Chemours Consent Order Toxicology Studies:

28-Day Immuno-Toxicity Study of NafionBP2 in Sprague Dawley Rats

NC Secretaries' Science Advisory Board Meeting
October 1, 2025

Frannie Nilsen, PhD
DEQ Environmental Toxicologist

Rodent Toxicity Studies

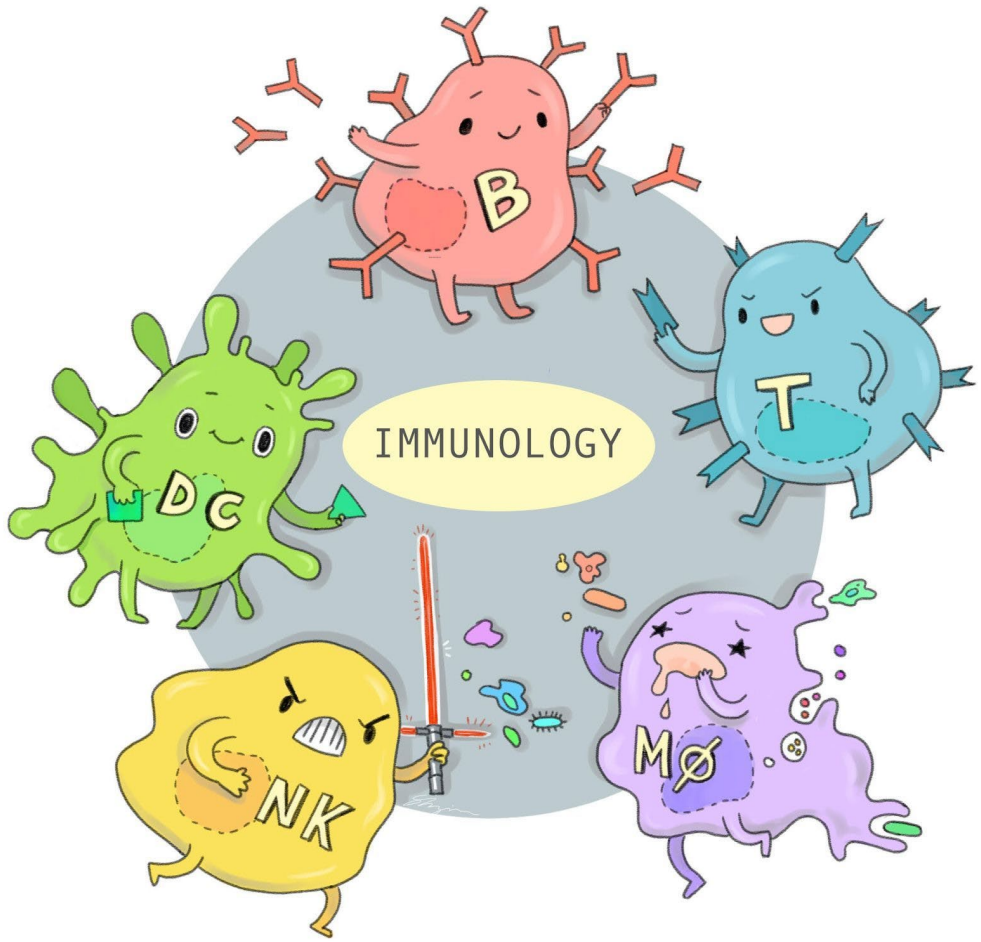
- Mice and rats have:
  - genetic similarities to humans,
  - ease of handling,
  - rapid reproduction rates.
- Crucial in advancing medical research.
- Animal studies allow empirical examination of PFAS exposure and the determination of isolated health effects.
- Toxicity is dependent on both intrinsic potency of the compound and its toxicokinetics.

NORTH CAROLINA Department of Environmental Quality DEQ

Immune System Toxicology

Immunotoxicology

- The study of the toxicity of foreign substances, and their effects on the immune system.
- These substances can alter both the innate and adaptive parts of the immune system, leading to immunosuppression, hypersensitivity, and autoimmunity.
- Immunotoxicity tests designed to evaluate immune function and disease resistance are carried out using in vitro methods, cultured mammalian cells, and rodent models.



28-Day Immunotoxicity Test

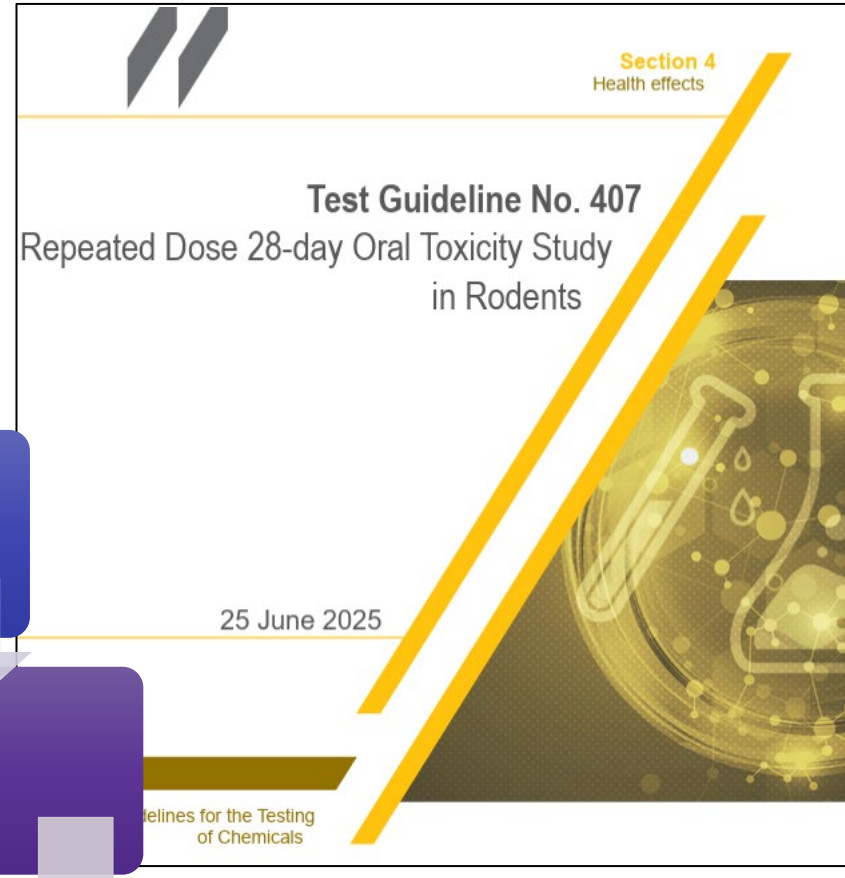
Based on an internationally recognized test method.

The test chemical is orally administered daily to groups of experimental animals for 28 days.

A 28-day study provides information on the effects of repeated oral exposure and can indicate the need for longer-term studies.

It can also provide information on the selection of concentrations for longer term studies.

The data should provide the dose response relationship and the determination of the No-Observed Adverse Effect Level (NOAEL).



NORTH CAROLINA
Department of Environmental Quality

Nafion BP2 28-day Immunotox Test

- The objective of this study was to determine the potential toxicity of Nafion©BP2 (CAS No. 749836-20-2), when administered by oral gavage for 28 days to Sprague Dawley rats.

- Final doses determined using available literature, a dose-range-finding pre-experiment, and agreed upon by DEQ, Chemours and Charles River Labs.

Group Number	Test and Control Materials	Dose Level (mg/kg/day)
1	Negative Control (diluent)	0
2	Nafion BP2	0.3
3	Nafion BP2	3
4	Nafion BP2	10
5	Positive Control (Nafion BP2)	50

Nafion BP2 28-day Immunotox Test

Potential immunotoxicity of the test substance were determined.

=

T-cell dependent antibody response (TDAR)

+

Others if possible:

Thymus/spleen weights, spleen immuno-phenotyping.

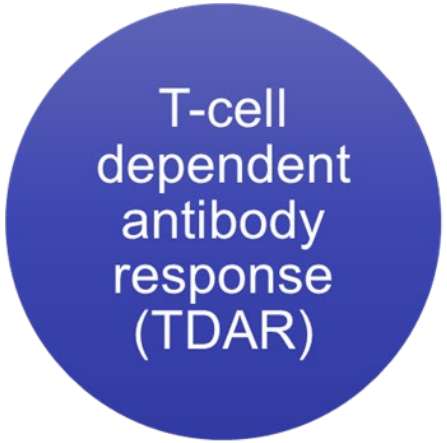
Experimental Design Details

Doses:	• administered once daily, for 28 consecutive days via oral gavage
Animal Care:	• Daily Observations and Measurements, Ophthalmic Examinations, Neurobehavioral and Motor Activity Assessments, • ***TDAR:*** animals immunized with sRBC via the tail vein on Day 24.
Samples:	• Blood and Urine, Necropsy Tissues
Sample Testing:	• Blood panel and clinical chemistry, urinalysis, Bone marrow evaluation, TDAR assay, Tissue Histology, organ weights, splenic phenotyping.

Observation/Test Results Summary

No Effect Reported

- Clinical observations
- Ophthalmic Examinations
- Activity Observations
- Autonomic Observations
- Excitability Observations
- Neuromuscular Observations
- Physiological Observations
- Sensorimotor Observations
- Motor Activity
- Coagulation
- Urinalysis
- Splenic Immunophenotyping



Observation/Test Results Summary
No Substance-related Effects
TDAR Assay

- No statistical differences in dose groups compared to control group.
  - Positive control demonstrated ability to detect an immune response
- Changes were observed that were not NBP2-related:
  - Lower mean IgM values in 0.3 and 3 mg/kg/day group females were unrelated to NBP2, due to lack of a dose-response.
  - Higher mean IgM values in the males was attributed to biological variation and indicated the lack of immunosuppressive effects.
- There were no NBP2-related effects on the T-Cell dependent antibody response at doses on 0.3, 3, and 10 mg/kg/day in males and females.

Observation/Test Results Summary
Reported Changes

Dose Level (mg/kg/day)	Sex	Reported NBP2-related Effects (Dose + Sex-Specific)	Reported NBP2-related Effects (Dose-Specific)
0.3	Males	Clinical Chemistry: ALT, TRIG	Slight Bone Marrow marker changes
	Females	*none*	
3	Males	Clinical Chemistry: ALT*, TRIG*, GLUC, CHOL	• Blood cell (RBC),reticulocyte (RETIC) and platelet counts (PLT) changes • Slight Bone Marrow marker changes
	Females	Clinical Chemistry: TRIG	
10	Males	• Slight Bone Marrow marker changes • Pale focus in liver with necrosis • Clinical Chemistry: ALT*, TRIG*, UREAN*, CHOL, TPROT*, ALB*, A/G, PHOS	Blood cell (RBC),reticulocyte (RETIC) and platelet counts (PLT), hemoglobin (HGB) and hematocrit (HCT) changes
	Females	Clinical Chemistry: TRIG	

* Statistically Significant

Observation/Test Results Summary
Reported Increases & Decreases

Dose Level (mg/kg/day)	Sex	Reported NBP2-related Effects (Dose + Sex-Specific)	Reported NBP2-related Effects (Dose-Specific)
0.3	Males	*none*	*none*
	Females	*none*	
3	Males	• Increased: Liver weight, size, and cellular changes [A] • Decreased: body weights	*none*
	Females	Increased: thyroid/parathyroid gland weights [N]	
10	Males	Decreased: body weights*, food consumption*	Increased: Liver weight size, and cellular changes [A]
	Females	Increased: thyroid/parathyroid gland weights [N]	

* Statistically Significant

N= non-adverse; no cellular changes; A=adverse, cellular changes observed

Nafion BP2 28-day Immunotox Test Conclusions

1. There were no NBP2-related changes in TDAR or splenic immunophenotyping parameters that indicated immunosuppressive effects.

2. Based on these results, the no-observed-adverse-effect level (NOAEL) was considered to be 0.3 mg/kg/day in males and 10 mg/kg/day in females.

Overall Summary

NBP2 did not elicit immuno-toxic effects in this 28-day test.

- TDAR and splenic immunophenotyping assays used.

The 28-day test did show NBP2-related changes that are not specific to the immune system:

- Decreased body weight,
- Increased liver weight,
- Changes to liver cells, and
- Clinical chemistry associated with liver damage.

These results will help us interpret the 90-day toxicity tests, which also include liver analyses.

Thank you

Department of Environmental Quality