U.S. FOOD & DRUG ADMINISTRATION
CENTER FOR FOOD SAFETY & APPLIED NUTRITION

FDA Safety Assessment of Natural Extracts Used as Color Additives: Focus on New Approach Methodologies

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Overview

- Brief review of the requirements for submission of a color additive petition to the Office of Food Additive Safety (OFAS)

- New Approach Methodologies (NAMs)
  - Alternatives to animal testing
  - Background
  - NAMs at FDA

- Case studies of NAMs in color additive petitions

Color Additive Petitions

- Color additive petitions submitted to FDA must provide evidence that the substance is safe for its intended use(s)
- Specific requirements, language is prescribed in 21 CFR 71.1:
  - Identity and composition
  - Amount proposed for use and color effect intended
  - Analytical methodology
  - Full reports of safety studies
  - Data to demonstrate relevant exposure
  - Proposed limitations, if needed
  - Rationale for batch certification exemption (21 CFR 71.18)
  - Prescribed fee for listing (21 CFR 70.19)
  - Environmental information

Reasonable certainty that no harm will result from the intended use of the color additive (21 CFR 70.3(i))

New Approach Methodologies: A Brief History

1959: The 3Rs: Replacement, Reduction, and Refinement

Published by William Russell and Rex Burch in the *Principles of Humane Experimental Technique*

1980s: Interest in animal alternatives increases

Center for Alternatives to Animal Testing (CAAT) established; OECD begins to discussing the 3Rs; legislative changes in Europe; *In Vitro Toxicology* established

1990s: Establishment of government-based centers

ECVAM (1992); ICCVAM (1994); NICEATM (1998)

2000s: National Academy of Sciences publications

Toxicity Testing in the 21st Century: A Vision and a Strategy (2007) and Science and Decisions: Advancing Risk Assessment (2009)

Stephens, Goldberg, and Rowan, 2001
Parish et al., 2020

New Approach Methodologies: Definitions

"NAMs are *in vitro*, *in chemico* or *in silico* methods and/or integrated approaches used to test for toxicological endpoints in place of traditional animal testing" (CPSC, 2022).

"NAMs are envisioned to facilitate the replacement of animal testing with combinations of predictive *in silico* models . . . *in vitro* assays . . . and computational models of external and internal exposure…" (Parish et al., 2020).

Predictive modeling, QSAR, read-across, 3-D/organotypic, 2-D/cell-based, cell-free, transcriptomics; microphysiological systems (i.e., organ-on-a-chip), high throughput/high content methods, and others

New Approach Methodologies: Frameworks for Use

Adverse Outcome Pathways (AOP)

"A model that identifies the sequence of molecular and cellular events required to produce a toxic effect when an organism is exposed to a substance" (NICEATM, 2022).

"AOPs are intended to help regulatory agencies and risk assessors utilise a broader range of mechanistic data concerning the effects of stressors on various test systems (e.g., *in silico*, *in vitro*, *in vivo*) for decision-making" (OECD 2021).

Integrated Approach to Testing and Assessment (IATA)

"Pragmatic, science-based approaches for chemical hazard or risk characterisation that rely on an integrated analysis of existing information in a weight of evidence assessment coupled with the generation of new information using testing strategies" (OECD 2017).

"An IATA can include a combination of methods and can be informed by integrating results from one or many methodological approaches…" (OECD 2017).

New Approach Methodologies at the FDA

- Most current alternative methods cannot predict effects that occur in highly complex interacting systems
- This is especially true for animal tests that include repeated-dosing and evaluation of multiple-endpoints
- Additional research is necessary before alternative methods are able to be used routinely for addressing such complex issues
- However, FDA has active efforts to promote the development of alternative test methods to support the replacement, reduction and/or refinement of animal testing

Advancing New Alternative Methodologies at FDA, 2021

New Approach Methodologies at the FDA

- FDA's Alternative Methods Working Group
  - Public-facing website contains FDA co-authored publications and presentations
  - *Activities are informational; do not serve as official regulatory guidance*

- FDA's Predictive Toxicology Roadmap (2017) and Advancing New Alternative Methods at FDA (2021)
  - Development and evaluation of emerging toxicological methods and incorporation into FDA regulatory review

- Partnerships
  - Tox21 Consortium, ICCVAM, OECD, EU-ToxRisk, and others

- Input and comments can be sent to alternatives@FDA.hhs.gov

New Approach Methodologies in OFAS: Color Additive Petitions

To date, most color additive petitions reviewed by OFAS have contained few new approach methodologies as part of the safety package.

However, two examples may be instructive as case studies.

- Soy leghemoglobin
- Silver nitrate

New Approach Methodologies in OFAS: Soy Leghemoglobin

Color Additive: Soy leghemoglobin (hemeprotein present in soybean root)

- Reddish-brown coloring in ground beef analog products

Safety Package: Weight-of-Evidence Approach

1. History of consumption: soy, soy leghemoglobin protein, and *P. pastoris*
2. Safety of *P. pastoris* as a production strain
3. Feeding studies (14- and 28-day) in rats
4. Mutagenicity and genotoxicity
5. Allergy assessment (significant part of the safety assessment)

"Old" New Approach Methodology: Bioinformatics and *in vitro* digestion

- Sequence alignment and support-vector machine (SVM) learning algorithm
- Pepsin degradation in simulated gastric fluid

New Approach Methodologies in OFAS: Silver Nitrate

Color Additive: Silver nitrate to color eyebrows and eyelashes

Safety Package (including, but not limited to):

1. Cosmetic product components: Narrative including published data and information
2. Cosmetic product: Intended use study in human subjects with evaluation by dermatologist and ophthalmologist
3. *In vitro* assays for ocular irritation

New Approach Methodology: OECD test guideline methods for eye irritation/eye damage; full study reports and narrative provided in petition

OECD Test Guideline 492: **Reconstructed Human Cornea-Like Epithelium (Rhce)** Test Method for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage

OECD Test Guideline 437**: Bovine Corneal Opacity and Permeability Test Method** for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage

New Approach Methodologies in OFAS: Silver Nitrate

The petitioner…

- Utilized validated test methods for *in vitro* assays
- Provided a discussion of the mechanistic understanding which underpinned the endpoints of the *in vitro* assays
- Utilized more than one *in vitro* assay
- Performed the Bovine Corneal Opacity and Permeability assay with optional histology
- ✓ Discussed traditional toxicology data **as well as** data from the NAMs

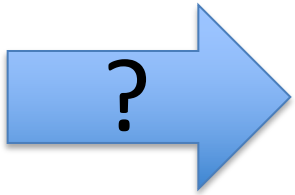
The FDA…

- The *in vitro* assays provided only limited value to the safety assessment → assay interference
- However, histopathological results from the bovine corneas did not reveal any significant physical effects or potential for damage

New Approach Methodologies: General Considerations

- The scope and depth of new approach methodologies is expanding, but acceptance by regulatory agencies takes time.
- The FDA Redbook recommends types of toxicological testing to support the safety standard for color additives.
- While specific types of testing are not required, the safety package must establish reasonable certainty of no harm for FDA scientists.
- FDA's product centers have different legal authorities for evaluating product safety.

What is the context of use?
What are the performance standards?



Safety Standard:
Reasonable certainty of no harm

New Approach Methodologies: General Considerations

Context of Use:

A clearly articulated description delineating the manner and purpose of the use of the assay/tool/method (i.e., when and how it will be used)

Performance standards:

- Applicability
- Limitations
- Relevance
- Reliability
- Reproducibility
- Sensitivity
- Others

FDA must be able to evaluate information about the assay/test/tool

Not intended to be guidance!

FDA's Predictive Toxicology Roadmap, 2017

New Approach Methodologies:
Bovine Corneal Opacity and Permeability

Applicability: Can correctly identify chemicals inducing serious eye damage as well as those not requiring classification for eye irritation or serious eye damage.

Sensitivity/reliability: When used to identify chemicals inducing serious eye damage

- Overall accuracy: 79% (85%)
- False positive rate: 25% (20%)
- False negative rate: 14% (8%)
- Compared to *in vivo* rabbit eye test

Limitations:

- High false positive rate for alcohols and ketones
- High false negative rate for solids
- Cannot evaluate reversibility of corneal lesions
- Does not allow for an assessment of potential for systemic toxicity with ocular exposure

OECD, 2020

New Approach Methodologies in OFAS: Color Additive Petitions

Final Thoughts

- With few exceptions, petitioners have not submitted dossiers for color additives containing data from new approach methodologies
- The safety standard remains the same regardless of the data package: **Reasonable certainty of no harm**
- The burden of proof is on the petitioner
- Use best practices; stay up to date with the state of the science
- Anticipate how to answer FDA's questions on alternative data based on the best available science
- A weight-of-evidence approach remains a good strategy

Engage with us!

Petitioners interested in submitting a dossier using a new approach methodology or other alternative data are invited to discuss their proposal with us during a pre-petition consultation.

Pre-petition consultations can be scheduled by emailing:

premarkt@fda.hhs.gov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