Developing metrics to track animal use and the impact of NAMs

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The development of metrics to track the use of new approach methodologies (NAMs) and their effects on animal use are critical to set goals, monitor progress, and provide accountability for resources spent on NAMs and tests on animals. Metrics further facilitate the identification of NAMs that are providing the greatest reductions in animal use and highlight gaps where NAM development and/or use should be prioritized. Currently, this tracking is virtually impossible in many sectors and countries, yet it is increasingly important as NAMs and the opportunities that they offer to better understand human health processes and outcomes continue to grow, prompting investment across sectors. However, for reasons from a lack of international regulatory acceptance of the animal-free approaches to a lack of reviewer awareness of the NAM, an increase in the number of NAMs does not necessarily translate to a reduction in animal use. Therefore, it's important to quantitatively demonstrate NAM implementation and their implications on the number of animals used in testing. Here, we outline the approach to define a tracking strategy that is defined in the article "Animal metrics: tracking contributions of new approach methods to reduced animal use". It establishes metrics for animal savings from *in silico*, *in chemico*, and *in vitro* methods; study waivers; and intelligent design based on the utility of the data.

TRACKING ANIMAL USE AND THE IMPACT OF NAMS

01

Define what types of animals and studies are in scope. Are just mammals included, or are all vertebrates, invertebrates, and/or fetal animals included? Are studies at all locations included–in-house and contracted or externally funded studies? Are animals used for breeding, tissue procurement, and reagents included?

03

Identify goals, which may shift over time, for example, as companies' product portfolios change. Goals may be to reduce animal use by a specific percentage or to conduct *in vivo* tests only to comply with regulatory requirements.

05

Measure the impact of NAMs on animal use by calculating 1) the absolute number of animal savings, 2) the percent reduction in animal use due to the application of NAMs, or 3) the percentage of toxicity information that comes from NAMs.

02

Based on the scope, with well-defined rules for inclusion and exclusion that can ensure consistency in future assessments, a baseline for animal use can be established. The absolute number of animals can be tracked annually by monitoring animal orders or tracking animals used in studies.

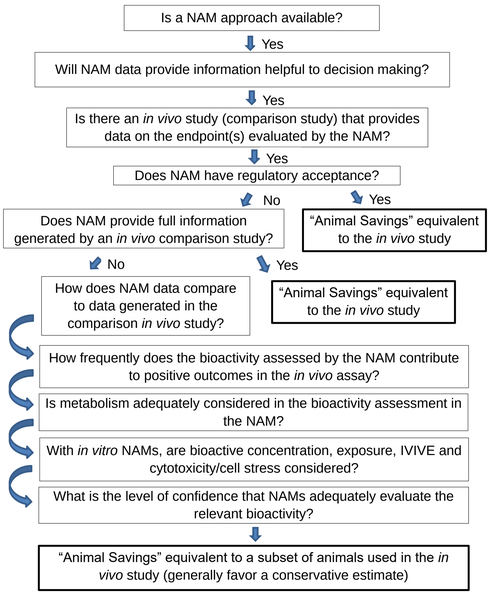
04

Establish metrics for animal savings from NAMs based on the utility of the data, or what the data is used for and its level of certainty.

ESTABLISHING METRICS FOR NAMS AND ANIMAL SAVINGS

Metrics for animal savings from NAMs were established based on data use (early screening/internal decision making, supporting data in a regulatory submission, or full substitute for an *in vivo* test) and the level of confidence in the data.

A decision tree to assign "animal savings" due to NAM use



ASSIGNING ANIMAL SAVINGS VALUES

In some cases, a NAM may completely replace animal use, resulting in animal savings equal to all animals used in the equivalent study. In other instances, NAM data may partially fulfill information generated by animal-based guideline studies or screening assays; in which case, the animal equivalent number for the NAM is a subset of the animal-based guideline study.

Conservative estimates of animal savings based on the extent to which a NAMs replaces an *in vivo* study were assigned per method per endpoint. The estimates can be adjusted depending on use of the information and level of certainty, Dow developed animal savings data for:

- *In silico* models for human health effects and ecotoxIcity
- *In vitro* methods for human health effects and ecotoxIcity
- *In silico* and *in vitro* models for metabolism/bioaccumulation
- Intelligent study designs
- Waivers

Animal use reductions due to the application of *in silico* (computer-based) NAMs to predict toxicity

Endpoint addressed by NAM	Corresponding *in vivo* test	No. animals *in vivo*	Animal savings using NAM	Rationale for percentage selected	No. animals saved by NAM use
Acute oral toxicity	OECD 425 (Acute oral toxicity – Up-and-down procedure; options: OECD 420 and 423)	5-8; 6-12 per Corvaro et al.	30%	• Most potent MOAs identified, but not all relevant targets have been modeled • Predictions are conservative and route agnostic • Evaluation covers parent and predicted highly toxic metabolites • Predicts potential MOA and GHS class	2.1
Acute inhalation toxicity	OECD 403 (Acute toxic class; other options: OECD 436 and 433)	40-42	10%	• Not all relevant targets have been modeled • Predictions are conservative and route agnostic • Evaluation covers parent and predicted metabolites • Predicts potential MOA but not GHS class	4

Animal use reductions due to the application of *in vitro* NAMs to predict toxicity

Endpoint addressed by NAM	Corresponding *in vivo* test	No. animals *in vivo*	Animal savings using NAM	Rationale for percentage selected	No. animals saved by NAM use
Reconstructed human cornea-like epithelium test method for eye hazard identification (OECD 492B)	OECD 405 (acute eye irritation/ corrosion)	1-3 (1 if corrosive)	100%	Replaces *in vivo* eye irritation assay	3
KeratinoSens™ or LuSens test (OECD 442D)	OECD 429 or 442A (LLNA) (options: 406)	28	33-50%	2-3 assays are needed; if both assays agree, then 50% animal savings for each assay. If all three assays are needed, 33% savings for each assay. Total animal savings equals 28 as these assays have regulatory acceptance and a combination can replace the LLNA.	28
DPRA or ADRA (OECD 442C)			33-50%		
h-CLAT, U-SENS™, or IL-8 Luc assay (OECD 442E)			33-50%		

MEASURING IMPACT

Tracking NAMs, animal savings, animal use, and purpose of studies allows one to understand NAM and animal use for various endpoints over a period of time or for research/development areas, and it provides the data needed to measure the impact of NAMs. One can:

- quantitatively monitor progress on NAM implementation and animal use reduction,
- assess the efficacy of programs and activities that aim to increase the use of NAMs,
- demonstrate accountability for resources spent on NAMs and tests on animals,
- identify NAMs that are providing the greatest reductions in animal use,
- and highlight gaps where NAM development and/or use should be prioritized.

Template to track NAMs

Endpoint	Study/waiver	Purpose	Number of studies/waivers	Species	Animal Use	Animals Saved
Sub-acute dietary (eco)	Waiver	Regulatory Requirement (U.S. EPA)	1	Bobwhite quail	-	60
Acute oral toxicity (eco)	Median lethal dose (LD50) test	Regulatory Requirement (U.S. EPA)	6	Bobwhite quail	360	-
Genotoxicity	Integrated mammalian erythrocyte micronucleus test and 28-day repeat dose study	Regulatory Requirement (ECHA)	3	Rat	75	75

For more information:
Marty MS, Andrus AK, Groff K. Animal metrics: tracking contributions of new approach methods to reduced animal use. *ALTEX*. 2022;39(1):95-112.

NEXT STEPS

This approach can be refined for use at other organizations. Key steps for consideration for developing and implementing a tracking strategy for NAM and animal use include:

- Establish a team to assess approaches to tracking NAMs and their impact on animal use.
- Set the scope and establish baseline animal use. Consider how to acquire data across businesses or international affiliates and from CROs, consortia, and other externally funded laboratories.
- Set goals for NAM implementation and a reduction in animal use.
- Assign animal use reduction numbers for individual NAMs.
- Track NAM use and animal use quantitatively.
- Periodically review progress towards goals.

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