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

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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 assay, 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, EC20, EC80), as specified by DEQ, are to be reported.
   1.2. Response statistics are to be performed using appropriate software. Concentrations – response plots are to be included for each reported endpoint. Concentrations–response plots manually plotted on graphical paper are not an acceptable data reporting method.
   1.3. Ideally, the highest test concentration should result in 100% effect response and the lowest concentration no observable effect (or less than 20% effect response). Not less than 5 test concentrations are recommended for all assays, where 5 or less treatments are specified in the method.
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 modeling (benchmark dose modeling).
3. Data that does not provide a dose/exposure-response suitable for modeling may require re-testing.
4. Due to the unique physicochemical properties of the test chemicals (PFAS) the concentration of the test substance should be measured, at a minimum, at the highest and lowest test concentration, at the beginning and end of the test. Control solutions are to be verified as PFAS-free for at the beginning and end of the test. PFAS measurements are to be completed using DEQ-approved analytical methods and obtain DEQ-approved PFAS sensitivity criteria. Less than ±20% variation is required.
5. Confirmation that all test apparatus is PFAS-free is required.
6. Test data reporting is to meet at a minimum the specifications in each method, with individual treatment or replicate data recording sheets made available at DEQ's request.
7. Assays reported to DEQ are to include the most recent quality control testing of the test organism cultures, culture media and test media.
8. For mortality endpoint tests (acute) an additional observation at 6-8 hours after organisms are exposed to the test and control solutions (i.e., at the end of the work day on the day of test

initiation) is recommended. At all observation periods report any observed mortalities or other observations of abnormal behavior or effects.

Comments on Specific Assay Specifications noted by Chemours

A. Mammalian Studies – no additional comments
B. Ecological Studies –
    a. Algal Acute Assay –
        i. Limit tests are acceptable only at the solubility limit of the specific PFAS in the test-specific medium, derived using appropriate methods approved by DEQ. The test solutions must be verified by analysis at the end of the exposure period. A minimum of 6 replicates are required for each treatment and controls.
        ii. Recommended test organism – Freshwater algae *Pseudokirchneriella subcapitata,* (formerly known as *Selenastrum capricornutum*)
    b. Daphnia Acute Immobilization Assay –
        i. Reported effect-levels (immobilization) at 24 and 48 hours are to include EC10, EC20, EC50 and EC80.
        ii. Lethal effect concentrations are to be reported for the same response levels and exposure periods as indicated for immobilization.
        iii. Behaviors, adverse effects or visible abnormalities in addition to immobilization are to be reported for all treatments/replicates at the prescribed exposure durations.
        iv. Limit tests must meet the specifications in the referenced test method (including minimum number of test organisms) and be conducted at the solubility limit of the specific PFAS in the test-specific medium. The solubility limit must be derived using appropriate methods approved by DEQ.
    c. Fish Acute Assay
        i. Limit tests must meet the specifications in the referenced test method (including minimum number of test organisms) and be conducted at the solubility limit of the specific PFAS in the test-specific medium. The solubility limit must be derived using appropriate methods approved by DEQ.
        ii. Recommended test species is *Oncorhynchus mykiss* (rainbow trout) or *Pimephales promelas* (fathead minnow). All 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.
    d. *Daphnia magna* Reproduction Assay
        i. Control and any one treatment accidental mortality of the parent animal shall not exceed 20%.
        ii. 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.

e. 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 most recent reference toxicity test data is to be reported with the PFAS assay data to document the ability of the testing lab to generate data of high quality and a consistent response of test organisms.
    iv. 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.
    v. 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. DEQ does not agree with the proposed modified 2-test concentration plus controls test design. 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 contract services for the development of PFAS test material if this will generate the needed volume of test material more quickly that the 1-year period anticipated for Chemours to generate the material.

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. Protocol design should not be delayed until the test material has been generated.

E. Mammalian Studies
    a. See comment 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.

F. Ecotoxicology Studies
    a. Six months to complete the 72-hour algae and fish acute assays seems excessive. Three months would seem to be more than adequate time to complete this work, with an additional 3-4 months for the Daphnia reproduction assays.

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 the pooled individual PFAS test material is available.
b. DEQ would urge Chemours to complete reporting of all rodent study data to DEQ no more than 24 months after receiving the test material.