

Statistical analysis supporting the development of the guidance on dermal absorption

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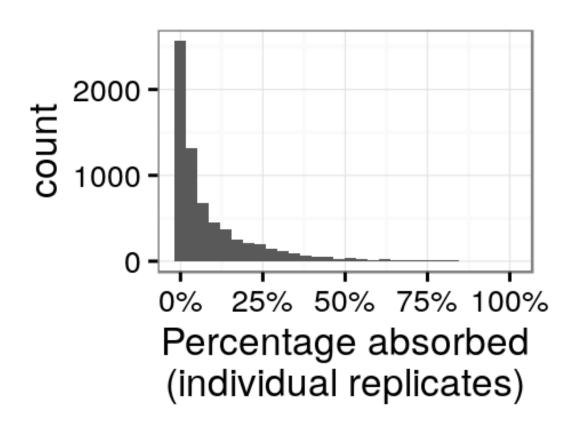


Variability of percentage absorbed

Highly skewed distribution

Lots of replicates near 0% absorption

Potentially tricky to use as response variable in regression





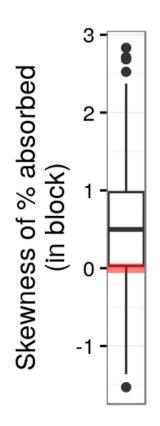
Variability within blocks: raw data

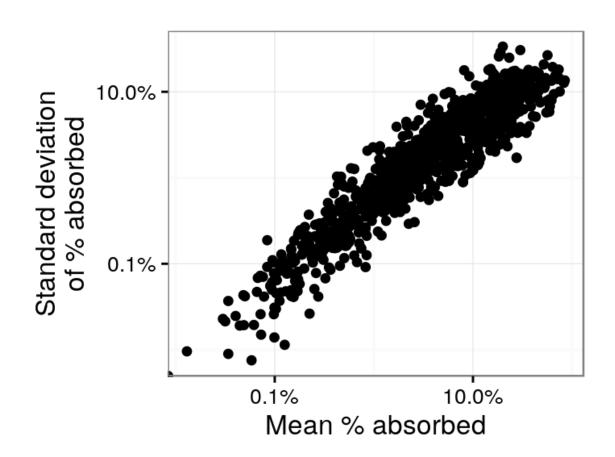
Each point in the figures summarises a block of replicates: an in vitro "study"

75% of blocks have positive skewness

Strong relationship in @location-scale" plot

Transformation needed



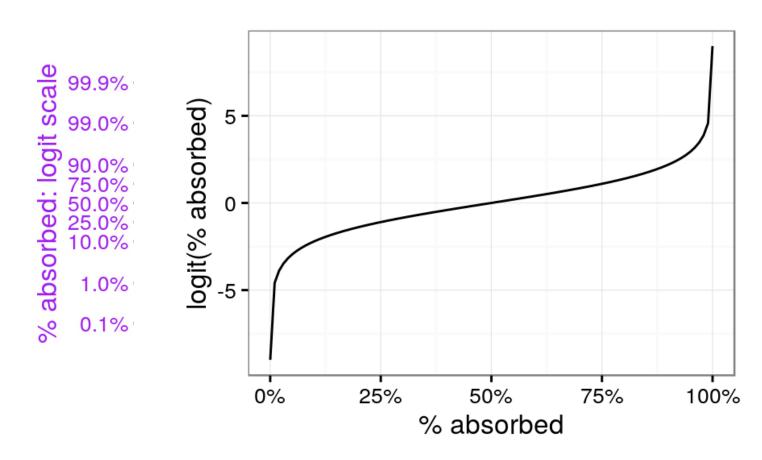




Logit transform

The logit transform stretches out values near 0 or near 1

Transformed data can be plotted with a "logit scale"



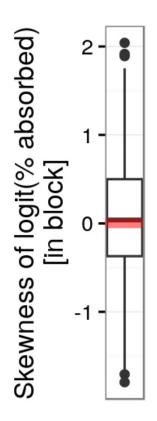


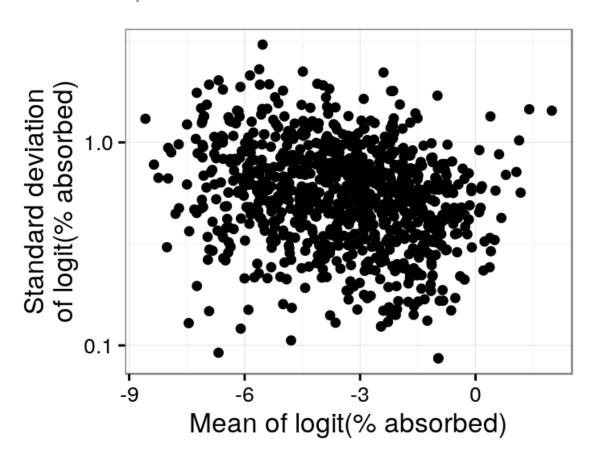
Variability within blocks: logit transform

Each point in the figures summarises a block of replicates: an in vitro "study"

No tendency to positive or negative skewness
Little relationship in "location-scale" plot

Transformation successful







Data transformations

Summary of conclusions

- Logit transform removes issue of skewness and of lack of homogeneity at different levels of absorption
- Logit-transformed data more suitable than raw data for use of standard statistical procedures, including confidence intervals and regression
- Other transformations were investigated but found to be less satisfactory: probit, arc-sine, log
- Existence of measurements with 0% absorption means that logit-transform needs a small adjustment to include those data, for example in regression modelling.



EFSA 2012 GD and OECD 2011 guidance notes:

- If variability low (standard deviation <= 25% of mean), use mean of replicates in risk assessment
- If variability high (standard deviation > 25% of mean):
 - EFSA 2012: preferred option is to add one standard deviation to the mean.
 - OECD 2011: consider using maximum measurement if n=4 and add one standard deviation to the mean when n>4

Making allowance for uncertainty, due to finite sample size, about mean absorption.



In the database, 85 % of blocks have standard deviation > 25% of mean: variability is usually "high".

EFSA 2017 GD:

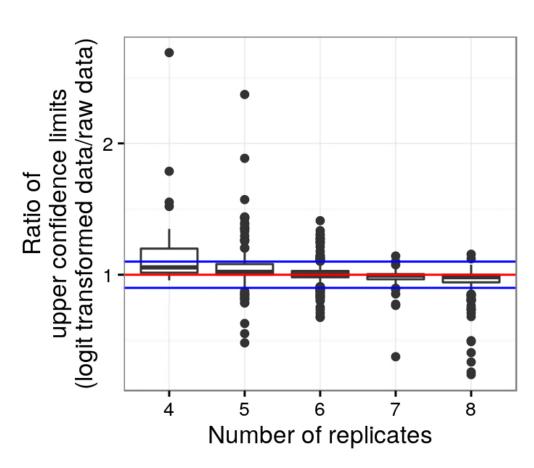
- Use approximate upper (97.5%) confidence limit for mean to allow for uncertainty due to finite sample size: $\bar{x} + k \times s$ where \bar{x} and s are the mean and standard deviation of the data and k depends on the number of replicates (standard confidence interval calculation).
- Rewards higher number of replicates by reducing multiple of standard deviation as sample size increases. Same as EFSA 2012 GD when n=6.
- Would theoretically be better to calculate confidence limit using logit-transformed data but was considered unnecessarily complicated and empirical performance was not very different.



Comparison of outcomes using raw data and transformed data upper confidence limits

Transformed data upper confidence limit typically a little higher for n=4.

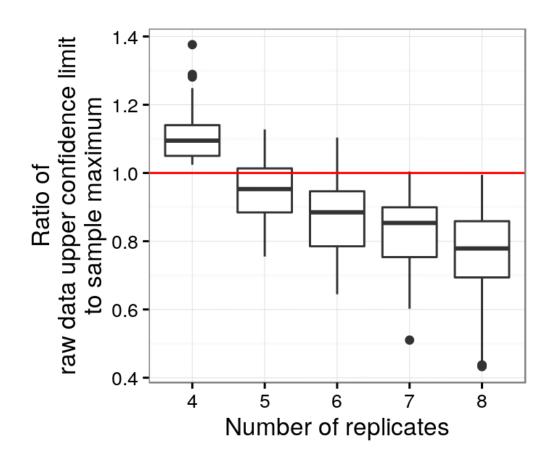
For n>4, very little difference in most cases (blue lines show +/- 10%)





Comparison of outcome using raw data upper confidence limit to outcome using sample maximum

Sample maximum does not allow sufficiently for uncertainty when n=4





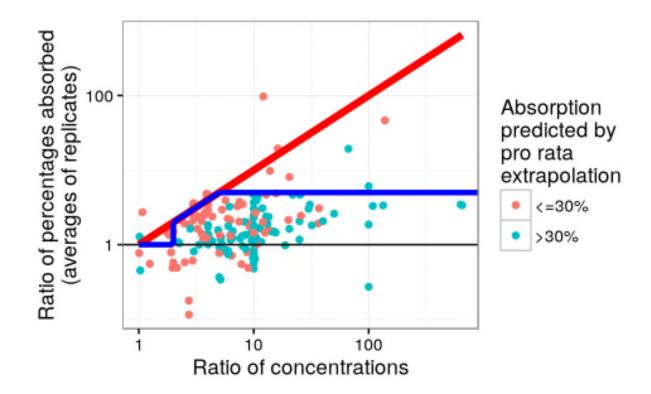
Pro-rata extrapolation

Each point represents a case where a substance was tested in the same formulation at different dilutions in the same study

Red line: pro-rata extrapolation

Blue lines: Aggarwal et al (2014,2015)

Pink/blue points: section 4.7 of Aggarwal (2014)





Explaining absorption variability

- Many potential explanatory variables but a lot of confounding.
- Two statistical modelling approaches used to try to understand which explanatory variables are more important:
 - Beta-regression (special regression methodology directly addressing the range limitation for absorption without transforming data). Explored all possible combinations of explanatory variables (without interactions).
 - Random-effects regression for logit-transformed absorption percentage.



Explaining absorption variability

Explanatory variables:

Physico-chemical properties (active substances) and formulation (product)	Active substance code, MW, log P _{ow} , water solubility, formulation type, concentration tested, concentration status (concentrate/dilution)
Skin sample	Skin type, sex, age, donor, skin source, skin region
Experimental conditions	Exposure duration, chamber type, mass balance recovery, receptor medium

MW: molecular weight



Number of explanatory variables	<u>Best</u> sub-model	Change in DIC
1	FormTypeConcDil	-
2	logMolConc + AScode	3122
3	logMolConc + ReceptMedium + AScode	314
4	logMolConc + Duration + ReceptMedium + AScode	174
5	FormTypeConcDil + logMolConc + Duration + ReceptMedium + AScode	111
6	•••	86
7	•••	35
8	•••	29
9		28
10	•••	24



Number of explanatory variables	Best sub-model without AScode and Receptor Medium	Change in DIC
1	FormTypeConcDil	-
2	FormTypeConcDil + Duration	306
3	FormTypeConcDil + SkinType + Duration	108
4	FormTypeConcDil + SkinType + Age + Duration	99
5	FormTypeConcDil + logMolConc + SkinType + Age + Duration	82
6	logMW + logPow + FormTypeConcDil + logMolConc + SkinType + Duration	46
7	•••	107
8	•••	22
9	•••	11
10	•••	-1.5

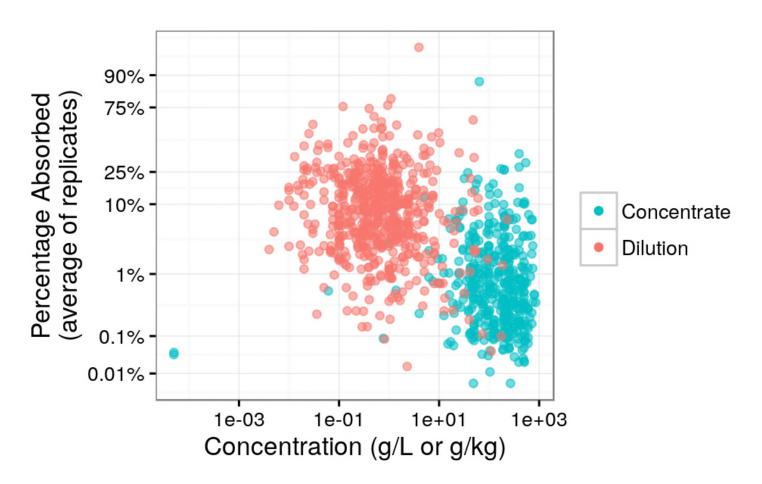


Conclusions from beta-regression

- Strong support for distinguishing between formulation groups and for distinguishing concentrates from dilutions
- Evidence that many other variables have some explanatory capability BUT that the dominant explanatory variable is active substance, i.e. the level of absorption varies greatly between substances.
- In particular physical/chemical properties of substances did not easily explain the same amount of variation.
- Need to find some way to directly address variability between substances
- Turned away from beta-regression due to issues with model fit, limitations of software used to fit models and difficulties with predictive calculations

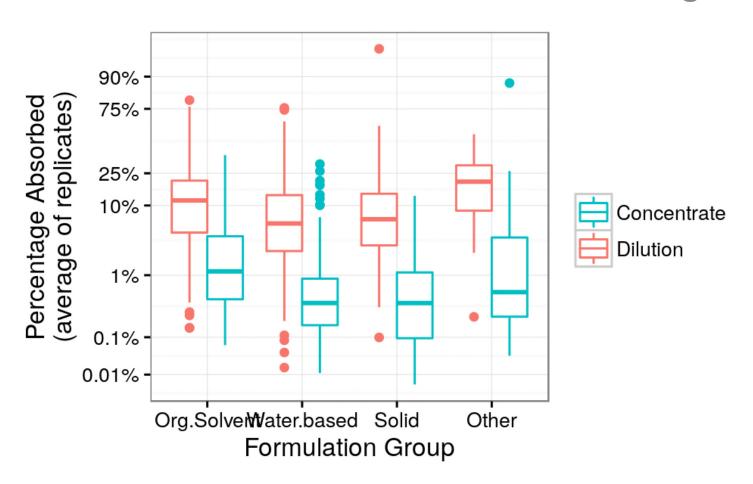


Difference between concentrates and dilutions but not much evidence of other dependence on concentration





Consistent difference between concentrates and dilutions and differences between formulation groups





Analyses of variance:

Source of variation	% of variation explained		
	All data	Data with	
		donor ID	
Dilution/concentrate	33	32	
Formulation group	3	3	
Active substance	28	28	
Active substance specific	7	7	
dilution/concentrate			
Study (on same substance)	9	9	
Different concentrations and	5	5	
formulations within study	5		
Replicates: between donors	15	10	
Replicates: within donors	13	5	



More about Analysis of Variance results

- A variety of analyses were performed, adding explanatory variables in different sequences
- Confirmed the conclusions of the beta-regression modelling
- In particular, dilution/concentrate, formulation group and active substance dominate.
- Other potential explanatory variables explain relatively little variation if added after active substance.
- This includes the data-set origin: ECPA versus BfR which is substantially confounded with active substance.

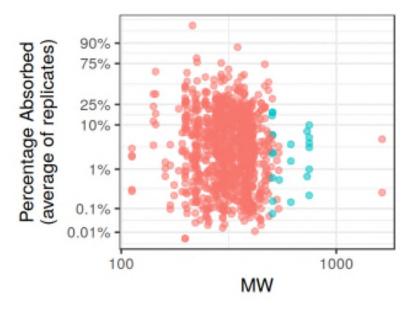


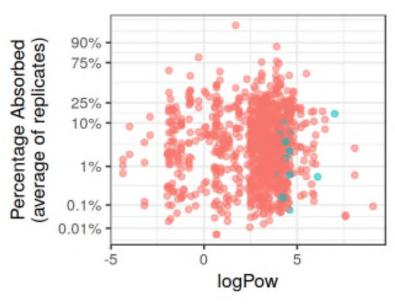
EFSA GD (2012): special case 10% DV?

EFSA 2012 GD: if log Pow < -1 or > 4 and MW > 500 a default dermal absorption value of 10% may be applied.

EFSA 2017 GD: no evidence found in the data to support this special case.

Points coloured blue in figures are those for which log Pow < -1 or > 4 and MW > 500:







Towards DVs: variability & uncertainty

Variability:

- Default values might be based on estimating a specified percentile of variability.
- Choosing which percentile to use is a risk management decision.

Uncertainty:

- All statistical estimates are subject to uncertainty.
- Uncertainty should be taken into account when setting default values.
- How much allowance to make for uncertainty is a risk management decision.



Towards DVs: empirical quantiles

Example of table of empirical 95th percentiles and approximate upper confidence limits

Formulation category	Dilution/ concentrate	Sample size	Data 95 th percentile	UCL for percentile
Organic solvent + other	Concentrate	1284	18	20
Water based + solid	Concentrate	1544	8	9
Organic solvent + other	Dilution	1658	51	<i>55</i>
Water based + solid	Dilution	2277	45	48



Towards DVs: empirical quantiles

- Merits of empirical quantiles
 - Easily understood
 - Relatively easy to implement
- Weaknesses:
 - Variability:
 - All sources are included. Inter-, intra-human should be excluded, perhaps more.
 - Each measurement in the dataset gets equal weight. No allowance for hierarchical structure: varying numbers of studies, experimental conditions and replicates
 - 95th %ile of what? Makes RM decision tricky
 - Uncertainty:
 - UCL method used is designed for random sample from homogeneous population not a hierarchical dataset.
 - How is uncertainty being addressed?

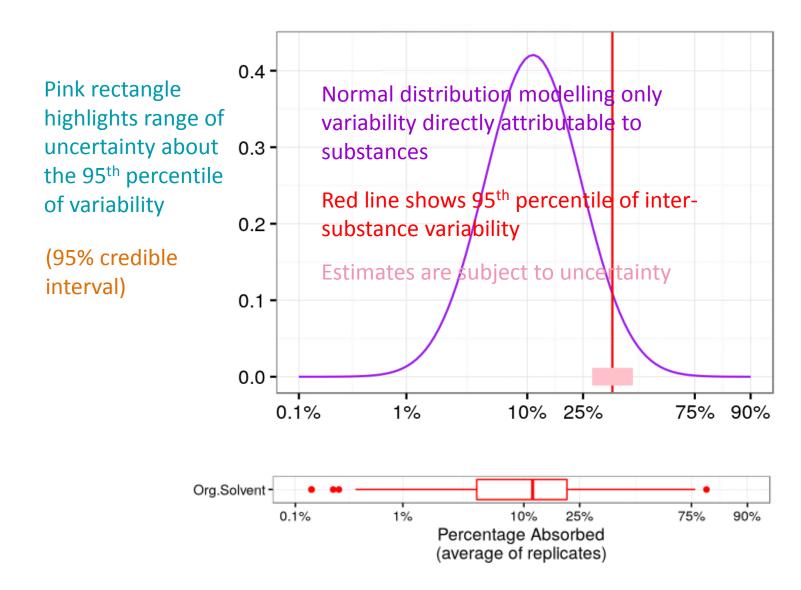


Random effects logit-regression:

Statistical meaning of "effect"

- Active substance "effects" seen as sampled from "population of substance effects".
- Model statistically as "random variability"
 - modelled by normal distribution
 - standard deviation of distribution controls amount of variation between substances
 - unexplained variation between substances also modelled as "random variability" when included in model
- Basis for default values:
 - "Effect" for substance without data for risk assessment is treated as random value sampled from the distribution.
 - Risk management decision: what percentile of variation of substances to use







A decision must be made about which sources of variation to include in the random effects part of the model: A or A+B or A+B+C

Source of variation	Effects group	
Dilution/concentrate	Fixed effects	
Formulation group	Fixed effects	
Active substance	Α	
Active substance specific dilution/concentrate		
Study (on same substance)	В	
Different concentrations/formulations in study	С	
Replicates: between donors	Not included	
Replicates: within donors		



Example of table of estimated 95% percentiles and upper credible limits

		Sources of variation to include					
		Α		A+B		A+B+C	
Form'n category	Dilut'n/conc.	Est. 95%	UCL	Est. 95%	UCL	Est. 95%	UCL
Org. solv. + other	Conc.	5	7	8	10	11	13
H ₂ O based + solid	Conc.	3	4	4	6	6	8
Org. solv. + other	Dilution	36	44	47	54	56	62
H ₂ O based + solid	Dilution	23	29	32	38	40	46



- Merits: addresses all identified weaknesses of the empirical approach
- Weakness: dependent on choice of statistical model. In particular, the model used:
 - assumes that random effects are homogeneous across the 8 (or 4) categories whereas the empirical approach treats each category separately;
 - makes distributional assumptions which may not be valid: random effects and replicate variation are both assumed to be normally distributed.
- Overall: Some identified modelling approach weaknesses could be addressed by further statistical analysis, whereas the empirical approach weaknesses are difficult to overcome.



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