National Centre for the Replacement Refinement & Reduction of Animals in Research

Dose setting and considerations for the 3Rs

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Workshop on Kinetically Derived Maximum Dose Concept to Refine Risk Assessment

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Pioneering Better Science

Dose setting and 3Rs consideration team

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Problem formulation statement

Lack of clear agreement on how to evaluate available data and approaches to determine top dose for repeated dose animal studies. The goal being to design dose-response studies that are **relevant to human exposures** and supportive of 3Rs principles.

Guidance states toxicokinetics should be ‘considered’ but limited information as to how this should be done.

NC 3Rs

The 3Rs

Reduction

Replacement

Refinement

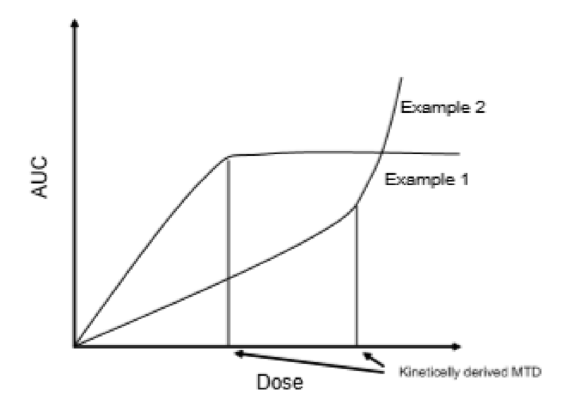
Why do things differently?

- Recognition that animals can be **poor predictors of humans**
- Potential to **reduce uncertainty** and **increase relevance** of safety assessments
- Development of **robust strategies** that exploit **all knowledge** currently available
- Address **societal concerns** related to the use of animals in toxicity testing
- **Meet legislative requirements** around the marketing of chemical products and **work towards global harmonization**
- Reduce **time and cost** associated with chemical safety assessment **without compromising human safety**

The 3Rs

	Standard	Contemporary
Reduction	Methods which minimise the number of animals used per experiment	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base
Refinement	Methods which minimise animal suffering and improve welfare	Advancing animal welfare by exploiting the latest *in vivo* technologies and by improving understanding of the impact of welfare on scientific outcomes
Replacement	Methods which avoid or replace the use of animals	Accelerating the development and use of models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals

Dose selection and the 3Rs



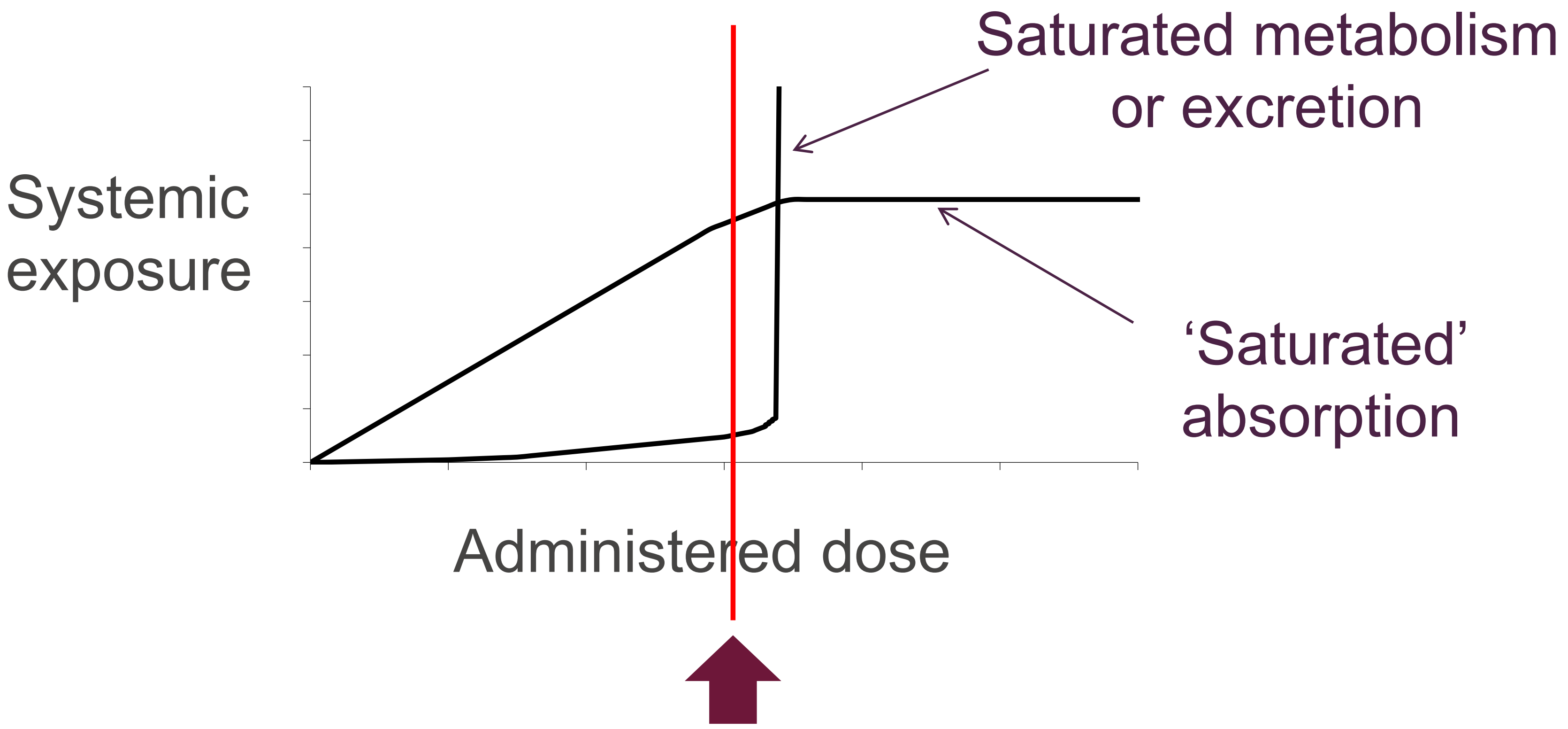
- 3Rs impact of inappropriate dose selection

- Top dose ‘too high’
  - Unnecessary animal suffering
  - Study may need to be terminated – or lose top dose group
  - Unreliable results – e.g. due to ‘biological stress’ / metabolic shift
  - Data not relevant – non-specific *vs.* chemical specific toxicity - may require further *in vivo* studies to explore MoAs occurring at doses far above realistic human exposures

- Top dose ‘too low’
  - Repeat studies may be required to demonstrate toxicity – additional animals used

- Critical to get the balance right and ensure the most scientifically appropriate doses are selected to add the most value

Dose Selection



- Doses above this generally exceed realistic dose scenarios
- Hazard finding related to biological stress occurring at high doses is not relevant to human exposures at much lower levels

Advice on use of TK for dose selection

- Advice on dose selection in OECD* Test Guidelines (TG 407, 408, 409, 451 and 453)
- 'Should' take into account any existing toxicity and TK data available

Additional information on specific OECD guidelines:

Study	Dose selection
OECD TG 409: 90 day non-rodents	▪ Non-rodent **should only be used where TK studies indicate** use of specific non-rodent species most relevant
OECD TG 451: 18 month carc	▪ Should be based on the results of shorter-term repeat dose or range finder ▪ **Should consider TK and dose ranges where metabolic induction, saturation, or non-linearity between external and internal doses occur.** ▪ **Should** consider known or suspected non-linearities or inflection points in dose–response.
OECD TG 453: 2 year chronic / carc	

*Organisation for Economic Co-operation and Development

Regulatory guidelines: TK and dose setting

Regulation	Summary of requirements/ recommendations
OECD GD116 (2012)	Design and Conduct of Chronic Toxicity and Carcinogenicity Studies (TG 451, 452, 453) suggests that TK should be considered in setting top dose (**linear vs. non-linear kinetics**).
OECD GD151 EOGRTS	If info on TK processes is known, dose selection can be based on that info (e.g. **highest dose does not exceed absorption or the setting of doses within and beyond linear metabolism**).
REACH Chapter R.7c	Use TK to support dose setting decisions for repeated dose studies. TK data, especially info on ADME are highly useful. Dose level corresponding to the inflexion point can be regarded as the kinetically derived maximum dose. **The highest dose-level should not exceed into the range of non-linear kinetics.**
EC/1107/2009	TK required in short and long-term studies. Dose level selection should take into account TK data such as saturation of absorption.
US EPA OPP HEDGD #G2003.2	Recommends 'use of innovative approaches'. **Highest dose tested should not be above a dose that results in saturation of absorption**.
US EPA EPA/630/P-03/001F:	TK should be considered to set top dose. High dose should not compromise study outcome through inducing inappropriate TK (e.g. overwhelming absorption, detoxification mechanisms). **Overt toxicity or qualitatively altered TK due to excessively high dose may result in tumour effects that are secondary to the toxicity rather than directly attributable to the agent.**

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High dose selection: pros and cons of different approaches

	Pros	Cons
Limit dose	▪ Historically used	▪ Arbitrary, not scientifically-driven
MTD	▪ Clearly identify adverse effects ▪ Simplifies hazard assessment	▪ Unnecessary animal suffering ▪ Ambiguity around tox endpoints used to determine MTD ▪ Effects may not be related to realistic human exposure ▪ May trigger additional testing (e.g., mechanistic data for effects at non-human relevant doses)
KMD	▪ Considers multiple lines of evidence ▪ Human relevant exposures ▪ Avoids additional testing (e.g., MoA) at non-human relevant doses	▪ May not be high enough for some jurisdictions (especially if no toxicity observed) ▪ May trigger additional testing

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- Optimum approaches may differ in the context of fit-for-purpose

Misconceptions

Overt toxicity needs to be observed at top dose

- Repeat dose studies are intended to assess the effects of (realistic) repeated exposures over time - overt toxicity does not necessarily need to be demonstrated
- Different interpretations of adversity
- Loss of ability to maintain homeostasis (i.e. saturation of kinetic processes) demonstrates ‘biological stress’ and is thought to be equivalent to bodyweight loss limits typically used to determine MTD
- Limited value in increasing dose above saturation of absorption – increase in applied dose will not lead to increase in internal dose
- Limited value in demonstrating more than ‘mild’ toxicity, provided it is outside of expected human exposure

Misconceptions

Use of KMD to set top dose in chronic toxicity study requires more animal use than traditional MTD

- Some cases *may* require additional dose levels of PK studies to determine KMD – but often balanced by benefits in overall package
- Incorporation of TK helps increase the available information to allow more informed decisions on dose selection
- Generally same studies and same numbers of animals used whether MTD or KMD approach
- Microsampling allows integration of TK and avoids the need for satellite groups
- Reduced chance of generating irrelevant toxicity data that may require additional mechanistic studies to explain relevance to humans

Use of toxicokinetics to inform dose selection

- Integration of TK into all studies maximises information available for dose setting
- Studies are often conducted in a specific order, so that at each stage more information is available to inform dose selection
- Consequence of inappropriate dosing increases with study duration and sample size

More data available

Repeat dose studies	DART studies	
DRF study		
28 day study		
90 day study	Probe dev tox	Probe repro study
2 year bioassay	Main dev tox	2-gen repro study

Longer duration, more animals

Repeat dose studies – animal numbers

Study	Species	OECD TG	Species	Animal numbers		
				Study design	Range	Typical
28 day	Dog	-	Dog	2 / sex / 4 doses	8	**8**
	Mouse	407	Mouse	5 / sex / 4 doses	40 - 60	**50**
	Rat	407	Rat	5 / sex / 4 doses	40 - 80	**40**
90 day	Dog	409	Dog	4 / sex / 4 doses	32 - 48	**32**
	Mouse	408	Mouse	10 / sex / 4 doses	80 - 100	**80**
	Rat	408	Rat	10 / sex / 4 doses	80 -100	**80**
18 month carc	Mouse	451	Mouse	50 / sex / 4 doses	>400	**400**
2 year chronic / carc	Rat	453	Rat	64 /sex / 4 doses	>512	**656**

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Huge impact of getting the dosing 'wrong' – especially if leads to repeat and/or additional investigational studies

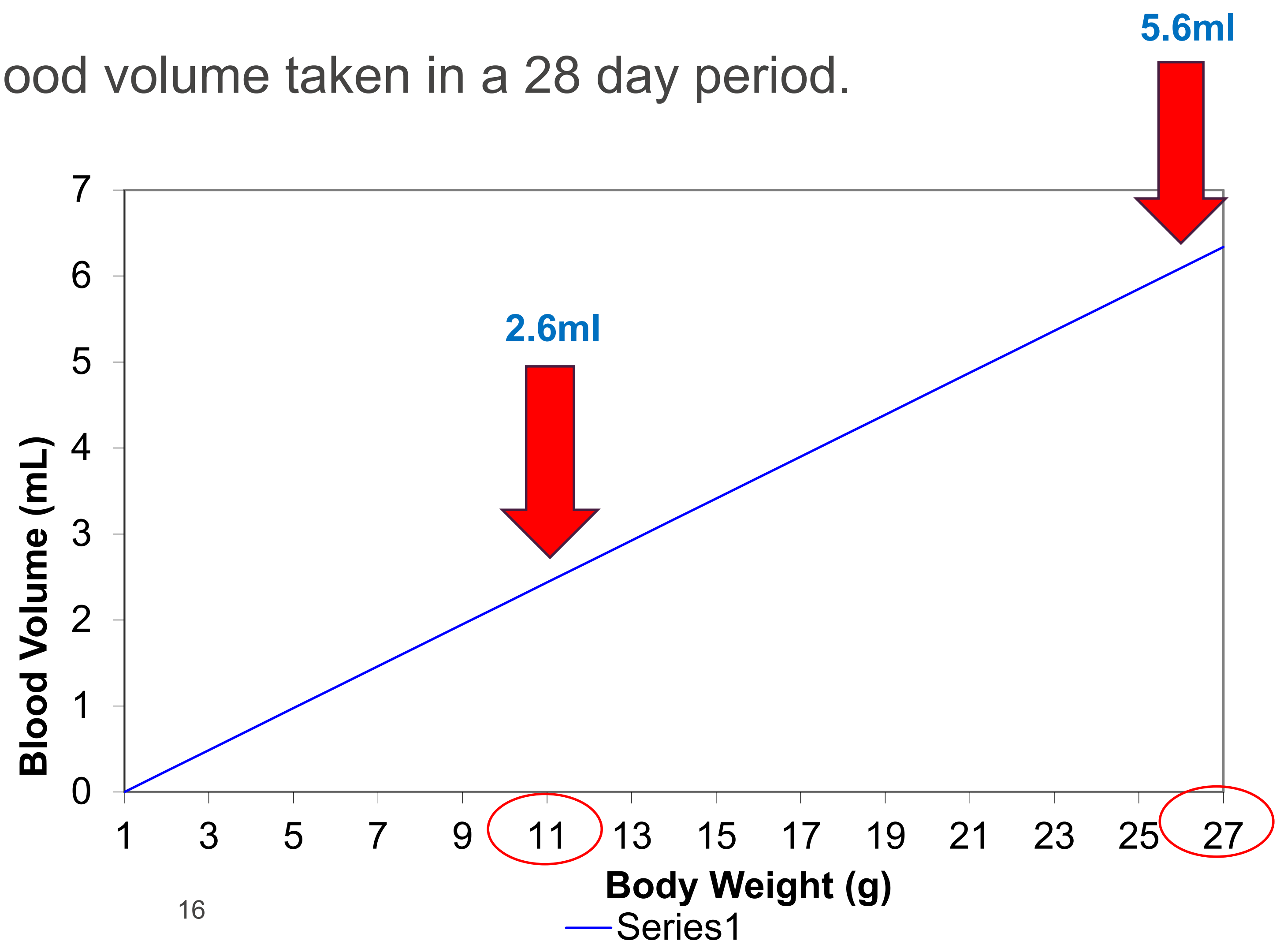
Examples of MoA studies

Supplemented with *in vitro* and *in silico* data (e.g. structure-activity relationships, SARs)

Study trigger / investigation	Study designs	Typical animal numbers used
Standard Liver MoA Liver tumours (in mouse and/or rat)	10 / single sex / 4 groups / 3 timepoints (days 1,7 & 28)	**120 – 480**
Standard Thyroid MOA Thyroid tumours (in mouse and/or rat)	15 / single sex / 4 groups / 3 timepoints (days 1,7 & 28)	**180 – 720**
To establish human (non) relevance for liver tumours, strain comparison for transfer to liver KO mouse study	4 / single sex / 5 groups (to assess PK linearity) / 2 timepoints (days 1 & 7 to account for limitation in mouse PK sampling) / 3 strains (e.g. CD1, KO WT & KO strains)	**120 – 240**
Liver KO mouse Study	10 / single sex / 4 groups / 2 timepoints (days 1 & 7) / 3 strains (e.g. CD1, KO WT & KO strains)	**240 – 480**

Integration of TK and blood sampling limits

- Up to 10% total blood volume taken on a single occasion from a normal, healthy animal.
- No more than 15% of circulating blood volume taken in a 28 day period.
- **Scenario:** to take 8 samples (~200 µl) at start and end of a toxicology study plus others for additional parameters (e.g. clin path) would require ~5.6 mL of blood – this is a rat weighing 650 g!
- Most rats weigh 250 g, with less blood!



How to reduce volume required per animal?

- **Fewer timepoints**
  - But may not achieve scientific objectives of study
- ***OR***
- **More animals – e.g. satellite group for TK**
  - Would enable sufficient conventional samples to build a TK profile
  - But can increase the number of animals by over 40%
- ***OR***
- **Microsampling**
  - 2 x 8 point profiles using 50 µl microsamples requires 0.8mL blood which represents around 5% of total blood volume

Reduction in animal use by using microsampling

Example: 90 day rat study with satellite animals

Dose group	Low	Medium	High	Control
Main study	10M+10F	10M+10F	10M+10F	10M+10F
TK satellite	6M+6F	6M+6F	6M+6F	3M+3F
Total for 1 study				122

Example: 90 day rat study with microsampling allowing smaller satellite groups

Dose group	Low	Medium	High	Control
Main study	10M+10F	10M+10F	10M+10F	10M+10F
TK satellite	3M+3F	3M+3F	3M+3F	3M+3F
Total for 1 study				104

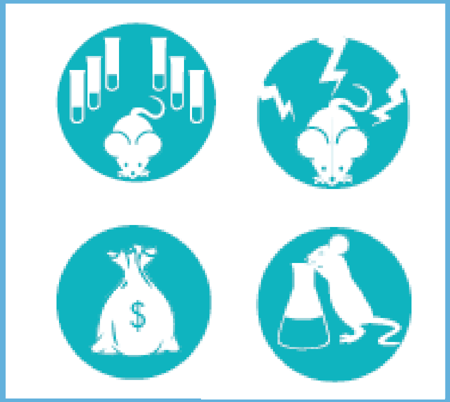
18 fewer animals

Example study design: 90 day rat study with microsampling of main study animals

Dose group	Low	Medium	High	Control
Main study	10M+10F	10M+10F	10M+10F	10M+10F
Total for 1 study				80

42 fewer animals

Microsampling



Improves the science and reduces and refines animal use simultaneously

1. Scientific benefit:
   - Comparison of data within the same animal at different time points – acts as own control and can track changes from baseline.
   - Allows direct comparison of different datasets e.g. exposure data and toxicology in same animal.
2. Reduced stress:
   - quicker, reduced or no warming
   - reduced handling and stress
3. Less blood loss, allows serial samples from same animal*
4. Use fewer animals overall
5. Less test item needed, less housing space/husbandry – financial savings

* Within limits of acceptable needle-stick punctures & animal burden

Common questions / concerns

- Microsampling is being used for agrochemicals and CROs are reporting an increase in TK sampling in these toxicity studies

- LCMS-MS sensitivity has increased over past decade - allows detection of low concentrations of analytes

- The European Bioanalysis Forum (EBF) have investigated and published recommendations / best practices to ensure scientific quality and reproducibility of microsamples

Special Report
Bioanalysis
For reprint orders, please contact: reprints@future-science.com

Feedback from the European Bioanalysis Forum liquid microsampling consortium: capillary liquid microsampling and assessment of homogeneity of the resultant samples

Zoe Cobb[1], Morten Rohde[2], Iain Love[3], Valerie Boutet[4], Katrin Schroeter[5], Glen Hawthorne[6], Lieve Dillen[7], Matthew Barfield[8], Abdullah Kandira[5], Marion Kranenborgh[9], Stephen White[8] & Philip Timmerman*[,10]

Regulatory Toxicology and Pharmacology 72 (2015) 429–439
Contents lists available at ScienceDirect
Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph
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Clinical and anatomic pathology effects of serial blood sampling in rat toxicology studies, using conventional or microsampling methods

CrossMark

Alexis Caron *, Christine Lelong, T. Bartels, O. Dorchies, T. Gury, Catherine Chalier, Véronique Benning
Sanofi-Aventis Recherche & Développement, Disposition, Safety & Animal Research, Paris, France

Regulatory Toxicology and Pharmacology 69 (2014) 425–433
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Regulatory Toxicology and Pharmacology
journal homepage: www.elsevier.com/locate/yrtph
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Assessment of haematological and clinical pathology effects of blood microsampling in suckling and weaned juvenile rats

Nicola Powles-Glover [a,*], Sarah Kirk [a], Lynne Jardine [b], Stephanie Clubb [b], Jane Stewart [a]
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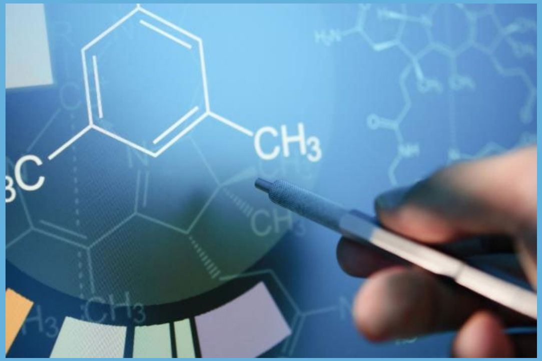
[b] Charles River Laboratories, Tranent, Edinburgh EH33 2NE, UK

- Clinical pathology parameters and functional measurements similar to vehicle animals, when microsampling from main test adult and juvenile animals included – bibliography with evidence on NC3Rs website

Incorporation of non-animal approaches



- Opportunities to integrate non-animal kinetic (*in vitro* or *in silico* data) and dynamic data to inform dose selection in repeated dose animal studies
- TK information can help inform IVIVE and ensure new approach methodologies (NAMs) use appropriate and relevant concentrations
- Allows more hypothesis/data-driven testing to be conducted
- Use of AOP-driven approaches to identify biomarkers for molecular initiating events to be tested in *in vitro* or early *in vivo* studies to avoid the need for future testing in animals, or testing at irrelevant high doses.

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Summary

Use of TK offers opportunities to both improve science and benefit the 3Rs

- 3Rs consequence of inappropriate dose selection
- TK can provide information on dose exposure relationship to inform more appropriate dose selection (i.e. to reflect effect of the compound following repeat exposure - not the effect on a ‘stressed system’)
- Highest dose should ideally be within linear kinetics
- Benefits include more informative and scientifically refined dosing, and offers 3Rs benefits - reduced suffering to animals (and fewer animals overall)
- Need a better understanding of when the KMD approach may/may not be appropriate for regulatory (and other) purposes
- Need guidance on how to present and communicate the data to regulators so that it is acceptable – what do they need to see?

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Thank you!

For more information

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Check out our microsampling resources:
www.nc3rs.org.uk/3rs-resources/blood-sampling

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