ROY COOPER
Governor

MICHAEL S. REGAN
Secretary

NORTH CAROLINA
Environmental Quality

May 3, 2019

Mr. Brian D. Long
Plant Manager
Chemours Fayetteville Works
22828 NC Highway 87 W
Fayetteville, NC 28306

RE: Comments on Draft Plan for Toxicity Studies

Dear Mr. Long,

The N.C. Department of Environmental Quality (DEQ) has reviewed Chemours' Proposed Toxicity Study Work Plan dated March 27, 2019 and submitted pursuant to paragraph 14 of the Consent Order. DEQ offer the comments in the attached document.

DEQ is open to a conference call to discuss any questions regarding these comments. We can discuss the need for a conference call on the May 8, 2019 standing weekly status call regarding the implementation of the consent order.

Sincerely,

Sheila C. Holman

Sheila C. Holman
Assistant Secretary for the Environment
NC Department of Environmental Quality

North Carolina Department of Environmental Quality
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Comments on Chemours' Proposed Toxicity Study Work Plan Pursuant to Paragraph 14 of the Consent Order, dated 3/27/2019

General Work Plan comments

1. The objective of this effort is to define a range of statistically significant sensitive adverse effect responses for each chemical and endpoint, i.e. dose-response or concentration-response characterization. No-effect levels or lowest-effect levels (NOEAC, LOAEC) alone will not meet the objectives of the prescribed toxicity testing program.
   1.1. NOAEC, LOAEC, 50% response levels and multiple other effect concentrations (i.e., EC10 to EC90 at 5-10% effect levels), as requested by DEQ, are to be reported.
   1.2. Response statistics are to be performed using appropriate software packages designed for this purpose. Concentration–response plots are to be included for each reported endpoint. Concentration–response plots manually plotted on graphical paper are not an acceptable data reporting method.
   1.3. Ideally, the highest test concentration results in an effect response statistically significantly different from the controls and, the lowest concentration elicits an effect level not statistically different from the controls. Refer to the individual toxicity assay method specifications for guidance on separation factors to design an appropriate geometric series of test treatments that will indicate a range of effects.
   1.4. All assays should include not less than the minimum number of test (PFAS) treatments and controls specified in the individual assay methods. Where the minimum number of treatments is less than 5, DEQ recommends at least 5 test (PFAS) treatments to better define effect-response relationships and to minimize the potential need to repeat assays.
   1.5. All controls should be run concurrent with the test (PFAS) treatments.
2. All test organism cultures, toxicity assays and associated procedures (e.g., culture and test environmental conditions, analyses of test solutions, statistical analyses and criteria) are to satisfy the minimum specifications, performance criteria, quality assurance and quality control requirements as specified in the referenced OECD, OPPT, USEPA or other method references, as well as meeting established GLP protocols.
   2.1. This includes not less than the minimum number of exposure (treatment) concentrations and replicates per treatment concentrations and controls, selected with the objective to provide a range of effect-levels suitable for dose/concentration-response.
   2.2. Any deviations from individual assay performance or QA/QC specifications as outlined in the current method references are to identified in the test-design specifications to be provided to, and approved by, DEQ before the toxicity assays are initiated.
   2.3. Statistical analyses of the treatment and control replicates must meet the performance criteria defined by the individual methods.
3. Data that does not provide a dose/concentration-response suitable for modeling or that does not meet the specifications needed for DEQ to develop regulatory levels will require re-testing.
4. Due to the unique physicochemical properties of the test chemicals (PFAS), the concentration of the test substance is to be measured, at a minimum, at the highest and lowest test concentrations, at the beginning and end of the assay. Control solutions are to be verified as PFAS-free at the beginning and end of the assay. PFAS measurements are to be completed using DEQ-approved analytical methods and are to meet DEQ-approved PFAS sensitivity criteria

(sample reporting limit). Less than ±20% variation is required for a test treatment concentration over the exposure period of the assay.

5. Confirmation by analyses that all test apparatus is PFAS-free is required.
6. Test data reporting is to meet at a minimum the specifications in each method. Individual replicate and treatment/control data recording sheets are to be made available at DEQ's request. All laboratory reports and associated QA/QC for supporting analytical are to available at request.
7. Assays reported to DEQ are to include the most recent quality control and reference standard tests for the test organism cultures, culture media and test dilution media.
8. For mortality endpoint tests (acute) an additional observation at 6-8 hours after organisms are initially exposed to the test and control solutions (i.e., at the end of the work day on the day of test initiation) is to be included with the reported data.
9. At all observation periods any observed mortalities or other observations of abnormal behavior or effects are to be recorded and reported.
10. All assays, including those using mammalian testing organisms, are to be made with previously un-exposed organisms.

Comments on Specific Assay Specifications noted by Chemours

A. Mammalian Studies – no additional comments
B. Ecological Studies –
    a. General comments on the Ecological Studies –
        i. The concentration of the test substance should be measured, at a minimum, at the highest and lowest test concentration and controls, at the beginning and end of the test and at each media change-over. Less than ±20% variation is required.
        ii. Reported effect-levels at each assays specified intervals are to include EC_x / LC_x levels over the range of observed effects, i.e., EC_{10} to EC_{90} in 5-10% effect levels.
        iii. The most recent reference standard toxicity test data is to be reported with each assay data to document the ability of the testing lab to sustain healthy, consistent cultures, to generate toxicity assay data of high quality, and to document the suitability and consistency of the artificial test medium.
        iv. Limit tests are not acceptable submissions to document a range of toxicity effect doses/concentrations. Multi-treatment concentration (PFAS) tests are required as specified above to support development of PFAS-related regulatory values.
    b. Algal Acute Assay –
        i. The test organism is to be the freshwater algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
    c. Daphnia Acute Immobilization Assay –
        i. Reported effect-levels (immobilization and mortality) at 24 and 48 hours are to include EC_x / LC_x levels over the range of observed effects, i.e., EC_{10} to EC_{90} in 5-10% effect levels.
        ii. In addition to the 24 and 48-hour observations, observations are to be recorded at the end of the work day on the day of test initiation.

iii. Behaviors, adverse effects or visible abnormalities in addition to immobilization are to be reported for all replicates at the prescribed exposure durations.

d. Fish Acute Assay
   i. Limit tests are not acceptable submissions to document a range of toxicity effect concentrations. Multi-treatment (PFAS) tests are required as specified above to support development of PFAS-related regulatory values.
   ii. Recommended test species is *Oncorhynchus mykiss* (rainbow trout) or alternatively, *Pimephales promelas* (fathead minnow). Assays for all PFAS are to be completed using a single species throughout these investigations, unless otherwise specified by DEQ.
   iii. A minimum of 10 fish per treatment and controls is recommended to provide additional statistical power.

e. *Daphnia magna* Reproduction Assay
   i. Control and any one treatment accidental mortality of the parent animal shall not exceed 20%.

f. Sediment-Water *Lumbriculus variegatus* Toxicity Test Using Spiked Sediment
   i. The concentration of the test substance in the lowest and highest test sediment concentrations is to be measured after sediment spiking at the beginning of the test.
   ii. The test substance concentration in the lowest and highest concentration sediment and water phases individually are to be determined at the end of the equilibration phase and at the end of the 28-day exposure, or at the end of the exposure period as dictated by organism mortality.
   iii. The laboratory must document the suitability of the un-spiked sediment substrate to provide a suitable environment for the test organism culture survival and reproduction.
   iv. 6 replicates are recommended for the control.

C. Dose Selection
   a. DEQ agrees that development and pooling of the full mass of test substance needed to complete the specified assays is a logical approach to eliminate lot-to-lot variations and artificial response variability.
   b. The proposed modified 2-test concentration plus controls test design does not meet the objectives of the PFAS toxicity testing required by DEQ. This test design will not provide the desired dose/effect-response data needed to adequately characterize the toxicity of the individual PFAS to the individual test organisms and provide the data desired for regulatory development. As specified above, the multi-test treatment design designated in each of the individual assays is to be followed, with not less than 5 treatment concentrations, or the minimal number specified in each assay if that minimum number of treatments (not including the controls) is more than 5.
   c. DEQ urges Chemours pursue independent, third-party contract services for the development of PFAS test material to generate the volume of test material. Third-party sources will likely generate the appropriate material more quickly and can provide a consistent reference material available to multiple entities. Characterization of the reference material is to be included with assay data, including full

qualitative/quantitative characterization of each material, including structural isomer specifications. Commercial suppliers of suitable PFAS reference materials for certain PFAS include:

1. SynQuest Labs
2. Wellington Laboratories
3. Fluoryx Labs

D. Work Plan Schedule
   a. DEQ urges that test protocol design and submittal to DEQ for approval be initiated as soon as possible to facilitate initiation of the individual PFAS assays as soon as adequate testing material is available for the Tier 1 assays.

E. Mammalian Studies
   a. See comments above regarding acquiring assay protocol design approval from DEQ in the interim period during which the PFAS test materials are being generated, so that the assays can be initiated soon (1-3 months) after the pooled material is received.
   b. Pre-test specifications to be submitted to DEQ for review and approval prior to assay initiation is to include the planned dosing regimen for mammalian assays (gavage/water/food, frequency per day, time during the day) along with the explanation of why this method was selected. PFAS concentrations in the dosing materials (gavage solution, water, feed) are to be verified for elast sentanach preparation of the low and high treatment concentrations and controls, and not less than weekly.

F. Ecotoxicology Studies
   a. DEQ requests an explanation of the timeline projected 7-14 months for the Tier 1 ecological assays to complete the testing and final reports so that we may better understand the potential issues and complexities anticipated with this effort. The proposed time frame to complete this work is longer than anticipated by DEQ considering the short-duration exposure periods for these assays (48 hours to 10-days).

G. Estimated Timeline
   a. As noted above, DEQ urges Chemours or their contractor to proceed with test design and submittal to DEQ for approval as soon as is feasible to allow for initiation of the toxicity assays to begin as soon as possible (within 1-3 months) after adequate individual PFAS reference materials are acquired, rather than delaying test design and DEQ-approval until after the pooled individual PFAS test material is available.