# Replacing, Reducing and Refining the Use of Test Animals

IAFNS supports the replacement, reduction, and refinement of the use of test animals – through our scientific programming in food and nutritional sciences.

The “Three R’s” principle for test animal research was launched in the early 1960s by two English biologists, Russel and Burch in their book “The Principle of Humane Experimental Technique.” The 3 Rs stand for **Replacement, Reduction and Refinement** of test animals.

**Replacement** alternatives refer to methods which avoid or replace the use of animals. This includes both absolute replacements (i.e., replacing animals by computer models) and relative replacements (i.e., replacing vertebrates, with animals having a lower potential for pain perception, such as some invertebrates).

**Reduction** alternatives refer to any strategy that will result in fewer animals being used to obtain sufficient data to answer the research question, or in maximizing the information obtained per animal and thus potentially limiting or avoiding the subsequent use of additional animals, without compromising animal welfare.

**Refinement** alternatives refer to the modification of husbandry or experimental procedures to minimize pain and distress, and to enhance the welfare of an animal used in science from the time it is born until its death.

- Today IAFNS continues to support the 3Rs with work in food and nutritional sciences.

Recent IAFNS publications on Alternatives to Animal Testing:

“State of the Science on Alternatives to Animal Testing and Integration of Testing Strategies for Food Safety Assessments: Workshop Proceedings,” (Karmaus et al., 2020).

“Incorporating New Approach Methodologies in Toxicity Testing and Exposure Assessment for Tiered Risk Assessment Using the RISK21 Approach: Case Studies on Food Contact Chemicals,” (Turley et al., 2019).

# IAFNS Support of 3Rs

Institute for the Advancement of Food and Nutrition Sciences

## Replace Animal Testing in Determining Protein Digestion

The current approach used in the US to calculate protein quality relies on digestibility measures that are typically taken in animal models. This is a major disincentive to food and beverage industry stakeholders developing alternative protein ingredients and foods. Moreover, the currently accepted rat bioassay, which is time consuming and costly, may not, in fact, be the best test model for foods intended for human consumption. Thus, advancing standardized and validated *in vitro* methods of assessing protein digestibility is an incentive to encourage food manufacturers to determine the protein quality of foods they produce.

The Food & Drug Administration's (FDA) current method to calculate protein quality (i.e., PDCAAS) relies on a digestibility factor and while these factors are available for many proteins, new alternative protein foods often do not have these values. However, developers should be encouraged to characterize protein quality. Health Canada could also adopt a similar approach to encourage more companies to measure and maintain protein quality of innovative new protein foods.

Currently, IAFNS is supporting a collaborative study to validate *in vitro* protein digestibility as standardized methods by the American Oil Chemists Society (AOCS). Join us with this first step toward regulatory acceptance of alternative methods for determining protein digestion.

Learn more at www.iafns.org - Protein Committee

## Refining Risk Assessments and Reducing Animal Testing

IAFNS support for reducing animal testing and advancing public health extends to dietary exposures that carry the potential for adverse health outcomes. An IAFNS-supported research group is developing a novel framework that evaluates whether dietary items have the potential to cause cancer. Currently, risk assessments rely on studies in test animals, but new approaches may allow risk assessors to curtail reliance on animal bioassays.

This framework will consider the relevance and inter-species extrapolation of exposure, toxicokinetics, as well as the toxicological mechanisms of action (MOA) underlying observed tumors. By reviewing multiple streams of evidence, reliance on test animals can be constrained. Additionally, the research team will apply the framework to case studies and comparing the accuracy of the new framework to currently available frameworks using a semi-quantitative approach. Chemicals for the case studies will include at least one chemical with a non-mutagenic MOA and one with a mutagenic MOA.

Learn more at www.iafns.org - Food & Chemical Safety

# Alternatives to Animal Testing

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**IAFNS and Animal Testing Alternatives**

IAFNS works collaboratively with regulatory agencies to advance the science and the implementation of animal testing alternatives. This includes working with US FDA to host multiple workshops on emerging non-animal alternative toxicological methods for use in food safety assessments. These workshops also addressed the scope and applicability of high-throughput screening (HTS) in weight-of-evidence approaches for food safety.

- State of the Science on Alternatives to Animal Testing and Integration of Testing Strategies for Food Safety Assessments: Workshop Proceedings

IAFNS also completed a project on Incorporating New Approach Methodologies in Toxicity Testing and Exposure Assessment for Tiered Risk Assessment Using the RISK21 Approach: Case Studies on Food Contact Chemicals, that focused on case studies of indirect food additive chemicals. ToxCast data were compared with *in vivo* toxicity data using the RISK21 approach.

- Two food contact substances, sodium (2-pyridylthio)-N-oxide and dibutyltin dichloride, were selected, and available exposure data, toxicity data, and model predictions were assessed.
- For sodium (2-pyridylthio)-N-oxide, bioactive concentrations in ToxCast assays corresponded to low- and no-observed adverse effect levels in animal studies, suggesting that similar results for other chemicals could be gathered without relying on test animals.
- For dibutyltin dichloride, the ToxCast bioactive concentrations were below the dose range that demonstrated toxicity in animals; however, this was confounded by the lack of toxicokinetic data, necessitating the use of conservative toxicokinetic parameter estimates..

- Incorporating New Approach Methodologies in Toxicity Testing and Exposure Assessment for Tiered Risk Assessment Using the RISK21 Approach: Case Studies on Food Contact Chemicals

**For more information on how IAFNS connects, collaborates, and catalyzes science that matters, please connect with us!** wendelyn@iafns.org.

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