Chemours' Proposed Toxicity Study Work Plan
Pursuant to Paragraph 14 of the Consent Order

I. IDENTIFICATION OF TEST SUBSTANCES

As stated in Attachment B to the Consent Order, the test substances identified for mammalian and ecological toxicity testing are as follows:

Common Name	Chemical Name	CAS Number
PFMOAA	Pefluoro-2-methoxyacetic acid	674-13-5
PFO2HXA	Perfluoro(3,5-dioxahexanoic)acid	39492-88-1
PFESA-BP2/Nafion BP #2	Nafion Byproduct 2	749836-20-2
PMPA	Perfluoro-2-methoxypropanoic acid	13140-29-9
PEPA	2,3,3,3-tetrafluoro-2-(pentafluoroethoxy)propanoic acid	267239-61-2

II. IDENTIFICATION OF TESTING GUIDELINES

In order to comply with Paragraph 14 of the Consent Order, Chemours proposes to use the following test guidelines when conducting the toxicity studies:

Toxicity Study	Identified Guideline
28-day oral immunotoxicity study in rats	OPPTS 870.7800
28-day oral immunotoxicity study in mice	OPPTS 870.7800
90-day repeated dose oral toxicity study in rats	OECD 408
90-day repeated dose oral toxicity study in mice	OECD 408
Algal acute (72-hour growth) toxicity study	OECD 201
Daphnid acute toxicity study	OECD 202
Daphnid chronic (reproduction) toxicity study	OECD 211
Fish acute toxicity study	OECD 203
Sediment 10-day freshwater invertebrates toxicity test	OECD 225

III. TEST GUIDELINE SUMMARIES

A. Mammalian Studies

1. Toxicity Study: 28-day oral immunotoxicity studies in rats and mice

Guideline Summary: EPA Health Effects Test Guidelines OPPTS 870.7800 Immunotoxicity

This guideline is intended to provide information on suppression of the immune system which might occur as a result of repeated exposure to a test chemical. In order to obtain data on the functional responsiveness of major components of the immune system to a T cell dependent antigen, sheep red blood cells (SRBC), rats and/or mice must be exposed to the test and control substances for at least twenty-eight days. The animals must be immunized by intravenous or intraperitoneal injection of SRBCs for approximately four days (depending on the strain of animal) prior to the end of the exposure. At the end of the exposure period, either the plaque forming cell (PFC) assay or an enzyme linked immunosorbent assay (ELISA) must be performed to determine the effects of the test substance on the splenic anti-SRBC (IgM) response or serum anti-SRBC IgM levels, respectively. In the event the test substance produces significant suppression of the anti-SRBC response, expression of phenotypic markers for major lymphocyte populations (total T and total B), and T cell subpopulations (T helpers (CD4) and T cytotoxic/suppressors (CD8)), as assessed by flow cytometry, may be performed to determine the effects of the test substance on either splenic or peripheral-blood lymphocyte populations and T cell subpopulations. When this study is performed, the appropriate monoclonal antibodies for the species being tested should be used. If the test substance has no significant effect on the anti-SRBC assay, a functional test for NK cells may be performed to test for a chemical's effect on non-specific immunity. For tests performed using cells or sera from blood (ELISA or flow cytometry), it is not necessary to destroy the animals, since immunization with SRBCs at twenty-eight days is not expected to markedly affect the results of other assays included in sub-chronic or longer-term studies. The necessity to perform either a quantitative analysis of the effects of a chemical on the numbers of cells in major lymphocyte populations and T cell subpopulations by flow cytometry, or a splenic NK cell activity assay to assess the effects of the test compound on non-specific immunity, should be determined on a case-by-case basis, depending upon the outcome of the anti-SRBC assay.

2. Toxicity Study: 90-day repeated dose oral toxicity study in rats and mice

Guideline Summary: OECD Test Guideline No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents

This guideline provides information on health hazards likely to arise from exposure to the test substance via oral administration. The determination of sub-chronic oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained from acute or repeated dose 28-day toxicity tests. The method is based on the repeated oral administration of the test substance over a prolonged period (one dose level daily during 90 days). This test guideline is intended primarily for use with rodents (preferably rats). At least 20 animals (10 female and 10 male) should be used for each test group. Three concentrations of the

test substance, at least, should be used. The test compound is administered by gavage or via the diet or drinking water. A limit test may be performed if no effects would be expected at a dose of 1000 mg/kg bw/d. The results of this study include: 1) measurements, including weighing at least once a week, as well as assessment of food and water consumption; 2) daily and detailed observations (i.e. of ophthalmological examination, haematology, clinical biochemistry and urinalysis), preferably collected at the same time each day; and 3) gross necropsy and histopathology. A number of endocrine-related measurements, particularly relevant to thyroid function, have been added in 2018. A properly conducted 90-day sub-chronic test should provide a satisfactory estimation of a no-effect level.

B. Ecotoxicological Studies

1. Algal acute (72-hour growth) toxicity study

Guideline Summary: OECD Test Guideline No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test

The purpose of this test is to determine the effects of the test substance on the growth of freshwater microalgae and/or cyanobacteria. Exponentially growing test organisms are exposed to the test substance in batch cultures, typically over a period of 72 hours. The system response is the reduction of growth in a series of algal cultures exposed to at least five concentrations of a test substance. Three replicates at each test concentration should be used. The response is evaluated as a function of the exposure concentration in comparison with the average growth of control cultures. The cultures are allowed unrestricted exponential growth under nutrient sufficient conditions (two alternative growth media: the OECD and the AAP) and continuous fluorescent illumination. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. The limit test corresponds to one dose level of 100 mg/L. This study includes: 1) the determination, at least daily, of the algal biomass; 2) the measure of pH (at the beginning and at the end); and 3) microscopic observation. This test guideline describes two response variables: average specific growth rate and yield.

2. Daphnid acute toxicity study

Guideline Summary: OECD Test Guideline No. 202: Daphnia Acute Immobilization Test

This test guideline describes an acute toxicity test to assess effects of chemicals on daphnids (usually Daphnia magna Staus). Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations (at least five concentrations) for a period of 48 hours. Immobilization is recorded at 24 hours and 48 hours and compared with control values. The results are analyzed in order to calculate the EC50 at 48 hours. Determination of the EC50 at 24 hours is optional. At least 20 animals, preferably divided into four groups of five animals each, should be used at each test concentration and for the controls. At least 2 ml of test solution should be provided for each animal (i.e. a volume of 10 ml for five daphnids per test vessel). The limit test corresponds to one dose level of 100 mg/L. The study report should include the observation for immobilized daphnids at 24 and 48 hours after

the beginning of the test and should also include the measures of dissolved oxygen, pH, and concentration of the test substance at the beginning and end of the test.

3. Fish acute toxicity study

Guideline Summary: OECD Test Guideline No. 203: Fish, Acute Toxicity Test

In this test, fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 percent of the fish (LC50) are determined where possible. One or more species may be used, the choice being at the discretion of the testing laboratory. At least seven fishes must be used at each test concentration and in the controls. The test substance should be administered to at least five concentrations in a geometric series with a factor preferably not exceeding 2.2. The limit test corresponds to one dose level of 100 mg/L. This study includes the observations of fish at least after 24, 48, 72 and 96 hours. The cumulative percentage mortality for each exposure period is plotted against concentration on logarithmic probability paper.

4. Daphnid chronic (reproduction) toxicity study

Guideline Summary: OECD Test Guideline No. 211: Daphnia magna Reproduction Test

This test method assesses the effect of chemicals on the reproductive output of Daphnia magna Straus. To this end, young female Daphnia are exposed to the test substance added to water at a range of concentrations (at least five). For semi-static tests, at least 10 animals at each test concentration and for flow-through tests, 40 animals divided into four groups of 10 animals at each test concentration are used. The test duration is 21 days. The total number of living offspring produced per parent animal that do not die accidentally during the test and the number of living offspring produced per surviving parent animal at the end of the test are reported. The study report also includes: 1) daily counting of the offspring; 2) daily recording of the parent mortality; 3) weekly measurement of oxygen concentration, temperature, hardness and pH values; and 4) determination of the concentrations of test substance. Optionally, other effects can be reported, including the sex ratio of the offspring. The reproductive output of the animals exposed to the test substance is analyzed by comparing it with that of the control in order to determine the lowest observed effect concentration (LOEC) and hence the no observed effect concentration (NOEC), and by estimating the concentration that causes a reduction in reproductive output by means of a regression analysis.

5. Sediment 10-day freshwater invertebrates toxicity test

Guideline Summary: OECD Test Guideline No. 225: Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment

This test guideline is designed to assess the effects of prolonged exposure to sediment-associated chemicals on the reproduction and the biomass of the endobenthic oligochaete Lumbriculus variegatus (Müller). The method is described for static test conditions. Worms of similar physiological state are exposed to a series of toxicant concentrations applied to the

sediment phase of a sediment-water system (artificial sediment amended with a food source and reconstituted water). Test vessels without the test substance serve as controls. The test animals are exposed to the sediment-water systems for a period of 28 days. The endpoints of this type of study are the ECx for reproduction and biomass. In addition, the No Observed Effect Concentration (NOEC), and the Lowest Observed Effect Concentration (LOEC) may be calculated. The purpose of the study, ECx or NOEC derivation, will determine the test design. At least five concentrations and a minimum of three replicates for ECx, or four replicates for NOEC/LOEC, for each concentration should be tested. The test is conducted with at least 10 worms for each replicate. The report should include the total number and the dry weight of the worms, as well as observations of abnormal behavior, mortalities, water characteristics within the test vessels, and the total organic content.

IV. DOSE SELECTION

The amount of test substance needed for the work outlined in Attachment B to the Consent Order is approximately 10 – 15 kg per chemical. Since none of these test substances are commercial products, each one will require a custom synthesis followed by a detailed analysis in order to establish the necessary levels and identify any impurities. Chemours estimates that the synthesis and analysis will take at least one year. In order to avoid complications from batch-to-batch inconsistencies, no toxicology studies can be performed until the full amounts are available.

Chemours proposes a modified study design using dose levels of 0.1 mg/kg/day and 1 mg/kg/day in both rats and mice for both the 28-day and 90-day studies. The 0.1 mg/kg/day dose corresponds to the rodent point of departure derived for the substance known as GenX in the development of the health advisory goal of 140 ppt by the North Carolina Department of Health and Human Services. Using these doses in the rodent studies accomplishes several goals:

- A substantial reduction (approximately 25%) in the number of rats and mice that must be killed
- A substantial reduction in the amount of test substance required (approximately 100 grams instead of multiple kilograms)
- The ability to start the toxicology studies sooner, and thus obtain data more quickly
- Quicker development of a drinking water standard

Using this proposal, a substance that shows no effect at 0.1 mg/kg/day (the GenX rodent point of departure) would be considered toxicologically similar to GenX itself. Therefore, the current health advisory goal value for Gen X (140 ppt), which was developed by experts in North Carolina based on an extensive toxicological data set, could be applied to these other substances for which less data is available. Substances that show a significant, human-relevant toxicological effect at a dose of 0.1 mg/kg/day would be candidates for additional work.

If no toxicity is observed at the higher dose of 1 mg/kg/day, then Chemours proposes that 1 mg/kg/day be used as a conservative point of departure for the development of a health advisory goal. Chemours notes that the actual no-observed-adverse-effect-level could be considerably higher (that is, less toxic) than 1 mg/kg/day, but in the interest of both a quick

turnaround time and animal welfare, Chemours accepts 1 mg/kg/day as the point of departure in this situation.

Using this approach will allow for a reduction in the amount of test chemical that must be synthesized, a faster time to study start, and a reduction in the number of rodents killed, but will still provide conservative points of departure for setting drinking water values for these substances.

V. WORK PLAN AND SCHEDULE

The implementation of the toxicity studies will depend on the availability of the test substances. As noted above, Chemours anticipates that it will take at least one year to complete the synthesis and analysis for each substance (i.e. the substances are not expected to be available before April 1, 2020). In addition, agreement with DEQ regarding the dose levels to be used for the mammalian toxicology studies will enable a more detailed schedule to be developed. Assuming that the contracting labs will be able to draft the appropriate protocol and obtain approval from DEQ for the protocol within 3 months of receiving a particular test substance, Chemours expects that the first toxicity tests will begin around July 1, 2020. The toxicity tests for each chemical may procced once sufficient quantities are available for that particular test substance. However, given the numerous factors that might affect the timeline of toxicity testing, it is difficult to provide a firm start and end date for the toxicity tests discussed herein. Chemours will continue to maintain an open dialogue with DEQ regarding the timeline for the toxicity studies and will provide updates to the estimated timeline as soon as Chemours learns the necessary and relevant information affecting the timeline. The chart at the end of this section provides a summary of Chemours' current estimated timeline.

A. Mammalian Studies

Within three months of the test substances becoming available in sufficient quantity and quality to complete all required studies, the mammalian studies will be contracted. For the mammalian studies, a single Contract Research Organization (CRO) that can perform all four study types will perform the contracted work. Chemours has proposed that Charles River Laboratories perform these studies; DEQ has approved.

The mammalian studies cannot be run in parallel because data collected in the 28-day study will be used to refine the protocol for the 90-day study. Therefore, for each given substance, the 28-day study will be completed prior to the start of the 90-day study. A 28-day study typically takes 6 to 9 months to complete from the time of dosing to the issuance of the final report. A 90-day study typically takes 9 to 12 months to complete from the time of dosing to the issuance of a final report.

When ready, a quantity of test substance sufficient to run all four studies for that particular substance will be shipped to the CRO. The CRO will draft protocols using the relevant OPPT/OECD guideline as a template and submit them to DEQ for approval. Once approved by DEQ, the study will be authorized by Chemours and the study dates will be finalized by the CRO. Chemours will provide a copy of the final protocol and the proposed study schedule to DEQ. Changes from the preliminary schedule resulting in significant delay will be

communicated to DEQ. The final report will be submitted to DEQ one month after finalization by the CRO.

B. Ecotoxicology Studies

Within three months of the test substances becoming available in sufficient quantity and quality to complete all required studies, the ecotoxicology studies will be contracted. For the ecotoxicological studies, a CRO that can perform all five ecotoxicology studies will be used. The studies can be run in parallel with the exception of the Daphnid chronic (reproduction) toxicity study (OECD 211), which is dependent on the results of the Daphnid acute toxicity study (OECD 202). Chemours has proposed that EAG Laboratories perform these studies and is awaiting approval from DEQ.

The algal acute (72-hour growth) toxicity study (OECD 201) and the fish acute toxicity study (OECD 203) each take approximately 6 months to complete from the time of dosing to the report. The Daphnid acute toxicity study (OECD 202) followed by the Daphnid chronic (reproduction) toxicity study (OECD 211) take a combined 10 months to complete (approximately 3 months for the acute study and 7 months for the reproduction study). The sediment 10-day freshwater invertebrates toxicity test (OECD 225) takes approximately 10 months to complete.

When ready, a quantity of test material sufficient to run all five studies for that particular substance will be shipped to the CRO. The CRO will draft protocols using the relevant OECD guideline as a template and submit them to DEQ for approval. Once approved by DEQ, the studies will be authorized by Chemours and the study dates will be finalized by the CRO. Chemours will provide a copy of the final protocol and the study schedule to DEQ. Changes from the preliminary schedule resulting in significant delay will be communicated to DEQ. The final report will be submitted to DEQ one month after finalization by the CRO.

C. Estimated Timeline

The chart below provides an estimated timeline for the start, duration, and completion of the toxicity studies, as well as submission of the related final reports to DEQ. The timeline assumes that the CRO will be able to draft its protocol, obtain approval from DEQ for the protocol, and find capacity within its labs to begin the studies 3 months after receiving any given test substance. Chemours reiterates that the timeline for the toxicity studies depends on many variables that are subject to change (i.e. availability of test substance, lab capacity, approval from DEQ). Nevertheless, the chart below reflects Chemours' current best estimate as to the anticipated timeline.

Toxicity Study	**Estimated Study Start (i.e. Dosing)***	**Estimated CRO Report****	**Estimated Final Report to DEQ**
28-day oral immunotoxicity study in rats	3 months after receiving test substance	12 months after receiving test substance (9 months after dosing)	13 months after receiving test substance
28-day oral immunotoxicity study in mice	3 months after receiving test substance	12 months after receiving test substance (9 months after dosing)	13 months after receiving test substance
90-day repeated dose oral toxicity study in rats	15 months after receiving test substance (3 months after completion of 28-day study)	27 months after receiving test substance (12 months after dosing)	28 months after receiving test substance
90-day repeated dose oral toxicity study in mice	15 months after receiving test substance (3 months after completion of 28-day study)	27 months after receiving test substance (12 months after dosing)	28 months after receiving test substance
Algal acute (72-hour growth) toxicity study	3 months after receiving test substance	9 months after receiving test substance (6 months after dosing)	10 months after receiving test substance
Daphnid acute toxicity study	3 months after receiving test substance	6 months after receiving test substance (3 months after dosing)	7 months after receiving test substance
Daphnid chronic (reproduction) toxicity study	6 months after receiving test substance (upon completion of acute study)	13 months after receiving test substance (7 months after dosing)	14 months after receiving test substance
Fish acute toxicity study	3 months after receiving test substance	9 months after receiving test substance (6 months after dosing)	10 months after receiving test substance
Sediment 10-day freshwater invertebrates toxicity test	3 months after receiving test substance	13 months after receiving test substance (10 months after dosing)	14 months after receiving test substance

*The study start date depends on availability of test substance, lab capacity, approval of protocol from DEQ, analytical capability, and other variables.

**Completion of the CRO's report depends on dosing start date, completion of any necessary prior studies, analytical capability, and other variables.