European Medicines Agency
Veterinary Medicines and Inspections

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OVERVIEW OF COMMENTS RECEIVED ON

REFLECTION PAPER ON ASSESSMENT OF BIOAVAILABILITY OF BOUND RESIDUES IN FOOD COMMODITIES OF ANIMAL ORIGIN IN THE CONTEXT OF COUNCIL REGULATION (EEC) NO 2377/90

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	IFAH-Europe	

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Table 2:Discussion of comments

GENERAL COMMENTS - OVERVIEW
IFAH-Europe welcomes this reflection paper that provides useful guidance on the assessment of bound residues. However it must be clear that assessment of these residues is not a regulatory requirement but an option that may be progressed at the discretion of the Sponsor. The benefit is to the Sponsor as bound residues may be discounted from those of toxicological concern thus improving compliance with the Acceptable Daily Intake (ADI) and possibly achieving a shorter withdrawal time. However, if the Sponsor does not wish to pursue these studies and is satisfied with the projected ADI, Maximum Residue Limits, and Withdrawal Period, it must be clear that there is no registration requirement to do so.
Outcome: Agreed. Submission of data demonstrating "bound and non-bioavailable residues" will be left at the discretion of applicants and will not become a new legal requirement.
SPECIFIC COMMENTS ON TEXT

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3. Definitions		
"Bioavailable Residues"	The term exocon should be defined e.g.: "portion derived from the parent" or "xenobiotic moiety".	The term exocon comes from terminology used in the IUPAC definition of bound residues[2]. "Exocon" refers to the part of the bound residue derived from a xenobiotic whereas the term "endocon" refers to the part derived from the natural molecule/macromolecule. The definition proposed by IFAH is acceptable.
Last paragraph	Apparently, this reflection paper does not allow the consideration of non-extractable residues as bound residue. This means the sponsor would need to provide information e.g. on covalent binding which is complicated and has rarely been done before. Evidence of covalent binding should not be regarded as pre-requisite for considerations of bound residues in the exposure assessment. Alternative approaches such as demonstration of "unextractability" using a set of standardized extraction methods (to ensure that methods used are	This distinction between "bound" and "non-extractable" residues is a key element of both the IUPAC and the Codex Alimentarius definition. It is clear that a direct mechanistic proof of covalent binding is usually not possible and what actually is measured in the experiment is lack of extractability of the residues. However, since the degree of extractability is highly depending on the extraction techniques used, the term

[1] Where applicable
[2] http://www.iupac.org/publications/pac/1998/pdf/7007x1423.pdf

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	sufficiently rigorous) should be allowed and included into the guideline. The last paragraph is somewhat confusing and appears contradictory to the definition of bound residue. To put it another way, while we understand the differentiation of the terms "bound residue" and "unextractable residue", it seems quite plausible that an unextractable residue could easily be non-bioavailable when tested in a Gallo-Torres model (or other model). Thus, it seems inappropriate to exclude these residues from possible discount in an exposure assessment as this guidance appears to do. "Experimental proof or at least strong evidence" may not be available but these residues may be completely non-bioavailable. The critical factor is whether the residues are bioavailable (or not) and not strictly how they are classified by an arbitrary definition.	"bound" is, according to both internationally recognized definitions, reserved to those non-extractable residues that cannot be removed from the matrix after all reasonable attempts have been made, i.e. by extensive/exhaustive extraction (using polar/nonpolar solvents, detergents, acid/base/enzyme hydrolysis etc, as described in the Reflection Paper). Demonstration of "non-extractability" under conditions that might not yield the most complete extraction is not considered suitable to identify "bound residues". There is general agreement that the validity of a "bound and non-bioavailable" claim should be supported by (at least) two complementary lines of evidence: One based on extensive/exhaustive physico-chemical information on the molecule (i.e. providing indirect mechanistic evidence of a strong and most likely 'covalent' binding) and the other on a biological assay (i.e. demonstrating lack of bioavailability of the bound fraction). Approaches that are not able to provide a reasonable "proof of binding" (e.g., non-exhaustive extraction) in combination with bioavailability data alone would not be considered robust enough to allow reduction of the residue of concern. Given the potential impact of "bound/non-bioavailable" residues on the outcome of a risk assessment, the SWP-V decided to adopt a conservative approach. Apart from that, use of exposure estimates based solely on the bioavailable fraction of the total residues of concern, as suggested by IFAH, would involve a significant change to the current approach: For practically all veterinary compounds, the ADI refers to a NOEL for exposure to a (total) oral dose rather than the systemically bioavailable dose.

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		Use of estimates of "bioavailable exposure" (instead of "total exposure") would therefore require parallel re-adjustment of the ADI, to represent a "bioavailable ADI".[3] The SWP-V agreed that this is a complex and interesting issue that probably deserves more discussion, but it was felt that development of guidance on how to integrate information on systemic bioavailablilty into the exposure assessment is obviously beyond the scope this reflection paper.
	Text change proposals: Please delete this paragraph: "~~*Where residues have not been fully defined as bound they should be termed as unextractable and the extraction procedures used should be specified. In the discussion on residue binding it is important to clearly differentiate between the term "bound", which requires experimental proof or at least strong evidence of covalent binding and the term "unextractable" which could simply mean that that the methods used to extract residues were not sufficiently rigorous (to solubilize, for instance, substances that are simply absorbed).*~~"	Deletion of the paragraph is not recommended for reasons set out above
	Text change proposals: N.B. Last paragraph, last word: it should be a**d**sorbed, since a**b**sorbed does not make sense in this context.	Agreed, it should read "a<u>d</u>sorbed"
"4. General principles and assumptions"		
Bullet points 2, 3, 4 and 5	This section seems to be sub-dividing the issue to a greater degree than necessary. We see little difference if the bound residues are a major portion or a minor portion of the residue profile. Since the ADI has been established, these residues are not considered more toxic than the parent drug and they have been tested by auto exposure during the battery of toxicological (or pharmacological) tests. If these residues are non-bioavailable, they may be discounted from the residues of concern, if not they remain included in the assessment. Bullet point 2, takes into account the situation where mutagenic/	It is agreed to delete bullet points 3 and 4. The most essential message and advice are already contained in bullet point 2 ("provide additional information where there is reason for a particular concern").

[3] this point was also recognized by IFAH's experts, see IFAH's comments ad Sect. 5 on p. 4

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	carcinogenic adducts may be formed, thus it is not necessary to carry over the issue to bullet points 3-5. Text change proposals: It is recommended that bullet points 3 and 4 be deleted as the essential aspect of this is captured by bullet point 5: "If a bound residue is not bioavailable, it can be discounted from the residues of concern."	
Bullet point 2	Text change proposals: Please amend this bullet point as follows: "*Full chemical.....extremely difficult to obtain, as many molecular species of bound residue are likely to be present and often in very small quantities, and will only be requested if there …*".	Amendment accepted
Bullet point 4	A guidance or definition should be given on what is regarded as "minor" in actual percent.	It is suggested to delete bullet point 4 (see above)
Bullet point 5 – Footnote 4	It remains unclear how regulators would apply the concept to anti-infective products. If a bound residue has been demonstrated to be non-bioavailable it is possible to discount the non-bioavailable portion from the residues of concern. However footnote 4 on page 4/6 states that this does not necessarily apply if the critical concern is related to non-systemic effects, i.e. local effects at sites of contact in the gastrointestinal tract and in such cases additional specific risk assessment may be required. As a reliable framework is required to be developed and register innovative antibiotic drugs, removing the footnote is strongly recommended. Text change proposals: Please delete footnote 4.	The reason behind the recommendation in "footnote 4" is as follows: A bound residue species that is not systemically bioavailable may nevertheless interact at the site of contact with the tissues of the gastrointestinal tract and cause gastrointestinal effects. Admittedly, this is probably a hypothetical scenario in most cases but it should, nevertheless, be taken into consideration, particularly for substances where the gastrointestinal tract is a known target for toxicological effects of a substance. As regards possible effects on gut flora, it is clearly stated in the reflection paper that microbiological hazard assessment is a separate issue, to be dealt with in separate guidance documents ("This paper does not include guidance on testing/ assessment of bioavailability of bound residues for bacteria of the human gut flora"). The deletion of footnote 4 is not supported.
Bullet point 6	It refers to endogenous incorporation. While we propose no change to this	Endogenous incorporation would mean the radiolabel can be detected in fat, carbohydrates or protein constituents of tissues

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	point, we would have interest in the future as to how the CVMP views evidence that "unambiguously" proves incorporation.	or in body water if tritiated compounds are used. If the term 'unambiguous' is thought to be redundant in this context, it can be deleted without loosing the meaning of the paragraph.
Last bullet point	This will be very difficult as bound residues can not be extracted by definition.	A bound residue is, by definition, a chemical complex that is unextractable by "methods which do not significantly change the chemical nature of these residues". This definition does not necessarily apply when a marker analyte is to be selected. A marker residue may also be the product of complete chemolytical breakdown of the bound residues or the exocon, respectively, and identification of suitable analyzable fragments. There are examples for monitoring methods (e.g nitrofurans) based on bound residue derived marker analytes[4]. Proposal for revised text: "*Apart from these ~~hazard~~ aspects, ~~structural identification~~ chemolytic fragmentation and identification of bound residues fragments may sometimes be required, if this information is needed to define a marker residue for residue control"*
"5. Calculations" and "6. Experimental Approaches"		
	Definitions should be the same in the whole document: "Information regarding free and bound residues ..." leaves extractable residues and the endogenously incorporated residue fraction unconsidered (see calculation on section 6). The document reflects on the bioavailability of bound residues. Free and extractable residues are ASSUMED to be bioavailable. The term "bound" assumes covalent binding and is dependent on "exhaustive solubilisation and extraction". The amount of bound residues may decline with increasingly rigorous methods. Thus, for a more robust bioavailability determination (and longer lasting validity of the bioavailability determination), the tests should	The reflection paper focuses on a very specific aspect which is "bioavailability of bound residues". It is not the mandate of this paper to provide recommendations on the assessment of "bioavailabilty" or "bioavailabilty factors" as possible components of the exposure assessment. The potential impact of "bioavailability" and use of refinement factors for "bioavailablilty" in the hazard characterization and exposure scenarios is certainly an interesting question that has not been systematically addressed so far in the guidance documents. The CVMP's present approach uses a (worst case) default

[4] E.g., http://www.teagasc.ie/research/reports/foodprocessing/4848/eopr-4848.pdf

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	preferably be done with TOTAL residues and not with bound residues only. This gives the real value of residue bioavailability with less assumptions and calculations. The fraction "bound" after "exhaustive solubilisation and extraction" would thus be subjected to no or only limited residue/metabolite identification and the % bioavailable of TOTAL residues determined experimentally would be used in MRL estimations. In this context it has to be mentioned that if the bioavailability of residues is used in estimating MRLs, the ADI should also be adjusted using the bioavailability of the active ingredient administered in the pivotal toxicity test to arrive at the "bioavailable ADI".	assumption of 100 % bioavailability of residues (except for residues that have been shown to be bound/non-bioavailable and, in case of antimicrobials, residues that are not bioavailable to the gut flora).
	Text change proposals: "*5. Calculations* *Information regarding ~~free and bound~~ bioavailable and non-bioavailable residues is considered in the assessment of potential consumer exposure and in conjunction with the estimation of MRLs. An estimate of the daily in take of residues of a drug that has a non-bioavailable ~~bound~~ residue component needs to take into account the bioavailability factor of the residues and (possibly) their toxicological potency." The bioavailability factor is the fraction of total residues found to be bioavailable."* *- Please also delete formulae*	Not all changes proposed by IFAH are acceptable, in particular the last sentence as it would change the focus and perspective of the whole paper. A compromise wording might be: *"Information regarding bioavailable and non-bioavailable bound residues is considered in the assessment of potential consumer exposure and in conjunction with the estimation of MRLs. An estimate of the daily intake of residues of a drug that has a bound/non-bioavailable residue component needs to take into account the bioavailability factor of these residues and (possibly) their toxicological potency." ~~The bioavailability factor is the fraction of total residues found to be bioavailable."~~* It is suggested to delete the first of the three formulae and the corresponding list of abbreviations. This equation has mainly been shown to illustrate calculations during internal discussions. It is not necessary to reproduce this level of detail in the final document. It is suggested, however, to keep equations 2 and 3. These two equations describe in simplified

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		form the two basic definitions underlying the approach. ~~Residue of concern = Σ TRti – (NBti + ERti) or Σ UB + (BR x fraction bioavailable)~~ Bound residue = total residue - (free residues + extractable residues + endogenously incorporated residue fraction) Bioavailable bound residue = (bound residue x bioavailablilty factor)
"6. Experimental Approaches"		
	"6. Experimental Approaches" *IFAH proposal for changes to the text:* *The objectives of the experiments are to~~: quantify the bound residues remaining after exhaustive extraction, to collect information on the nature of the associated complex and to develop correlations with bioavailability of bound residues and, where necessary, their possible biological significance.~~* *• solubilise and extract the residues with different methods complementary in their action in order;* *• quantify the non-extractable and extractable residues after those different methods;* *• try and identify the nature of the residues after those different methods;* *• try and identify the nature of binding of the residues;* *• try and show similarity between bound residues in the (major) target animal and the (major) toxicology animal (often the rat) if identification of the nature of the residues is not possible;* *• determine the bioavailable fraction of ~~total~~ bound residues.*	The text proposal is agreed except for a (small but important) change to bullet point 6 where the word "total" should be changed to "bound".

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	The testing strategy and study design are usually developed on a case by case basis. *A combination of chemical analytical data with biological in vitro and/or in vivo methods has been shown to be the most suitable way to provide the necessary information.* ~~*A variety of different techniques and methods might need to be used for quantitative determination, characterization and bioavailability testing of bound residues.*~~ *This investigation can normally only be* ~~*achieved by use of*~~ *conducted using radiolabelled substance.*"	
"6.1. Extraction and physico-chemical characterization"		
1st paragraph	"Solvent viscosity" as a method is unknown to us. This is probably included in the use of various solvents. "Solvent extraction" should also include detergent treatment, which can extract by breaking covalent bonds. A paragraph on enzymatic release is required. Text change proposals: "*<u>Enzymatic</u>* *<u>Release of free conjugates:</u>* *Residues covalently bound to small biomolecules, such as glucuronides and sulfates, can be released by treatment with appropriate enzymes, such as sulfatases, glucuronidases, esterases, peptidases, etc. Such conjugates are typically readily extractable from the tissue under mild conditions, as described above, and in general, an extraction process needs to be conducted before enzymatic treatment.*"	Agreed
2nd paragraph	"Exocon-endocon" as this term is not often used, it would be good to have a	A definition will be included in the paper (as discussed above)

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	definition. There should be clear differentiation between different types of enzyme treatment: Ones that break free conjugates such as sulfates or glucuronides, and others which break up binding to macromolecules, such as peptidases. Text change proposals: Please add a definition of "exocon-endocon". Please amend this paragraph as follows: "*Hydrolysis/Enzyme hydrolysis <u>to release bound residue</u>* *A complementary strategy to release bound residues may be via (strong) acid hydrolysis ~~or specific enzymatic hydrolysis by cleaving enzymatically degradable exocon-endocon links (e.g. by sulfatases, ß-glucuronidase, ß-glucosidase, esterases)~~ or by* *solubilisation of the entire matrix via breakdown of large macromolecules into their constituent parts for releasing protein bound residues (e.g. protease/peptidase treatment, <u>or by other means</u>).*"	Proposed amendments (strikethrough/underlined) are accepted
"6.2. Methods to determine the mechanisms of binding"		
Second line	Replace "analyzed" by "investigated" or "studied". Text change proposals: *"… metabolism studies in laboratory animals and target species are <u>investigated</u> ~~analyzed~~."*	Proposed amendments (underlined) accepted