Towards a recommendation for tissue analysis

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on behalf of the EBF TT24

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Outline

1. Background
   - Why is EBF discussion tissues?
   - Some critical questions driving quality
2. Survey results
3. Some scientific reflections
   - Taking a representative sample
   - From ‘tissue’ to ‘homogenate’
   - Isolate drug from the homogenate
   - How to document stability in tissues and homogenates
   - The analysis
4. Towards an EBF-recommendation

Background

Tissue analysis:

- Remains hot topic in many BA labs
- Continued scientific challenge to perform in correct/smart/efficient way
- Ambiguity on regulatory requirements resulting in risk of over-validation
- Needs clear Guidance

EBF community committed to share experience, engage with HA and provide recommendation on scientific/process

The TT Team

- Nathalie Mokrzycki (MSD), lead
- Eva Erbach (Bayer)
- Irene Lentheric (Harlan)
- Marc De Meulder (Janssen)
- Morna McIntosh (CRL)
- Pascal Delrat (Servier)
- Pawel Dzygiel (Roche)
- Philip Timmerman (Janssen), sponsor

Understand interplay between GLP and Method Validation or Regulated Bioanalysis*

- GLP
  - preclinical safety studies (OECD1-15, 21CFR58)
  - no requirements for specific for Bioanalysis, but relates to:
    1. Resources: organization, personnel, facilities and equipment.
    2. Rules: protocols and written procedures (SOPs).
    3. Characterization: test items and test systems.
    4. Documentation: raw data, final report and archives.
    5. Involvement of Quality assurance unit.
- Regulated bioanalysis:
  - Relates to FDA2001, EMA2011, ANVISA2012
  - When applied in a GLP study, needs to comply with GLP Guidelines on "1 to 5" in above bullet

** From EBF-presentation: "*Regulated Bioanalysis or GLP Bioanalysis?" *presented in APA, Boston, September 2008*

Some critical questions driving quality:
Why analyse tissue (homogenates)?

Document exposure of dosed drug (or metabolites):	Recommended level of Bioanalytical rigour
1. as unique endpoint of PK/safety/PD in topical dosing (e.g. skin, lung,..)	*See recommendation slide at end of presentation*
2. in relation to a priori identified safety assessment in a GLP study	
3. in tissue (homogenates) in a PK study, mechanistic/GLP tox./PD study *	
4. in relation to understanding relative tissue distribution of dosed drug (or metabolites)	

* via protocol: desire to know tissue levels was *a priori* defined or GLP study or via amendment: desire to know tissue levels was *a posteriori* defined

EBF-Survey

- 57 Questions (Qs), divided over following chapters
  - General Qs
  - Qs around quality and GLP status of analysis
  - Qs on origin and types of tissue and reason for analysis
  - Qs related to tissue sampling
  - General Qs related to Method Establishment (ME)
  - Method specific Qs related to ME
  - Qs related to protocol and reporting
  - Some questions allowing room for comments

- On the next slides and interspersed in the continuation of the slide deck…some results of the survey
- Visit our posters and learn full details of survey

Do we have experience as a group?

Does your lab perform tissue analysis?

Answer Options	Response Count
Yes, go to Q2	16
No or rarely but I will give my opinion/share previous experience	7
No and I cannot give my opinion	7
answered question	30

= 23

Does your lab analyze small ('S') or large ('L') molecules?

Answer Options	Response Count
'S' using LC-MS/MS	21
'L' using LBAs	5
'L' using LC-MS/MS	4

> 23, so some do both

How often does your lab perform tissue analysis?

Answer Options	Response Percent
Weekly	22%
Monthly	9%
Sporadically	61%
Other (please specify)	9%

Q: Which tissue is most frequently analyzed?

	Drug Discovery	Drug development
Liver	11	12
Brain	16	14
Lung	6	6
Kidney	3	2
Heart	4	4
Muscle	1	0
Tumor	5	2

How to read these numbers:
- each company could assign a score of 1-3 to the 3 most frequently analyzed tissues/most evident reason.
- The sum of this ranking order is displayed above

Q: From which species do you analyze tissues?

Answer Options	Drug Discovery phase	Drug Development phase
Mouse	16	12
Rat	17	17
Dog	6	12
Non human primates (NHP)	2	7
Human	0	3

Compliance aspects?

Q: We apply following regulations when performing tissue analysis

Answer Options	Response
Fully GLP	4
According to GLP principles i.e. same quality but non-GLP	3
Using the principles of qualified assays	9
A mixture of above 3 depending on stage of development	4
Other (please specify)	3

Q: Why perform analysis under GLP conditions?

Answer Options	Response Percent
The data are generated as part of a GLP study	73%
At customer request	60%
The data are used on pivotal decision other than safety	7%
Driven by the Drug Development stage	7%

Q: Does your company have experience with filings of studies incl. tissue to HA?

Answer Options	Response Percent
Yes	38%
No	62%

Q: Did you get questions from HA on the status of sample analysis and the MVAL for these tissue data?

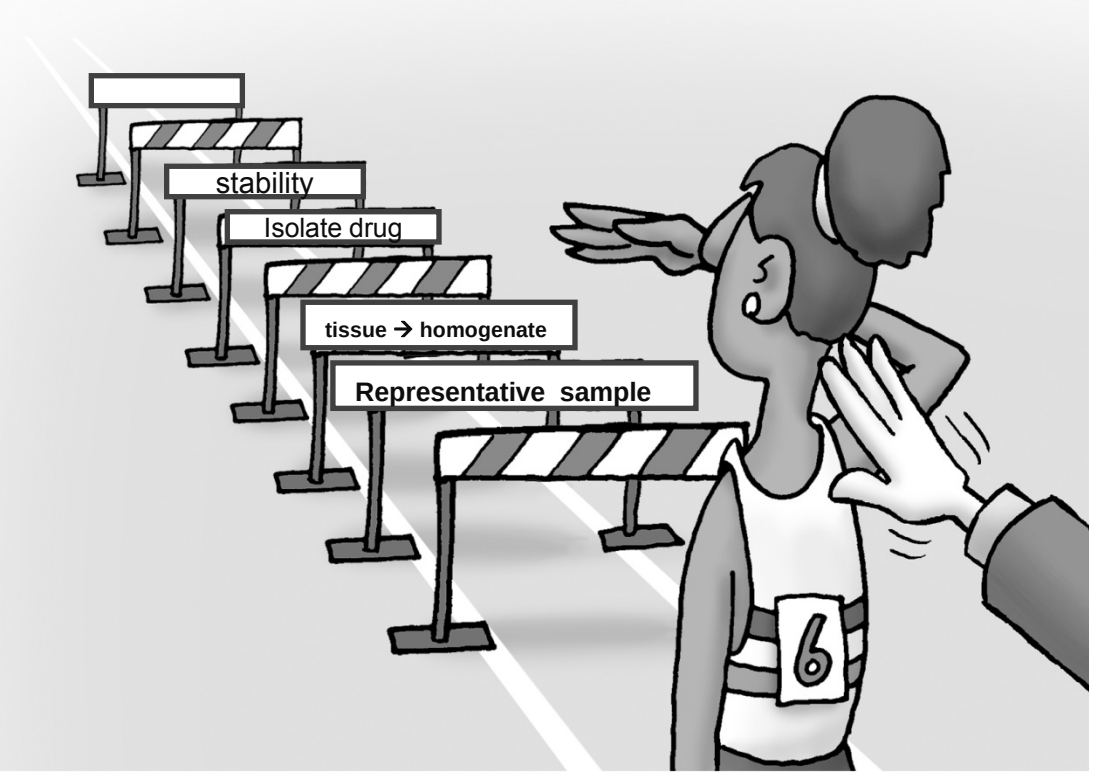
Answer Options	Response Percent
No	94%
Yes, because the authorities didn't agree with the non-GLP status of tissue analysis performed	0%
Yes, because the authorities didn't understand the details of tissue analysis performed	6%

A lot of more detail from the survey

- See our posters

TT still has a lot to discuss…

- But first…let's discuss and get your input to take some scientific hurdles



Scientific points of attention

- Taking a representative sample
- From ‘tissue’ to ‘homogenate’
- Isolate drug from the homogenate
- How to document stability in tissues and homogenates
- The analysis

Scientific points of attention:

Taking a representative sample

- Prevent contamination from other tissues or blood
  - Ensure accurate dissection of tissue of interest
  - Removal of contaminant tissue
    - Fat, gal bladder/bile, gut content, residual blood, small glands
    - Special care when sampling tissues after topical application (e.g. inhalation, application to skin,…)
- Ensure sample represents question in study
  - Procedure may depend on questions asked: document regional distribution *vs.* average tissue concentration
    - Regional distribution: sub-fraction of 1 organ
      - how to dissect tissue-section of interest?
    - Average: sense or non-sense of average organ concentration
      - Doable for smaller animals or smaller tissues, a real challenge for larger animals/tissues/organs
      - Reassess the rationale for average tissues concentrations?

Scientific points of attention: **From tissue to homogenate**

- Points of attention when generating an homogenate
  - Right choice of apparatus
    - Evaluate soft tissues *vs.* hard tissues

Some interesting data from the Survey

Q: What is your preferred technique for tissue homogenization (small animals)?

Answer Options	soft tissue (liver, lung,)	hard tissue (bone, eye, skin)
Mechanical (e.g. grinding, blending , Sonication,..)	20	17
Chemical (Saponification…)	0	1
Biochemical (Enzymatic treatment…)	0	1

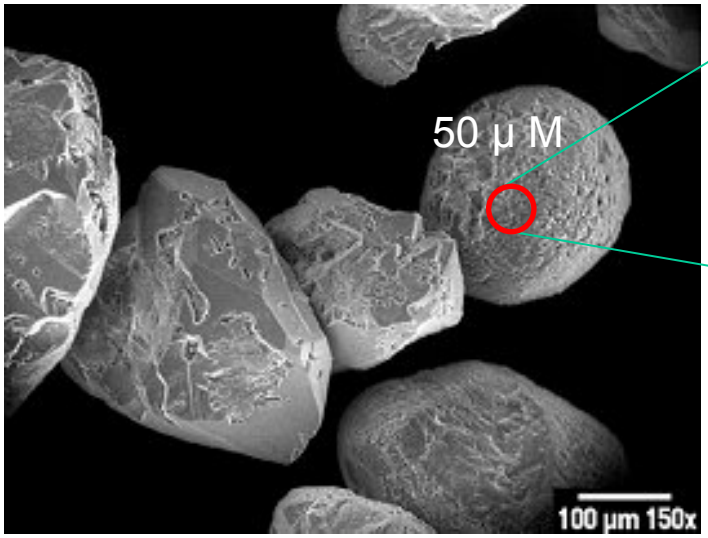
Q: What is your preferred technique for tissue homogenization (large animal / large tissues)?

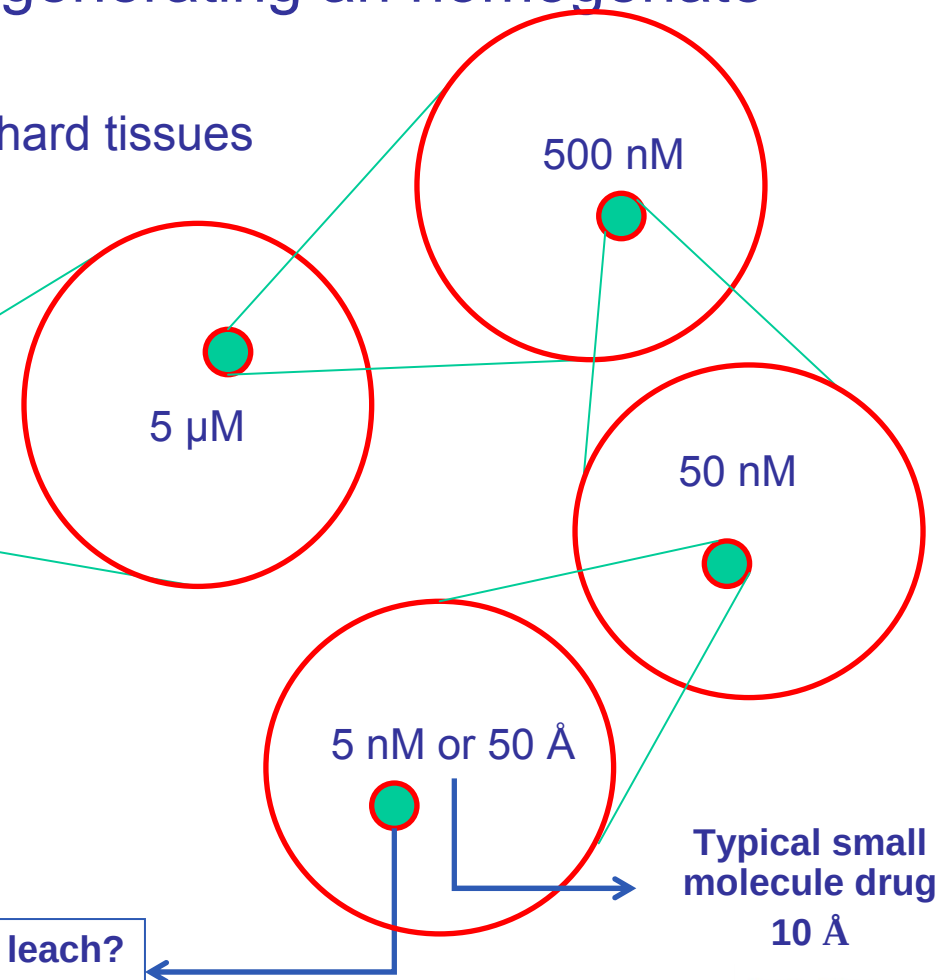
Answer Options	soft tissue (liver, lung,)	hard tissue (bone, eye, skin,)
Mechanical (e.g. grinding, blending, Sonication, ..)	19	17
Chemical (Saponification…)	0	1
Biochemical (Enzymatic treatment…)	0	1

Scientific points of attention: **From tissue to homogenate**

- Points of attention when generating an homogenate
  - Right choice of apparatus
    - Evaluate soft tissues *vs.* hard tissues

Grinded hard tissue…





Will molecule leach?
How is molecule incorporated in hard tissue?

Scientific points of attention: **From tissue to homogenate**

- Points of attention when generating an homogenate
  - Right choice of apparatus
    - Evaluate soft tissues *vs.* hard tissues
    - Ensure proper cleaning procedures of apparatus between different samples
  - Considerations to prevent cross contamination:
    - Consider potential high concentration differences between tissues when homogenizing different tissues in 1 study
    - Consider PK profile when homogenizing tissues for full PK
    - Special care when homogenizing tissues after topical application (e.g. inhalation, application to skin,…)
  - Considerations timing of homogenization:
    - Immediately after sampling (→ freezing of tissue homogenate?)
    - Immediately prior to analysis (→ freezing of tissue?)
    - May also have stability implications

Q: Do you prefer to have the tissue homogenized immediately after harvesting?

Answer Options	Response Percent
Yes, for convenience	14%
Yes, we feel this is a scientific requirement	9%
Yes, but we have difficulties in managing the logistics of this preferred process	14%
Yes, but only for soft tissue. Less a requirement for hard tissues	0%
No, for convenience, we prefer to freeze the tissue prior to homogenization	50%
No, for scientific reasons, we prefer to freeze the tissue prior to homogenization	9%
Other (please specify)	5%

Some recommendations on homogenization process

- Reflect on convenience *vs.* science
- Ensure tissue is not contaminated with residual blood/other tissue debris prior to homogenization
  - Will require certain level of experience/knowledge to identify issues
  - 'GDP': 'Good Dissecting Practices'
- Ensure use of appropriate apparatus to generate a tissue homogenate from which the compounds of interest can be isolated/extracted
  - Can be different for hard/soft tissues
- Ensure appropriate cleaning of apparatus in between tissue
  - Evaluate cleaning solvents to prevent carry over/cross contamination.
  - Is Water/buffer the right choice? Organic solvents needed?

Scientific points of attention:

Isolate drug from the homogenate

- Use appropriate bioanalytical science to isolate drug from tissue homogenate
  - Reflect on physicochemical process of extraction or getting the drug into solution
    - Understand logP/LogD *vs.* pKa *vs.* solvent strength interplay in choice of solvents
  - Consider different challenges for tissue homogenates and plasma
    - E.g. ppt with acetonitrile may be appropriate for plasma but inappropriate for tissue homogenate
  - What about inter- *vs.* intra-cellular concentrations
    - relates to choice and timing of homogenization

Scientific points of attention:

Stability considerations in tissue (homogenates)

Stability:

- Understand difference between stability in tissue - (currently difficult (if not impossible) to generate - and tissue homogenates
- During sampling
  - Immediate snap freezing *vs.* immediate homogenization
    - Choice may depend on compound class or tissue type
    - Immediate homogenization may introduce instability caused by release of enzymes
      - » Ensure Cal/QC match sampling and homogenization process of incurred samples
  - Understand stability implications of 'time of homogenization'
  - Understand analogies and differences with established plasma/blood assay
    - Most assessments will be equal to plasma
    - Most assessment for plasma not needed for tissues, due to the specific rationale for tissue analysis (e.g. LTS, F/T cycles,…)

Scientific points of attention: **the analysis**

- Straightforward for validated assays
  - use in house validation SOPs as template/source of inspiration
- designing a analytical run should be flexible for all other assays types:
  - Important parameters when designing a analytical run:
    - Ethical considerations: blank matrix = sacrificing compound naive animals
    - Matrix effects → choice of matrix of cal/QCs, matrix matching
    - Scarce matrix → surrogate matrix,…
    - differences in concentrations between different tissues or tissue/plasma → choice of dilution (begging for choice of matrix to dilute in)
  - Above reflections define your injection sequence run and acceptance strategy
  - Survey results and some examples on next slides
    - See poster or for review when we publish slides

And finally…To validate or not?

From the Survey

Q: what type of MVAL or qualification do you apply?

Answer Options	Drug Discovery	Drug Dev. nonGLP studies	Drug dev. GLP studies
screening methods..relative ratios	6	0	0
qualified methods - widened acceptance criteria	12	11	4
qualified methods - acceptance criteria cfr BMV	3	9	3
validated methods - widened acceptance criteria	0	2	8
validated methods - acceptance criteria cfr BMV	1	2	8

Note: Qualified and validated methods generate absolute concentration values
N=22 companies responding

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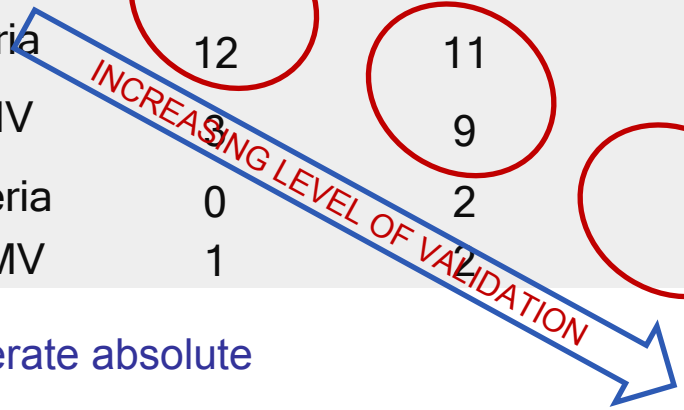

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But…

- Does '*validate or not?*' distracts us from the real bioanalytical challenges/questions?
  - Is it really possible to analyze a tissue?
    - **We do everything on tissue homogenates…so not on tissues!!**
  - Timing of homogenizing tissues is potentially a critical factor
  - How does applying plasma validation methodology to tissue homogenates distract us from the scientific challenges
  - Are we giving a smart answer to the questions related to tissue analysis
- Proposal to rethink the need and process for tissue analysis – from "getting in the question' to 'providing the answer'

A first step towards a recommendation on next slide

Proposal for Recommendation on validation:
starting from the critical questions driving quality

Document exposure of dosed drug (or metabolites):	Recommended level of Bioanalytical rigour
1. as unique endpoint of PK/safety/PD in topical dosing (e.g. skin, lung,..)	**Consider validated assay** for tissue homogenates. Consider a priori widening of the acc. and precision acceptance criteria (e.g. from 4-6-15 to 4-6-20
2. in relation to *a priori* identified safety assessment in a GLP study	
3. in tissue (homogenates) in a PK study, mechanistic/GLP tox./PD study *	**Use a qualified assay** Use widened acceptance criteria
4. in relation to understanding relative tissue distribution of dosed drug (or metabolites)	**Using alternative simplified bioanalytical processes** (Evaluate need of absolute conc. above relative ratios). Use widened acceptance criteria.

* via protocol: desire to know tissue levels was *a priori* defined or GLP study or via amendment: desire to know tissue levels was *a posteriori* defined

Future plans

- Get further input from:
  - All of you today
  - Continued discussions in the Topic Team and EBF members prior to publication
  - Publish the survey results as a separate paper
  - Publish a recommendation on EBF's view on challenges and bioanalytical qualification or validation required for tissue homogenate analysis in the same issue of Bioanalysis *(including GLP vs. nonGLP question)*

Acknowledgement

- The team
- EBF for proving the data for the survey
- All of you !!