out in such a way that it can serve as a dose-finding study for the longer term investigation." In order to minimise the animal usage, some different strategies may be applied to choose the appropriate design of toxicity studies of three months duration or longer. Proposed change : Remove this sentence.	The sentence commented upon is added in the last version. The proposal of EFPIA is accepted with a different wording.
7.1 P 4, lines 1-4	1	Comments: The guideline should refer the reader to ICH M3, and the text should be consistent with the recommendations	See above.

		made there. "When toxicity studies of three months duration or longer are needed, it is recommended that a repeated dose toxicity study of two or four weeks duration is carried out in such a way that it can serve as a dose-finding study for the longer term investigation. "In order to minimize the animal usage, some different strategies may be applied to choose the appropriate design of toxicity studies of three months duration or longer – these studies could be GLP or non-GLP. The possibility for a recovery arm in the study should be mentioned with recommendations on how to set its duration. Proposed change : Add new sentence after first sentence: **"For detailed guidance, see Note for guidance on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals …"** Remove the sentence "when toxicity. investigation."	
Section 7.1	6	Comments: Guidelines CPMP/ICH/286/95 and CPMP/ICH/300/95 are relevant to the considerations in this paragraph and should be re-inserted. It is important that repeated dose studies conform to national requirements in all regions so that re-testing is not requested or carried out. Consideration should also be given to the increased use of *in vitro* (i.e., basal cytotoxicity) and other non-	The Guidelines are now included.

		animal techniques for dose-setting purposes. Proposed change: Revise: "The duration of repeated dose toxicity studies depends on the duration of the proposed therapeutic use in humans (see CPMP/ICH/286/95: *Note for guidance on Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals* and CPMP/ICH/300/95: *Duration of chronic toxicity testing in animals (Rodent and non-rodent toxicity testing)).* When toxicity studies of three months duration or longer are necessary, efforts should be made to determine appropriate dose levels (e.g., by read-across, *in vitro* methods, or if necessary, a limited subacute range-finding study *in vivo*)."	
Section 7.2	6	Comments; The guideline should discourage testing by more than one exposure route, or by routes other than the anticipated clinical route. Proposed change: Revise: "~~In general,~~ ***T***he medicinal product should be administered by the same route as that intended for humans. ***Testing by more than one route of administration, or by a route other than the anticipated clinical route, requires compelling justification.***"	The text of the guideline is sufficiently clear.
Section 7.4	7	Comments: Dose levels. It would be helpful to include a statement	Dose limits are defined in ICH M3 (rev.2). No need to repeat this here.

		on a limit dose, if possible, e. g. 2000 mg/kg for rodents and 1000 mg/kg for non-rodents (doses related to the active, not to the salt form). A dose of 2000 mg/kg is the generally accepted limit for acute toxicity studies with single dose administration only. Proposed change: Page 4, after final sentence: Generally, 2000 mg/kg for rodents and 1000 mg/kg for non-rodents (doses related to the active, not to the salt form) are regarded as limit dose.	
Section 7.4., 1st bullet point.	4	Comments: The last sentence is unspecific *"Ideally ….significant multiple…"* Must be more specific e.g. What is a significant multiple? The guideline does not address maximum feasible dose levels and other routes of administration e.g. inhalation where a basis for inhaled dosing may be a multiple of anticipated human dose. Nor is palatability in the case of diet admix studies discussed. With the respect to the maximum feasible dose and animal welfare, it would be helpful to specify a limit value for repeat dose toxicology studies by the oral route, if possible, e.g. 2000 mg/kg for rodents and 1000 mg/kg for non-rodents (doses related to the active, not to the salt form). A dose of 2000 mg/kg is the generally accepted limit for acute toxicity studies with single dose administration only. Proposed change :	The revised Guideline ICH M3 (R2) has specified the limit dose. The guideline will refer to this.

		After final sentence: *Generally, 2000 mg/kg for rodents and 1000 mg/kg for non-rodents (doses related to the active, not to the salt form) are regarded as limit dose.* Proposed change :	
Section 7.4	6	Comments: Consideration should be given to the inclusion of a limit test for substances considered unlikely to be highly toxic. Proposed change: Add: "If a test at one dose level (equivalent to 1000 mg/kg body weight/day) using the procedures described for this study, produces no observed adverse effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used."	Dose limits are defined in ICH M3 (rev.2). No need to repeat this here.
7.4 Dose levels	1	Comments: "a high dose, selected to enable identification of target organ toxicity or other non-specific toxicity, or until limited by volume of dose." This sentence is not clear. To help in the choice of high dose, an absolute limit may be necessary. Proposed change : "a high dose, selected to enable identification of target organ toxicity or other non-specific toxicity, or other non-specific toxicity, until saturation of absorption or	A reference is made to the revised ICH M3 (R2) Guideline.

		limited by maximal feasible dose.”	
Section 7.4 Dose levels, first bullet	5	Comments: Some guidance/elucidation could be given on the special cases where a positive control group should be considered. Proposed change :	See above on positive controls.
Section 7.4 Last line p4/9	5	Comments: More guidance should be given on what is considered a "significant multiple" of the anticipated clinical systemic exposure. The severity of effects observed in repeat dose nonclinical studies and the feasibility to monitor the effect in clinical studies plays an important role. Moreover the safety margin derived from repeat dose studies for the first human single dose study could be different than the safety margin reached in chronic nonclinical studies - which are executed at a stage of development where human safety data are also available.	See above.
Section 7.4 Last line p4/9	5	Comments: More guidance should be given on what is considered a "significant multiple" of the anticipated clinical systemic exposure. The severity of effects observed in repeat dose nonclinical studies and the feasibility to monitor the effect in clinical studies plays an important role. Moreover the safety margin derived from repeat dose studies for the first human single dose study could be different than the safety margin reached in chronic nonclinical studies - which are executed at a stage of development where human safety data are also	See above.

		available.	
Section 7.4	5	Comments: Discussion should be added as to the selection of a high dose that avoids overt or excessive toxicity when possible for animal welfare concerns. For example, a high-dose that produces no toxicity should be acceptable if a slightly higher dose was demonstrated to be overtly toxic in a separate study.	See above.
Section 7.4	5	Comments: The last sentence states: "Special care should be taken to eliminate contamination of the control group with the compound under study." Contamination of the control group with the compound under study could invalidate a study and reference for more guidance is made to CPMP/SWP/1094/04. Proposed change :	The guidance document is referred to in another section.
Section 8	6	Comments: It is imperative that a strategy for adhering to humane endpoints be prepared before a study commences, including procedures for daily monitoring of the welfare of animals and agreement on the clinical and/or other signs that should be used to determine the point at which termination from the study should be considered – preferably before morbidity occurs. Specific reference should be made to OECD Guidance Document 19 as well as a related ILAR report on this subject.	In section 8.2 a paragraph has been added. The prescribed number of clinical observations is up to sponsor, and therefore deleted.

		Proposed change: Insert prior to 8.1: "Pain and distress in animals should be prevented or alleviated by determining, prior to commencement, the earliest endpoint that is compatible with the scientific objectives of the study. At the onset of adverse reactions, a minimum of two or three clinical observations should be made daily. Criteria for making the decision to kill animals who are experiencing severe pain or distress, and guidance on the recognition of predictable death, are the subject of an OECD guidance document and ILAR report on humane endpoints." References: ILAR Journal (2000) 41(2). Humane Endpoints for Animals Used in Biomedical Research and Testing. OECD Guidance Document 19 on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluations (http://www.olis.oecd.org/olis/2000doc.nsf/LinkTo/NT00002E46/$FILE/00087372.PDF)	
8.4 Terminal monitoring	5	Comments: The guideline states that "Autopsy must be conducted on all animals". The STP believes that there are instances when a full, routine necroscopy may not be warranted. For example, when rodents die within 24 hrs after the first dose, especially when there are CNS signs, necroscopy is often uninformative. Also, rodents in toxicokinetic arms of a study (when not evaluated as a separate study) are often not necropsied, but discarded, as the toxicity groups of the study provide the relevant toxicity data for unscheduled death	Not changing the text. Nobody has raised thus far any question. Of course it is not needed to do autopsy at day one. Deviations from guidelines are acceptable if justifiable, and justified.

		animals. Proposed change : Autopsy must be conducted on all scheduled sacrifice toxicology group animals. Animals that die or are sacrificed early may not have an autopsy conducted when scientifically justified.	
8.4 Terminal monitoring	1	Comments: "Autopsy must be conducted on all animals." Exception to this rule should be possible, if a clear scientific rationale is provided. Proposed change : "As general rule, autopsies should be conducted on all animals. Under specific circumstances exceptions are possible, but a clear scientific rationale has to be provided."	See above.
8.4 Terminal monitoring	1	Comments: "In non-rodent species where small numbers of animals are used, histopathology on the organs and tissues listed (Appendix A) should be conducted in all animals at all dose levels. In rodents, histopathology should be performed on all organs and tissues in Appendix A from the high dose and the control groups." Evaluation of rodents and non-rodents should be similar. Proposed change : Replace the sentence by:	Why should rodents and non-rodents handled in the same way? There are many more rodents than non-rodents.

		"In rodents, histopathology should be performed on all organs and tissues listed (Annex A) from the high dose and the control groups. If drug-related changes are identified in the high dose group, then affected tissues from lower dose groups should be examined to clarify the exposure/response relationship. In non-rodents, histopathology can be performed on all organs and tissues listed (Annex A) from the high dose and the control groups, as described for rodents.. If drug-related changes are identified in the high dose group, then affected tissues from lower dose groups should be examined to clarify the exposure/response relationship. Alternatively, it is also acceptable for evaluation of all animals in all dose groups for non-rodent studies. Alternatively, it is also acceptable for evaluation of all animals in all dose groups for non-rodent studies."	
8.4 Terminal monitoring	1	Comments: "Examination of the lower dosed groups may be restricted to those organs and tissues showing gross pathological changes at autopsy. Furthermore, if histopathological changes are identified in the high dose group, lower dose groups should be examined to clarify the exposure/response relationship." Proposed change : Replace the sentence by: "Additional examination of the lower dose group may be restricted to those organs and tissues showing gross pathological changes at autopsy"	The second sentence has been rewritten to make clear that histopathology should be done anyway in case of gross lesions. Later on it might be decided to do additional tissues in the lower dose groups if histopathological lesions are seen only (without gross lesions).

		The modified sentence should be placed after the sentence dealing with potential drug-related changes in the low dose groups.	
8.4 Terminal monitoring	1	Comments: "In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected directly during treatment because of the receptor binding profile of the substance or other substance related pharmacodynamic effects (in addition to the structures listed in appendix A). If there are findings suggesting a specific neurotoxicity then further investigations should be conducted to identify and assess the damage and its functional consequences." Proposed change : Replace the sentence by: "In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected directly during treatment because of the receptor binding profile of the substance or other substance related pharmacodynamic effects (in addition to the structures listed in appendix A). If there are data suggesting a specific pattern of neurotoxicity in the brain, then representative regions including the target cell populations should be evaluated. If there are histomorphologic findings of specific neurotoxicity then further investigations should be conducted to identify and assess the damage and its functional	The comment of the company is accepted. By deleting the little words "the" it is indicated that not all target cells should be studied, but that an impression should be get about the impact on these cell population.

		consequences."	
8.4 Terminal monitoring	3	Comments: The guideline states that: "In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected…" The STP believes that this may be too broad, and that "representative target cells or regions" should be sampled. This thickness of the samples of rat and mouse brains for paraffin blocks can make it impractical to evaluate all potential sites there are many target cells or regions – even assuming that target cells can be identified with complete accuracy. Proposed change : "… should be extended to representative target cells or representative regions of the CNS…"	See above.
8.4 Terminal monitoring	1	Comments: "All tissues (see Appendix A) from all animals in the study should be conserved and wax blocks should be prepared. This material should Proposed change : Replace the sentence by: "All tissues (see Appendix A) from all animals study should be conserved"	Accepted.
8.2	4	Comments: The paragraph currently implies all parameters should be monitored on all studies. Is this necessary (e.g. are urine analysis or ECG's required on every study).	It is decided not to change this wording. It is always possible to do this only for the pivotal studies. Scientific justification is possible for all cases.

		There is a recommendation that all animals that die or are sacrificed during the study should be autopsied and if feasible should be subjected to microscopic examination. ▪ There are instances where this practice/procedure is not appropriate. For animals in the toxicologic assessment groups, animals are generally necropsied and tissues are examined microscopically. If animal death/morbundity occurs in satellite groups such as TK groups in rodent studies, other less thorough practices are generally implemented as animals may be examined only to establish cause of death. Strategy for examination of recovery animals should be included, namely, assessment limited to the reversibility of treatment-related changes as identified in the main study. Proposed change : It is suggested to amend the sentence with a view to allowing flexibility based on scientific rationale e.g. urine analysis, ECGs should be monitored on studies considered appropriate by the applicant during the toxicology programme. Clarification is necessary on when this recommendation (necropsy on animals during the study) is applicable.	
8.4 Paragraphs	4	Comments: ▪ A: "*Autopsy must be conducted on all animals*." Exception to this rule should be possible, if a	See above.

1&2		clear scientific rationale is provided. Proposed change : Replace the sentence "A" by: *"As a general rule, autopsies should be conducted on all animals. Under specific circumstances exceptions are possible, but a clear scientific rationale has to be provided.*	
8.4 Paragraph 4	4	Comments: Include after the sentence "Further histopathological examination..." the following text: "Bone marrow smear are prepared from all animals, but only examined if treatment-related changes are suspected in tissues/organs or in peripheral blood. The smears will be examined by visual assessment for cellularity, distribution and morphology and an assessment of the myeloid:erythroid ratio."	Accepted.
Section 8.4 Paragraph 5	4	Comments: The statement "In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected directly during treatment." should be modified. It is not always possible to identify precisely the specific CNS regions or target cells involved. In the case of very small regions, an extended histopath evaluation may be technically challenging. In addition, pharmacological activity might not be related to specific toxicity. Further investigation of specific neurotoxicity identified by terminal monitoring could follow identification of histomorphologic changes.	Accepted.

		Proposed change : It is suggested to modify the statement as follows: "*In the case of CNS active substances, routine histopathological examinations of the CNS are conducted (appendix). If there are data suggesting a specific pattern of neurotoxicity in the brain, then representative regions including the target cell populations not already covered by routine examination should be evaluated. If there are histopathological findings indicating specific neurotoxicity further investigations should be conducted to identify and assess the damage and its functional consequences."*	
8.4 Paragraph 6	4	Comments: There should be some overall reference to collection of organ weight data. Proposed change : A core list of tissues to be weighed can be included in this guidance document.	It is decided not to include such as differentiation, as this can be justified easily based on experience.
8.4 Paragraph 7	4	Comments: The recommendations to immunotoxicity assessments "If needed, bone marrow cellularity, lymphocyte subsets etc." are not sufficiently helpful or specific as a recommendation. It is better to indicate that if immunologic effects are anticipated with the compound or if there is evidence of immunologic activation or inhibition in repeat dose toxicity studies, immunotoxicity of the compound should be explored in consideration of the recommendations provided in the Guideline on Immunotoxicity of Human Pharmaceuticals	There is a misunderstanding around this sentence. This has been reformulated.

		(ICH S8). **The current draft guideline is not in line with ICH S8.** Proposed change : Immunotoxicity assessment is sufficiently covered in other guidelines and should not be repeated here.	
Sec 8.4 Paragraph 8	4	Comments: The guideline states that *"all tissues from all animals in the study should be conserved and wax blocks should be prepared".* In many cases only control and high dose groups are microscopically examined and thus it seems unnecessary to embed tissue from all animals. This should be optional, depending on the working procedures in the individual pathology labs. It is suggested to add e.g, *"from animals that should be examined histopathologically"* Storage of tissues in formalin should be an option. Proposed change : It is suggested to replace the sentence by: *'All tissues **(see Appendix A)** from all animals in the study should be conserved.'*	Accepted.
Section 8.3, 3rd sentence	5	Comments: Analysis of blood samples of the control group "should be considered". This is a very vague statement. It would be helpful to give more detailed information then this is.	Reference has been made to the contamination guidance.

Section 8.4	5	Comments: The second sentence of this section states: "In non-rodent species where small numbers of animals are used, histopathology on the organs and tissues listed (Appendix A) should be conducted in all animals at all dose levels." In contrast to this statement, it is commonly accepted practice to perform histopathological evaluation on the organs and tissues of the high-dose and control animals in non rodent as well as rodent studies and to read target organs in the mid- and low-dose groups down to the no effect level. To change this practice has significant implications. Proposed change : Strongly suggest that the agency reconsider this recommendation.	See above.
Section 8.4	5	Comments: Last sentence reads: "All tissues (see Appendix A) from all animals in the study should be conserved and wax blocks should be prepared" This is similar to the preceding comment. It is commonly accepted practice to do this for high-dose, controls and target organs. Suggest elaboration of the following statement "For specific guidance on the evaluation of the male genital tract, reference is made to the Note for Guidance on the Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5)." Proposed change :	Accepted.

		Suggested addition: The reproductive organs should be examined histologically. Histological assessment of the testis and epididymides is informative for the detection of effects on spermatogenesis. For specific guidance on the evaluation of the male genital tract, reference is made to the Note for Guidance on the Detection of Toxicity to Reproduction for Medicinal Products & Toxicity to Male Fertility (CPMP/ICH/386/95; ICH S5).	
Section 8.4	5	Comments: It is stated that "In the case of CNS active substances, systematic histopathological examinations should be extended to the target cells or the CNS regions that are affected directly during treatment because of the receptor binding profile of the substance or other substance related pharmacodynamic effects (in addition to the structures listed in appendix A)." It is typical practice to do routine survey brain sections rather than specific assessment of particular CNS regions/cells based on the receptor binding profile of the substance unless there is evidence of clinical signs of CNS toxicity or signs of pharmacological effects that might be associated with CNS morphological lesions. This makes sense in that many pharmacological CNS effects are mediated by mechanisms other than those that would be manifest by morphological alterations apparent on histologic assessment. Proposed change : Suggest that the section be revised to:	Accepted.

		In the event of clinical evidence of CNS activity or CNS toxicity, it is advisable to perform a more exhaustive histopathologic examination of CNS tissue across all major regions of the brain	
Appendix A	1	Comments: "Blood smears are part if a haematology evaluation and should not be required at the terminal monitoring. Some tissues on the list in Appendix A could be evaluated only when there are gross observations at necroscopy. The rationale has been detailed in an STP position paper (Bregman C. et al, Toxical. Pathol.31, pp 252-253, 2003). These tissues should be removed from the list in Appendix A. Proposed change : Remove from Appendix A; Tongue, ureters, oviducts, larynx, and blood smears. Revise: 1. joints to add (rodents only) 2. salivary glands (mandibular, parotid or sublingual) 3. femur bone with bone marrow (rodent only) 4. rib bone with bone marrow (non-rodent)	"blood smear has been removed". The list is nearly the same as the STP list. The list is not sorted alphabetically. The STP list has been included now, with al the notes that are needed.
Appendix A	3	Comments: The STP has published a recommended tissue list for toxicology studies (Bregman CL, et al. (2003) Recommended Tissue List for Histopathologic Examination in Repeat-Dose Toxicity and Carcinogenicity Studies: A Proposal of the Society of Toxicologic Pathology (STP), Toxicol. Pathol. 31(2):	See above.

		252-253). The STP believes that this list is appropriate for toxicology studies and provides the rationale for not routinely including some of these tissues. In addition, the immunotoxicology section has been removed from the document upon acceptance of ICH S8. However, the tissue list has not been harmonised with ICH S8.	
Appendix A	3	Comments: Peyer's Patches are included in the Appendix A as occurring in the large intestine, Payer's Patches occur in the small intestine. In addition, Peyer's Patches are listed as tissue to be evaluated in the ICH S8 guidance only for oral studies.	There is in addition included: where relevant. The location should be changed of course.
Appendix A	3	Comments: Blood smears are listed on the tissue list. Blood smears are part of the clinical pathology sampling and may not be at autopsy. In addition, blood smears are not routinely evaluated at many laboratories, but may be evaluated if there are abnormalities on the automated hemogram.	Accepted.
Appendix A	4	Comments: The list of tissues should be harmonized with previously established lists in internationally recognized guidelines in order to have homogeneous terminology (OECD for example or see Bregman CL, et al.; Society of Toxicologic Pathology (2003) Recommended tissue list for histopathologic examination in repeat-dose toxicity and carcinogenicity studies: a proposal of the Society of Toxicologic Pathology (STP). Toxicol Pathol 31: 252–253.).	Accepted.

Appendix A	4	Comments: A generic organ weight list should be included - Reference to STP paper could be given.	Accepted.