

# Nonclinical Toxicity Assessment of Oral Tobacco- Derived Nicotine Products: IV. *In Vitro* Regulatory Testing

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**CORESTA Smoke-Techno (SSPT) Conference - [ST 60]**

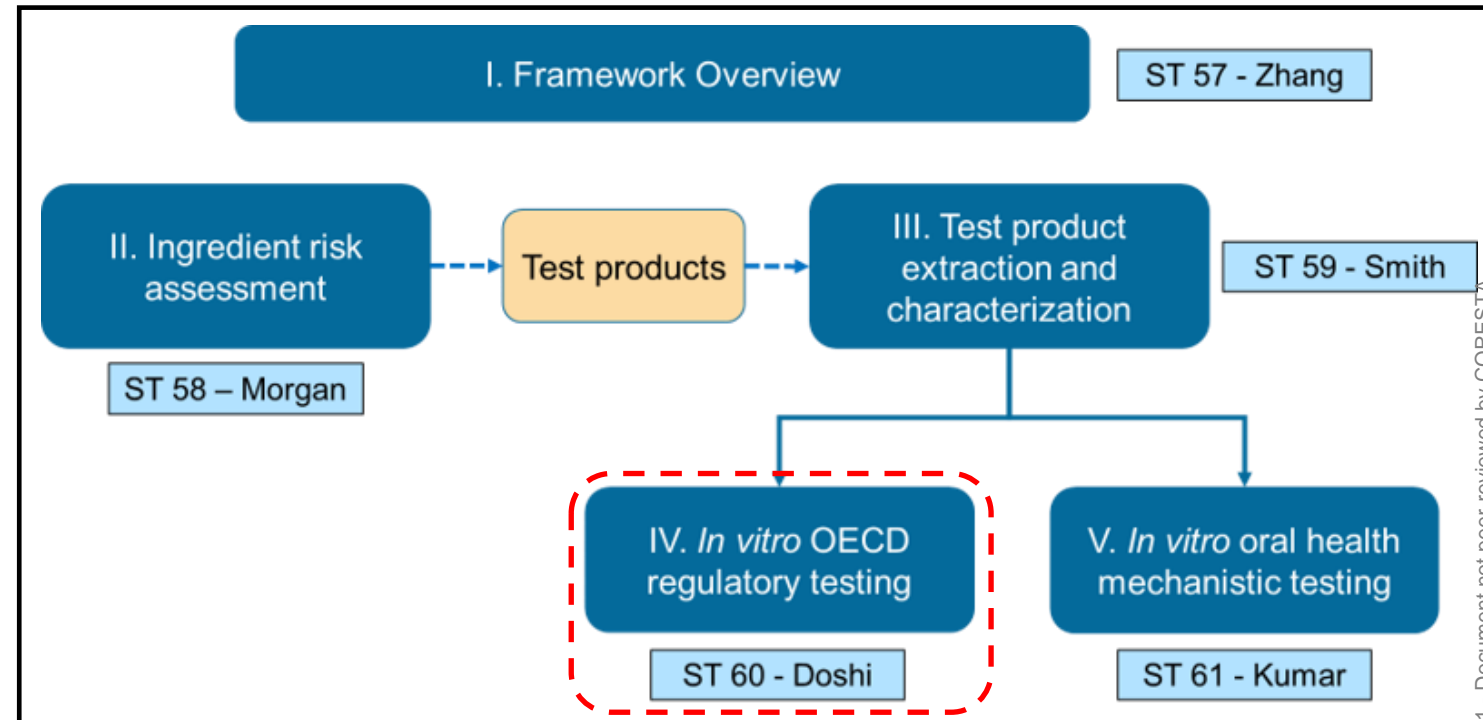
October 27, 2021





# Agenda

- Introduction
- *In Vitro* Assays
  - Test Articles & Study Design
  - Results
- Summary



# Tobacco Harm Reduction Framework



## The Product

- Manufacturing and quality control
- Physical and chemical characterization
- Product stability
- Hazard assessment

## Exposure and Health Risk

- Literature reviews
- *In vitro* studies
- *In vivo* studies
- Risk assessments
- Relative risk comparison across and within tobacco product categories
- Clinical studies

## Impact on the Population

- Secondary analysis of national data
- Risk perception, behavioral intention and product appeal
- Actual use study
- Product instructions comprehension
- Population modeling
- Post-market surveillance

# Introduction: *In Vitro* Regulatory Assays

- The Oral Tobacco-Derived Nicotine (OTDN) product category offers alternative oral nicotine products that do not contain tobacco
- Biological activity of the whole product (extract) is evaluated for cytotoxic and genotoxic potential
- Standard regulatory *in vitro* battery
  - Cytotoxicity: e.g., Neutral Red Uptake (NRU)/MTT Assay-mouse fibroblast cells (3T3)<sup>1</sup>
  - Genotoxicity
    - Salmonella Mutagenicity (Ames) Assay<sup>2</sup>: 5 strains ± S9 (TA:98,100,102,1535,1537)
    - Micronucleus (MN) Assay<sup>3</sup>: human lymphoblast cells (TK6), 3 conditions (4h±S9, 27h-S9)
- Objectives
  - Evaluate toxicity of oral products using different methods of extraction
  - Compare toxicity profile of various product extracts across categories and to cigarette smoke

# In Vitro Assays: Test Articles & Study Design

## Ethanol Extracts

- Test Articles:
  - 3R4F, CRP 2.1 (reference MST), CRP 1.1(reference Snus) & OTDN-6 mg nicotine (mint)
- Maximum Dose
  - Ames: 100  $\mu$ L/plate
  - MN & NRU: 1 % v/v of extract

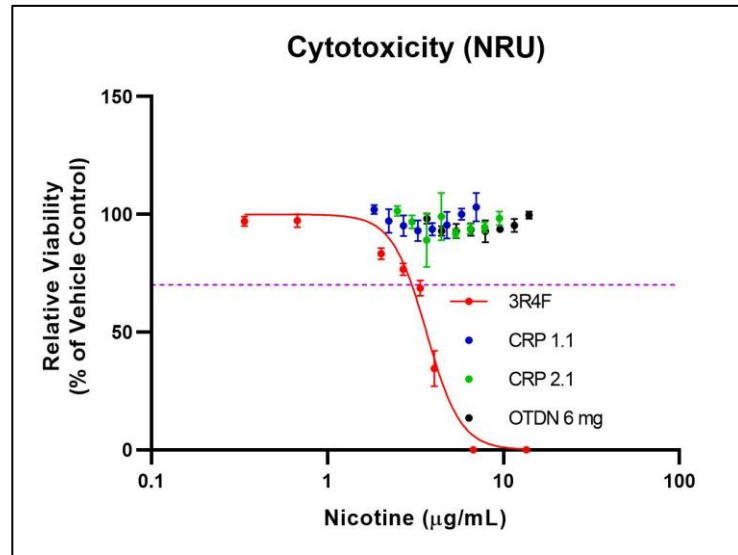
## Artificial Saliva Extracts

- Test Articles:
  - 3R4F\*, CRP 2.1, CRP 1.1, OTDN-6 mg & **OTDN-2 mg (citrus):** NRU & MTT
  - CRP 2.1, OTDN-6 mg, **OTDN-2 mg:** Ames & MN
- Maximum Dose
  - Ames: **250  $\mu$ L/plate**
  - MN & NRU: **25 % v/v of extract**

\* 3R4F was collected in ethanol, but dilutions were prepared in 1% EtOH/24% AS containing media

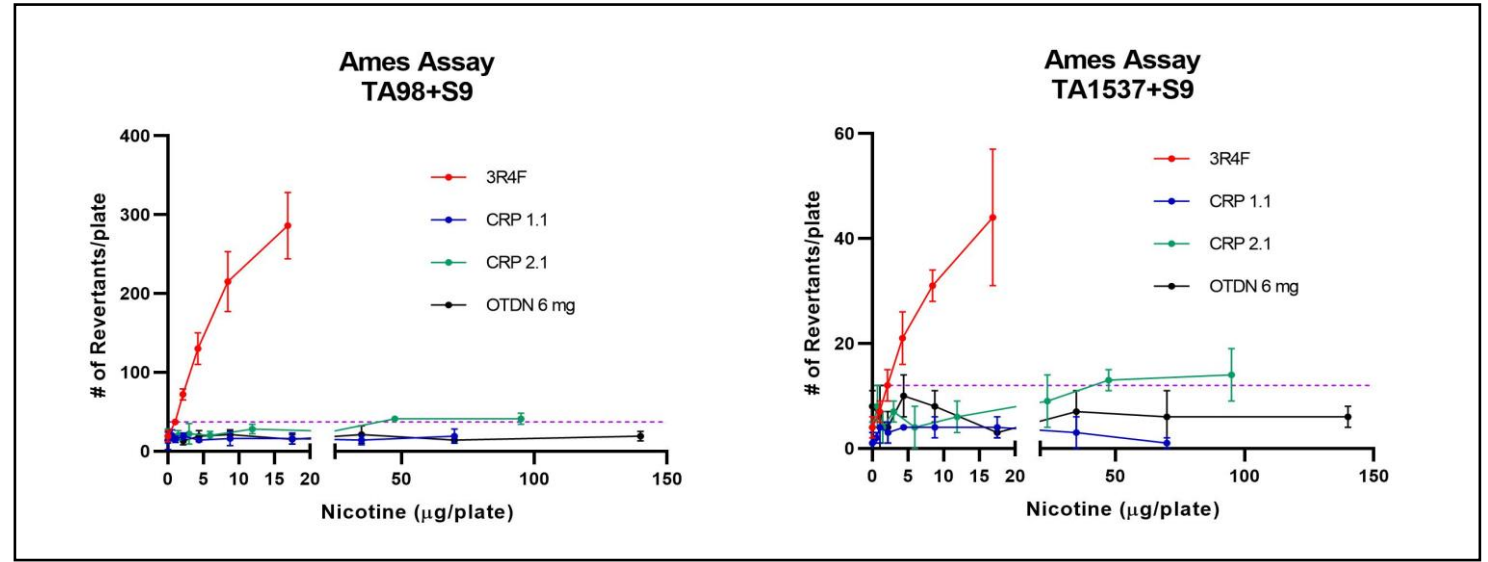
# Results: Ethanol Extracts

## ■ Cytotoxicity & Mutagenicity Assay



--- 70% toxicity

3R4F >>> Oral



--- 95% CI-upper limit for vehicle control

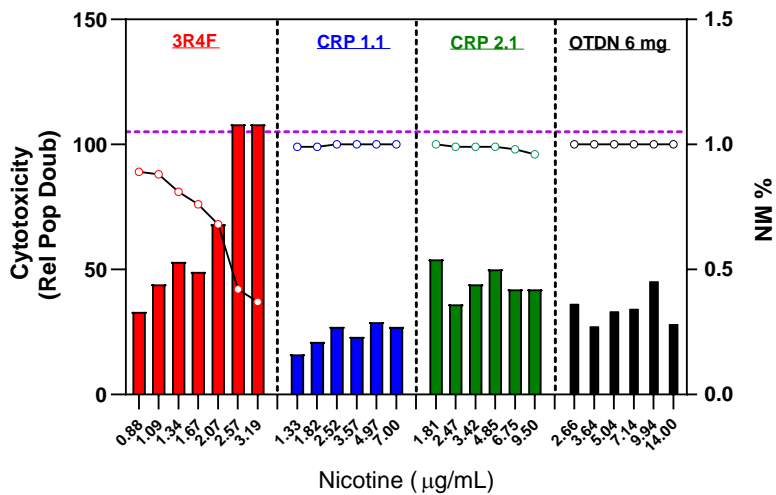
3R4F >>> CRP2.1 > CRP1.1/OTDN\*

\* Based on tested OTDN products

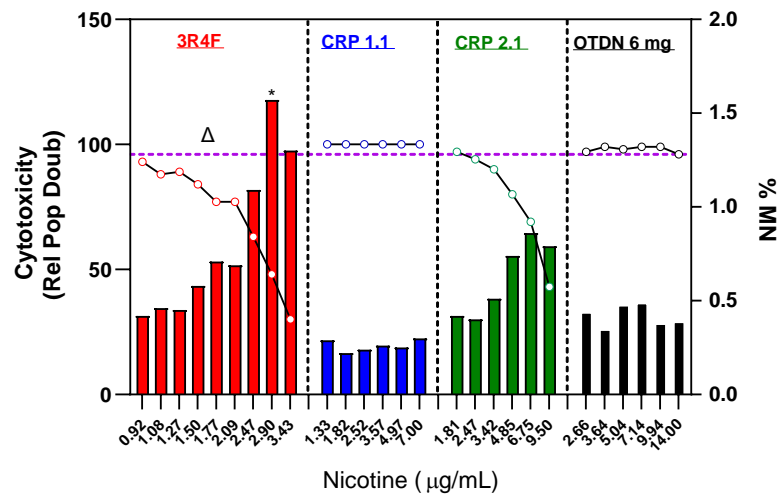
# Results: Ethanol Extracts

## Genotoxicity (MN)

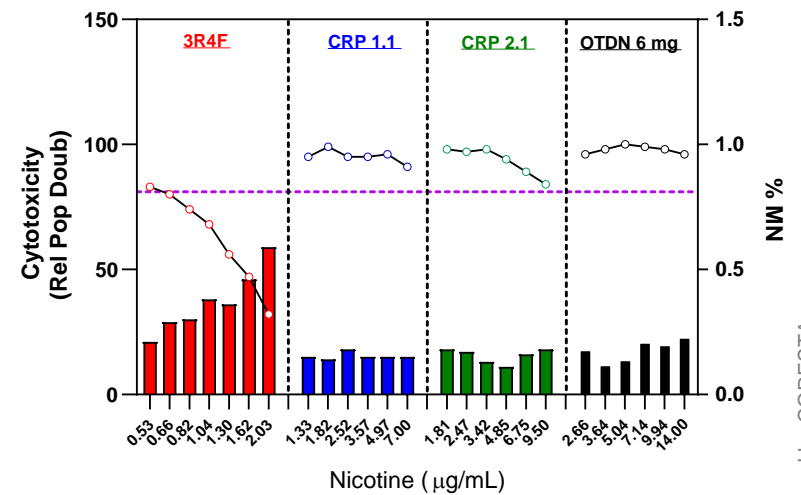
**Micronucleus Assay  
4h-S9**



**Micronucleus Assay  
4h+S9**



**Micronucleus Assay  
27h-S9**



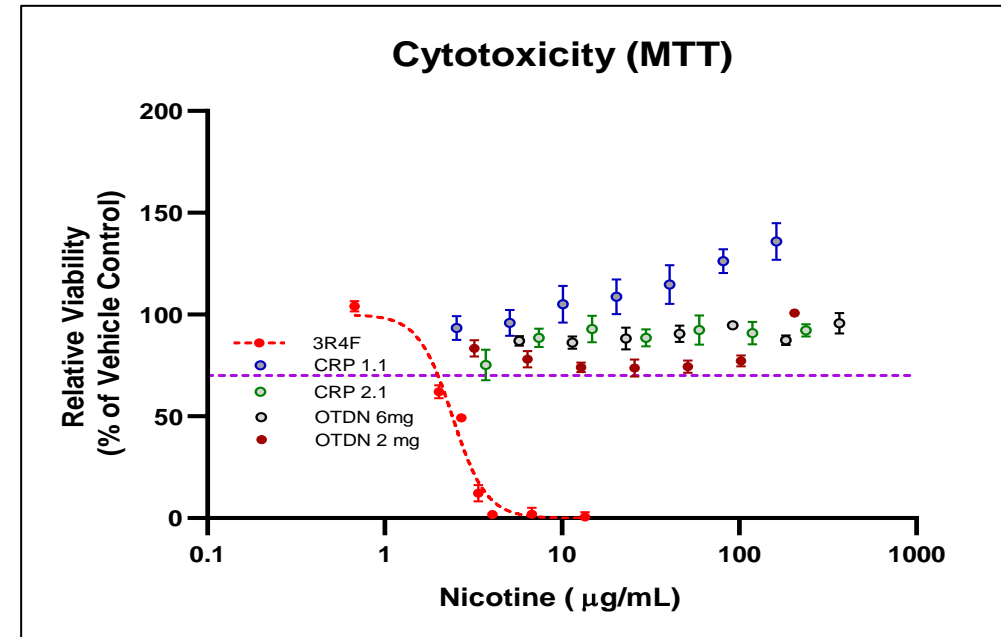
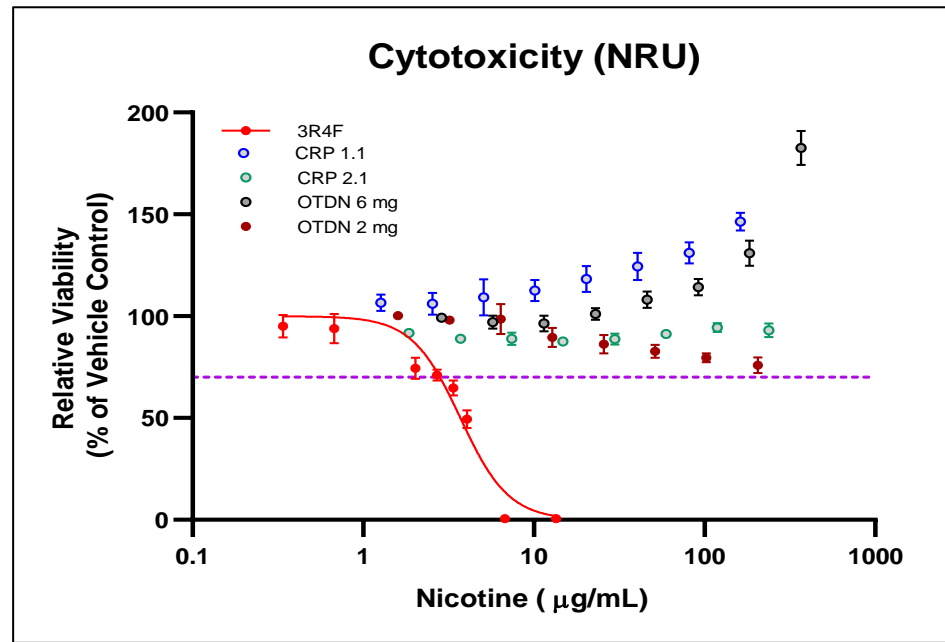
\* Statistical Significance compared to vehicle control; Δ Dose response (trend); - - - 95% CI-upper limit for vehicle control

**3R4F >>> CRP2.1 > CRP1.1/OTDN\***



# Results: Artificial Saliva Extracts

## ■ Cytotoxicity

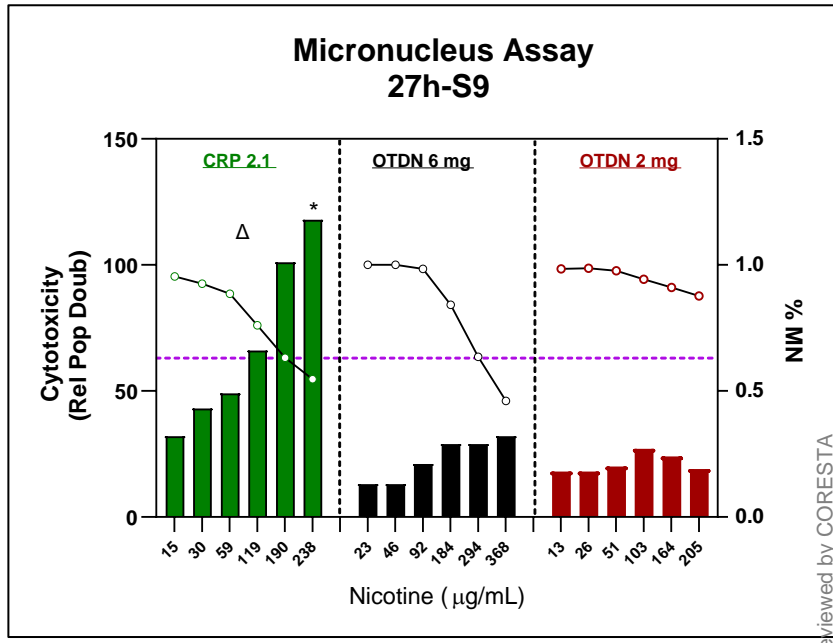
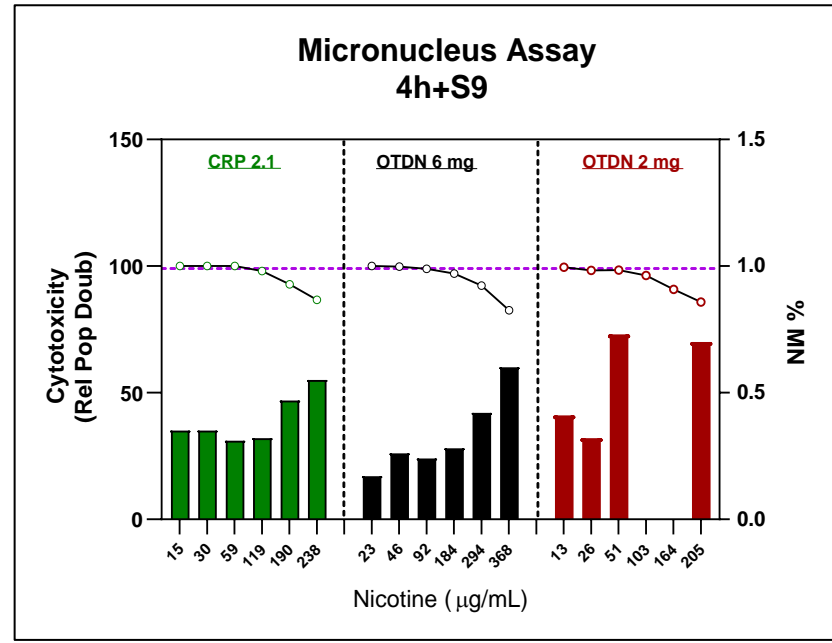
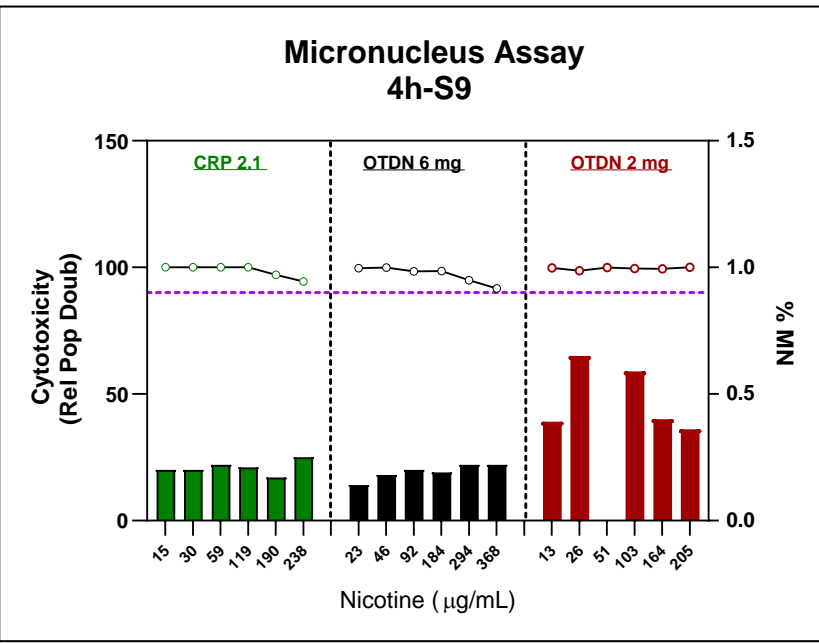


## ■ Mutagenicity: All 3 test articles were negative in the Ames assay



# Results: Artificial Saliva Extracts

## ■ Genotoxicity (MN)



\* Statistical Significance compared to vehicle control; Δ Dose response (trend); --- 95 % CI-upper limit for vehicle control

CRP2.1 > OTDN\*



\* Based on tested OTDN products



# Summary

- Different extraction methods were used to evaluate product comparisons in *in vitro* testing of inhalable and oral tobacco products
- Differences in biological activities between combustible cigarettes and oral tobacco products are demonstrated
  - 3R4F is mutagenic, genotoxic and cytotoxic
  - CRP 2.1 is mutagenic (EtOH extract) and genotoxic (AS extract)
  - Snus and OTDN (2 market products) are non-mutagenic, non-genotoxic and non-cytotoxic at the concentration tested
- Based on the market products tested, experimental *in vitro* regulatory testing results demonstrated substantially lower biological activity of OTDN products, in comparison to cigarette smoke condensate.



# Reference

1. OECD. (2010). OECD Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests
2. OECD. (2020). OECD Guideline for Testing Chemicals Test Guideline 471, Bacterial Reverse Mutation Test
3. OECD. (2016). OECD Guideline for Testing Chemicals Test Guideline 487, In vitro Mammalian Cell Micronucleus Test

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**Questions? Email us at  
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