

Utility of the Diversity Outcross mouse model in toxicology studies.

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These are some notes developed to try to convey some of the challenges and issues to consider with use of the Diversity Outcross (DO) mouse model in toxicology studies and where this resource might be useful to add value in a toxicological assessment of agents of public health concern. They are based on outcomes from informal internal discussions between staff at NIEHS, recent NTP experience from the assessment of benzene in the DO mouse (French, Gatti, et al 2014, <http://www.ncbi.nlm.nih.gov/pubmed/25376053>,) and past consideration of mouse multi-strain approaches in toxicology discussed as part of the NTP “Stocks and Strains” workshop (http://ntp.niehs.nih.gov/ntp/meetings/2005/agenda20050617_508.pdf) held in June 2005. These comments do not reflect a specific consensus of opinion with NTP or NTP policy or that of NIEHS/NIH/DHHS.

Why a DO mouse study?

- Improved assessment of population variability in hazard estimation;
 - Suspected cases of toxicities in humans not predicted by animal studies
 - Eg Known human hazards but inbred strains are refractory (ie a false negative) – Identify sensitive strains/haplotype for subsequent further analysis- eg MOA, dose response, possible interventions
 - Cases where “margins of exposure” between toxic effects and human exposures are “low” and there is a need to determine whether default safety factor assumptions of 10-fold to account for human variability are too small.
- Aid to Mode of Action assessment
 - Identification of the genes associated with a quantitative trait locus (QTL) may inform on species concordance of the potential mode of action (MOA) of the agent of concern.
- Identify genetic basis for a response
- To develop a mouse population model to address the issue of false negatives for a known human toxic response
 - Some inbred strains with defined genomic architectures are refractory to specific chemical exposures (ie a false negatives). Use of the DO resource may minimize the chance that any one inbred strain is refractory to a specific chemical exposure

- The DO could be used to identify sensitive haplotypes that be can be used for subsequent further analyses- eg MOA, dose response, possible interventions.

Implicit assumptions in use of the DO mouse.

- The genetic variability in the DO mouse is comparable, if not greater, than that seen in human cohorts.
- Phenotypic differences are in part due to host genetic/epigenetic differences.
- Inter-individual basis of response between individual mice is an appropriate surrogate for the inter-individual basis of response between individual humans.
- Genetic variability may lead to both toxico-kinetic and toxico-dynamic differences.

***A priori* design considerations.**

- It is useful to think of a DO mouse study design more like that of a human “prospective cohort study” rather than a traditional toxicological assessment in a specific defined mouse strain.
- In contrasts to traditional short term toxicology studies that use n=5-20 per exposure group, a sample size of 75 per exposure group is likely the sample size needed for a “pilot study” to estimate the variability in response and be able to adequately design subsequent focused studies using the full potential of the DO resource.
- Subsequent studies to identify QTLs may require in excess of 300 animals per exposure group, depending on the outcome of the assessment of population variability in response, to ensure adequate statistical power to define the QTLs.

What agents/test articles should be studied.

- Given the larger group sizes required for use of the DO mouse, agents should ideally be a known toxicant with known effects of concerns. The DO is likely not a good model as a initial “discovery” research tool for identification of agents that may pose a hazard.
- Known significant “population” exposure to justify resources needed.

Endpoint considerations.

- The endpoint should ideally be a **continuous** variable. (Mapping with binary/dichotomous traits can be used, though this would require different statistical approaches versus a quantitative trait and may not be as powerful for genetic mapping.)
- Some expectation of a population variability
- “Within strain” variability of the agent-response should be low; otherwise apparent differences between genetically heterogeneous individuals in the DO mice may simply be function of endpoint variance and not due to the underlying genetic differences.
- Toxicodynamic/time course stability; a highly dynamic response may lead false negative “non-responders” due to small differences in time course.
- Dose response dynamics; a very steep dose response may lead to false negative “non-responders” due to small shifts in potency.
- Non invasive assessment; this will allow “paired” analysis. Given the unknown variability in cohort responses, greater power can be obtained but will require using paired analysis of the endpoint in the “individual animal”, i.e. before and after exposure. There is no “control group” in the usual sense in traditional mouse toxicology studies, only cohorts of individuals.

Approach;

Use of the DO resource within a toxicological assessment of an agent is anticipated to require 3 phases.

- Phase 1: Hazard ID.
 - Agents should have a known hazard. As noted above and in Gatti et al 2013 (<http://www.ncbi.nlm.nih.gov/pubmed/25237114>) in general much larger sample sizes are required when using adverse population based models. As such they are best not used for initial “hazard identification” but more for characterization of a hazard. As such while a DO mouse model may be anticipated, it would be better to use an inbred or defined strain for initial hazard identification studies in Phase 1.
 - Past statistical simulations conducted as part of the NTP “Stocks and Strains” workshop (http://ntp.niehs.nih.gov/ntp/meetings/2005/agenda20050617_508.pdf) showed that single strain approaches generally have similar power for hazard ID as a multi-strain approach, though multi-strain (ie use of a more diverse genetic population of mice) have higher power when the effect is very heterogeneous and the effect is strong in sensitive strains. In the absence of such information to know that this *a priori*, a single strain approach in phase 1 is more appropriate from an animal use point of view.
- Phase 2. Characterization of population variability in response.
 - The best sample size to effectively use the full genetic mapping power of the DO resource is not known, as it depends on the population variability and penetrance of the effect. If population variability is small, subsequent studies will need to be very large to allow mapping of low penetrance effects.
 - If the questions under consideration are only an assessment of the population variability, Phase 2 may be all that is required.
 - If the endpoint can be assessed before and after exposure, the use of a non-exposed cohort may not be needed as each individual animal serves as its own control.
- Phase 3. Assessment of the genetic basis of population variability.
 - Once a robust is identified that is phenotypically variable in the, a larger study powered appropriately based on Phase 2 will be necessary to investigate the haplotypes that may be associated with either susceptibility or resistance.
 - Depending on phase 2 this could require in excess of 300 animals, (sequenced or SNPed) per exposure group.