Variability in organ-level effects in repeat dose animal studies

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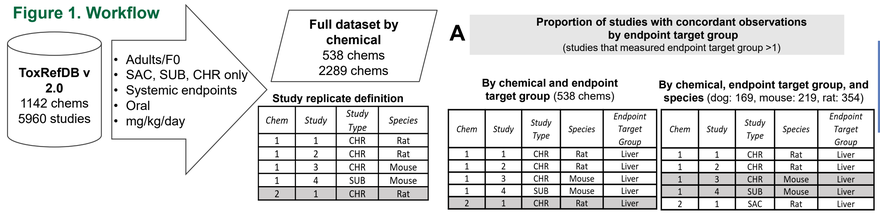
Building scientific confidence in the use of new approach methodologies (NAMs) in safety assessment may include performance comparison to *in vivo* study outcomes. This work defines the variability in organ-level effects and suggests qualitative and quantitative benchmarks for maximum NAM performance for prediction of organ-level effects in repeat dose studies of adult animals. Previous work suggests that the root mean square error (RMSE) for study-level lowest effect level (LEL) values (on a $\log_{10}$-mg/kg/day basis) approaches 0.5 $\log_{10}$-mg/kg/day†.

Observations of liver, kidney, stomach, spleen, thyroid and adrenal gland from the Toxicity Reference database (v2.0) were included in this analysis to understand the reproducibility of organ-level effects.

Abbreviations Key
CHR=Chronic; SUB=Subchronic; LEL=Lowest effect level; AED=Administered equivalent dose

Figure 1. Workflow



A **Proportion of studies with concordant observations by endpoint target group**
(studies that measured endpoint target group >1)

By chemical and endpoint target group (538 chems)

Chem	Study	Study Type	Species	Endpoint Target Group
1	1	CHR	Rat	Liver
1	2	CHR	Rat	Liver
1	3	CHR	Mouse	Liver
1	4	SUB	Mouse	Liver
2	1	CHR	Rat	Liver

By chemical, endpoint target group, and species (dog: 169, mouse: 219, rat: 354)

Chem	Study	Study Type	Species	Endpoint Target Group
1	1	CHR	Rat	Liver
1	2	CHR	Rat	Liver
1	3	CHR	Mouse	Liver
1	4	SUB	Mouse	Liver
2	1	SAC	Rat	Liver

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B **Variance analysis on subsets by endpoint target group**
(studies that measured endpoint target group >1)

Method: Multilinear regression (MLR)

Descriptors used for LEL data by organ:
- Study type
- Species
- Administration method
- Dose number
- Dose spacing
- Substance purity
- Study year

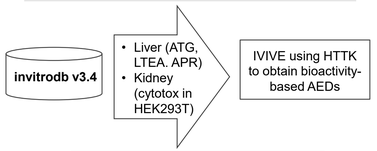
Used to calculate total variance =
Unexplained variance (MSE)
+
Explained variance

C **Analysis of differences of SUB and CHR findings by endpoint target group, paired by chemical**

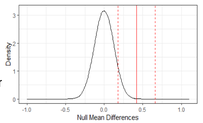
Method 1: Odds Ratios
- For each of the 6 endpoint target groups and species, filter by chemicals that have both study types present.
- Calculate the odds ratio for CHR and SUB.
- Convert odds ratio to probability of a positive in CHR given a positive in SUB.

Method 2: Paired Randomization Test
- For each of the 6 endpoint target groups, filter by chemicals that have both study types present.
- Calculate log10 differences of LELs.
- Perform a paired randomization test to check for significant differences in the distributions of SUB/CHR LELs.

D **Comparison of PODs for liver and kidney for bioactivity-based AEDs**

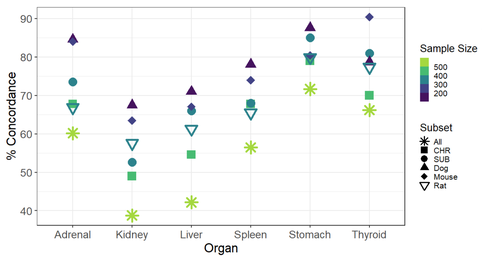


Method: Paired Randomization Test
- For liver and kidney, gather chemicals that have both LEL and AED data.
- Calculate log10 differences between LELs/AEDs.
- Perform a paired randomization test to check for significant differences in the distributions of SUB/CHR LELs.



A: Qualitative reproducibility of organ-level effect observations in repeat dose studies of adult animals was 33-88%, depending on grouping.

Figure 2. Repeated concordance of organ-level findings



$$\%\ Concordance = \frac{chemical\ with\ positive\ finding\ in\ all\ studies + chemicals\ with\ negative\ finding\ in\ all\ studies}{total\ chemicals\ tested}$$

- Organs associated with more negative chemicals (stomach, thyroid, adrenal) had higher rates of concordance in this range.
- Within-species concordance tended to be greater than within-study concordance

B: Variance in organ-level effects in repeat dose studies was smaller than study level variance, but the organ-level effect RMSE was similar to study-level RMSE and approaches ~0.5 $\log_{10}$-mg/kg/day.

Table 1. Results of MLR to estimate unexplained and explained variance in organ LELs

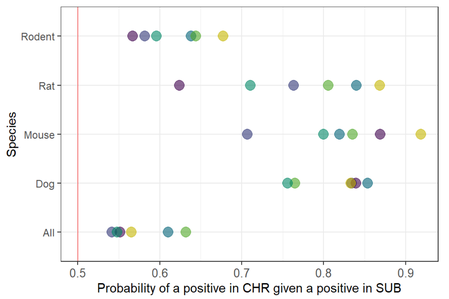
$$organLEL \sim b_0 + chemical * b_1 + species * b_2 + study\ type * b_3 + administration\ method * b_4 + dose\ spacing * b_5 + number\ of\ dose\ levels * b_6 + study\ year * b_7 + \%\ substance\ purity * b_8$$

Endpoint Target Group	Chem	N	Var	MSE	RMSE	% var explained
adrenal	81	208	0.756	0.349	**0.591**	53.8
kidney	263	790	0.765	0.316	**0.562**	58.7
liver	359	1318	0.745	0.355	**0.596**	52.3
spleen	127	336	0.671	0.318	**0.564**	52.6
stomach	55	146	0.553	0.173	**0.416**	68.7
thyroid	73	198	0.721	0.378	**0.615**	47.6

Chems = # chemicals; N = number of studies; Var = total variance; MSE = mean square error on the model; RMSE = root residual mean square error; % var explained = % of total variance explained.by study descriptors

C. Organ-level findings in SUB appear qualitatively predictive of organ-level findings in CHR studies (ignoring adversity).

Figure 3. Probability of a positive CHR outcome by organ/species subgroups



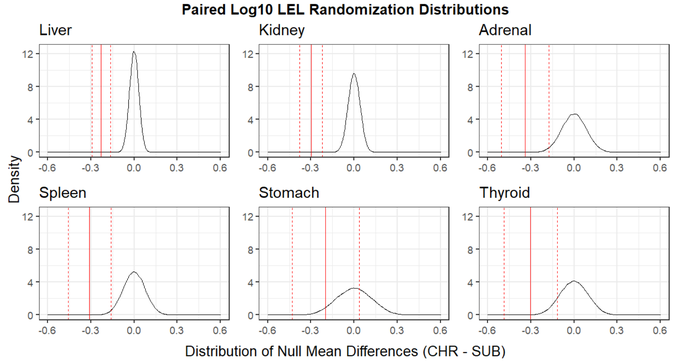
- Odds ratios are converted to probabilities using the formula:

$$P = \frac{OR}{1 + OR}$$

- The red line represents 0.5 probability, which would indicate having information about SUB would inform CHR no better than random.
- Note that "All" and "Rodent" sample sizes were significantly reduced because of the matching procedure.
- An "All" chemical must have been present in all species groups and both study types.

C. SUB organ-level LEL values are typically within 0.5 log10-mg/kg/day of CHR organ-level LEL values; for some organs, available data suggests that SUB and CHR studies produce similar LEL values.

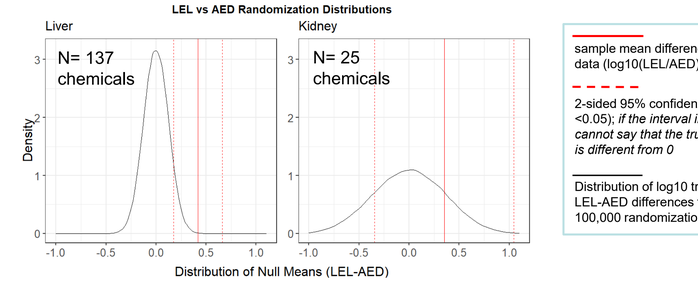
Figure 4. Matched randomization distributions for CHR/SUB LELs by organ



- Liver, kidney, spleen, and thyroid have significantly different log10 LEL difference from 0 at the α = 0.05 level [95% confidence interval (red dotted lines) does not include 0].
- Differences between CHR and SUB organ-level LELs may be within estimates of replicate study variance (0.5 log10-mg/kg/day).

D. *In vivo* LELs are higher than bioactivity-based AEDs on average for liver, and they may be within 0.5 $\log_{10}$-mg/kg/day for liver and kidney predictions.

Figure 5. Matched randomization distributions for LEL/AED differences



- Liver LEL and AED values are significantly different at the α = 0.05 level [95% confidence interval (red dotted lines) does not include 0], but the difference is well within estimates of variance in organ-level LELs (~0.5 log10-mg/kg/day).
- No significant difference for LEL-AED in kidney, the interval overlaps both 0 and 0.5, but N is small.
- Chemical diversity is limited for this preliminary result. The primary ToxPrint chemotypes represented are benzene ring and other aromatic bonds and chains.

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†Based on Pham LL, Watford S, Pradeep P, Martin MT, Thomas RS, Judson RS, Setzer RW, Paul Friedman K. Accepted. "Variability in in vivo studies: Defining the upper limit of performance for predictions of systemic effect levels." *Computational Toxicology*. https://doi.org/10.1016/j.comtox.2020.100126