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To Members of the Secretaries' Science Advisory Board,

This letter follows up on my previous letter of 26 January 2018 with more specific suggestions on deriving a Provisional Health Goal for GenX in North Carolina waters.[1] As set forth below, I have reviewed the available data and calculated possible Provisional Health Goal values using a number of different assumptions. These values are summarized in Table 1, with greater detail provided in the text and in Table 2.

The main point to consider is that the Provisional Health Goal for GenX should be based on the 2-year chronic rat study that is available for GenX. This study represents not only the best available science, but as the EPA states, subchronic studies are used to set chronic values ***"when a chronic study is not available."***[2] This is not the case for GenX, where there is a complete 2-year chronic rat study that meets all the criteria established by EPA for use in setting a chronic (lifetime) Provisional Health Goal. The State of North Carolina properly used this 2-year chronic study when it set the original Provisional Health Goal value of 71,000 ng/L (shown in Table 1, column 2). The State of North Carolina subsequently revised the Provisional Health Goal using subchronic study data and other assumptions to arrive at a value of 140 ng/L (shown in Table 1, column 3). There is no scientific rationale to ignore the chronic 2-year study on GenX and substitute it with the subchronic study data.

A second critical point to consider is that one of the assumptions used by the State of North Carolina for both the original and current Provisional Health Goal values is an incorrect application of the EPA Risk Assessment Guidelines and has no scientific basis. The State of North Carolina based a lifetime exposure scenario of GenX on the weight and drinking-water intake of a bottle-fed infant. The EPA does not base lifetime exposure on an infant because a human is an infant for only a small fraction of its entire lifespan.

Using the same proper 2-year chronic study data used by North Carolina in its original assessment, and using the correct lifetime exposure scenario, I provide two values of 70,000 ng/L and 233,300 ng/L that should be considered for the Provisional Health Goal for GenX (shown in the green columns 7 and 8 below). For comparison purposes only, I also have listed three other values derived using the subchronic study data and different assumptions including one calculation using the EPA benchmark dose method (shown in yellow below). Additional details and assumptions for my calculations are provided below in the text and in Table 2, and I would be happy to provide further details upon request.

[1] Chemours has retained me to review technical information regarding GenX and the possible human health effects associated with GenX. The views I express in this letter are my own and no endorsement by my employer, NCSU, is implied.

[2] https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf (p3-9)

Table 1. Summary of Provisional Health Goal values that can be derived based on available data.

	Original NC Assessment (chronic)	Current NC Estimate (subchronic)	Current NC Estimate *Adjustment 1* (subchronic)	Current NC Estimate *Adjustment 2* (subchronic)	EPA Benchmark Dose Method (subchronic)	Lifetime Exposure Assessment (chronic)	Lifetime Exposure Assessment *Adjustment 1* (chronic)
Provisional Health Goal (ng/L)*	71,000	140	700	7,000	16,100	70,000	233,300

*All values listed are in nanograms-per-liter (ng/L) in water, which is equivalent to parts-per-trillion (ppt)

The discussion below provides additional explanation for my calculations and incorporated assumptions.

Principal risk assessment decisions

There are several decisions that must be made regarding assumptions, appropriate data and other information to use for deriving the Provisional Health Goal for GenX including:

1. The appropriate receptor (population/subpopulation) for lifetime exposure
2. The appropriate relative source contribution (RSC) for possible exposure pathways
3. The appropriate toxicity study duration for lifetime exposure analysis
4. The identification of *adverse effects* in toxicity studies to set the point of departure (POD)
5. Subchronic-to-Chronic Uncertainty Factor (UF)
6. Interspecies Uncertainty Factor (UF)
7. The use of no observed adverse effect level (NOAEL) versus the EPA Benchmark Dose (BMD) method

The appropriate receptor (population/subpopulation) for lifetime exposure

The intention of the GenX Provisional Health Goal is to protect against adverse effects associated with lifetime exposure.[3] In deriving both the original and current Provisional Health Goal, the State of North Carolina used the body weight and drinking-water intake rate of a bottle-fed infant to assess lifetime exposures rather than for an adult. This method does not follow EPA guidance nor does it have any scientific basis. The EPA does not base lifetime exposure on infant body size and drinking water intake because a human is an infant for only a small fraction of its entire lifespan. The EPA default assumption for an adult lifetime exposure is a weight of 70 kg with a water intake of 2.0 L per day.[4] These are the values North Carolina should use in deriving the Provisional Health Goal.

[3]Presentation by Dr. Zack Moore, NC State Epidemiologist to the Secretaries' Science Advisory Board (SAB) on 4 December 2017. https://files.nc.gov/ncdeq/GenX/SAB/GenX%20Health%20Studies%20and%20Advisories%20SSAB%2012_4_2017.pdf

[4] https://www.epa.gov/risk/human-health-risk-assessment

The relative source contribution (RSC) for possible exposure pathways

A Provisional Health Goal may include an adjustment to account for other possible sources of GenX besides drinking water (such as food, inhalation, and dermal absorption) by using a relative source contribution (RSC) to apportion exposure to different sources. Given the chemical properties of the environmentally-relevant form of GenX (e.g., highly water soluble) one would not expect significant amounts of GenX in the food of the general population. Also, as pointed out by the Centers for Disease Control (CDC), GenX is unlikely to be absorbed through the skin.[5] Thus, assuming that the majority of GenX exposure is through drinking water is probably accurate for most people. However, since we do not have data on all relevant exposure pathways, for the purpose of this letter, I use the more protective assumption that only 20% of GenX exposure is through drinking water until additional data become available. This would set the RSC value for water to be 20% (RSC = 0.2) in the risk equation.

The appropriate toxicity study duration for lifetime exposure analysis

The EPA risk assessment process uses the 1- or 2-year chronic study as the standard for assessing potential adverse health effects for a lifetime exposure duration and allows for subchronic studies to be used ***"when a chronic study is not available"***.[6] For GenX, there is a complete 2-year chronic and carcinogenicity rat study that meets all the criteria established by EPA for use in a lifetime exposure analysis.[7] As discussed in my earlier letter, there is no scientific justification to substitute the subchronic or subacute studies for the 2-year chronic study, especially when the shorter duration studies do not reveal any different types of toxicity or modes of action that might require additional consideration.

The identification of *adverse effects* in the toxicity studies to set the point of departure (POD)

Since properly designed toxicological studies identify doses at which test substance-related changes occur, decisions must be made on which observed changes are considered *adverse effects*. Repeated exposure to GenX in laboratory animals is associated with peroxisome proliferator-activated receptor alpha (***PPARα***), but the lack of human relevance of this pathway (***PPARα-dependent*** toxicity) is very well established.[8,9] Thus, the endpoints of reduced serum lipids, hepatocyte hypertrophy, increased liver weights, and liver tumors in the rodent studies of GenX are not relevant to humans. A strong argument also can be made to treat single-cell necrosis (found only in the subacute and subchronic studies) as a non-adverse effect because 1) the effects were minimal-to-mild, 2) not seen at higher doses or at the lower doses at longer time points, and 3) are likely a result of the adaptive and reversible hypertrophy that is known to be PPARα-dependent and thus not relevant to human health. As the cell enlarges due to the PPARα-dependent hypertrophy, minimal-to-mild single-cell necrosis

[5]Presentation by Dr. Zack Moore, NC State Epidemiologist to the Secretaries' Science Advisory Board (SAB) on 4 December 2017.
[6]https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf (p3-9)
[7]https://echa.europa.eu/registration-dossier/-/registered-dossier/2679/7/6/2/?documentUUID=84f751a2-e4d0-418c-8103-6f0e18cd7069.
[8]Corton C, Peters JM, Klauning JE. 2017. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Archives of Toxicology. https://doi.org/10.1007/s00204-017-2094-7.
[9]Felter SP et al. 2018. Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regulatory Toxicology and Pharmacology. 92:1–7.

would be expected. However, a non-PPARα-dependent pathway for single-cell necrosis cannot be ruled out at this time and single-cell necrosis is usually considered adverse when correlative enzyme activation is observed.[10] Thus, to be conservative, one could consider the minimal-to-mild single-cell necrosis as an adverse effect if chronic study data were not available and the subacute/subchronic toxicity data are used.

Subchronic-to-Chronic Uncertainty Factor (UF)

The issue of what subchronic-to-chronic UF to use is a moot point if the 2-year chronic rat study is used to derive the POD. However, if the subchronic study data are used then consideration must be given to the value of this Uncertainty Factor. The EPA states "*A default value of 10 for this UF is applied to the NOAEL/LOAEL or BMDL/BMCL from the subchronic study on the assumption that effects from a given compound in a subchronic study occur at a 10-fold higher concentration than in a corresponding (but absent) chronic study*."[11] This condition is not true for GenX, since a complete 2-year chronic rat study is available for GenX. Moreover, the rationale for using a subchronic-to-chronic UF of 10 is to account for possible effects "*at a 10-fold higher concentration*" in a subchronic versus chronic study. However, in the case of GenX the opposite is true. In the mouse studies, there is no single-cell necrosis progression or lower NOAEL/LOAEL with the longer exposure duration study (90 days) compared to the shorter duration studies (28 days and 70+ days) and there is no single-cell necrosis at all in the 2-year chronic rat study. Thus, the subchronic data do not support the use of a default subchronic-to-chronic UF of 10. Rather, the data support a UF of 1 given that longer duration and higher dose did not demonstrate increased severity or incidence of any adverse endpoints.

Interspecies Uncertainty Factor (UF)

The EPA uses a default interspecies Uncertainty Factor of 10 under the assumption that humans may be more susceptible/sensitive to a chemical than the rat or mouse used in the toxicity study, but the EPA also allows for this 10-fold UF to be reduced if there is evidence that that humans are less sensitive.[12] Although definitive human data are lacking, there is good reason to assume that humans are less sensitive than rats and mice to the reported GenX toxicities (single-cell necrosis in subchronic studies and centrilobular necrosis in chronic study) due to the very strong ***PPARα-dependent*** toxicities that are not relevant in humans. This would justify setting the interspecies UF at 3.

The use of no observed adverse effect level (NOAEL) versus the EPA Benchmark Dose (BMD) method

The EPA risk assessment process includes an analysis of the dose-response relationship between exposure and adverse health-related outcomes and follows a two-step process: (1) defining a point of departure (POD) and (2) extrapolating from the POD for relevance to human exposure. The NOAEL has been used as the POD for many years, but recognizing the limitations of this approach, the EPA has adopted an alternative approach called the benchmark dose (BMD) method[13]. The BMD method

[10]Thoolen B et al. 2010. Proliferative and Nonproliferative Lesions of the Rat and Mouse Hepatobiliary System, Toxicologic Pathology, 38: 5S-81S.

[11]https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf (p4-45)

[12]https://www.epa.gov/risk/human-health-risk-assessment, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

[13]https://www.epa.gov/bmds/benchmark-dose-bmd-methods

involves statistical modeling of dose-response data and is particularly helpful at incorporating data from multiple related studies and extrapolations near the low end of the exposure range. The BMD analysis results in a BMDL (benchmark dose lower confidence level) that is used as the POD instead of the NOAEL. Not all data sets are amenable to BMD modeling and different models within the BMD software can provide slightly different results, so care must be taken in performing this analysis and the process should be well documented. I performed BMD modeling on the data available for GenX using the latest EPA BMD software.[14] A brief summary of this analysis is provided below; details of this analysis can be provided separately. BMD analysis was performed on males only because in all studies males were more sensitive than females and would provide the lowest BMDL and POD.

The 2-year chronic rat study data were not amenable to BMD modeling owing to the lack of observed effects at the lower doses, resulting in a best-fit BMDL of 38.2 mg/kg/day (for liver centrilobular necrosis) which is much higher than the NOAEL. Thus, the appropriate POD for the 2-year chronic study is the NOAEL of 1 mg/kg/day.

For the subchronic mouse studies, the data for male mice were combined for the 90-day subchronic[15] and 70+ day DART[16] studies because the exposure durations were similar, the mice were the same strain and single-cell necrosis in the liver was the common adverse endpoint reported by both studies. These data were amenable to BMD analysis and resulted in a best-fit BMDL of 0.23 mg/kg/day. If the more appropriate chronic rat data were ignored and subchronic mouse studies were used instead, the 0.23 mg/kg/day BMDL has a stronger scientific basis for use as the POD than the 0.1 mg/kg/day NOAEL.

Derivation of Provisional Health Goal for GenX

Using the above discussion as a basis for deriving a Provisional Health Goal for GenX, possible values are listed in Table 2 along with the parameters and assumptions used to derive the value. The values listed in green columns are based on the 2-year chronic study in the rat and thus are scientifically defensible in matching the lifetime exposure Provisional Health Goal to the lifetime chronic study in the rat. The original North Carolina Assessment (71,000 ng/L) was derived using this chronic study and is shown in column 2.

The values listed in the yellow columns are based on subacute/subchronic studies and thus are not appropriate to use when a chronic study is available, but they are listed here for comparison and because North Carolina used the 28-day subacute mouse study to derive the current North Carolina Estimate (140 ng/L; shown in column 3). If this 28-day study were to be used, it would need to be adjusted to account for lifetime exposure to the entire lifespan of a human using the body weight and daily water intake of an adult, yielding a value of 700 ng/L (column 4). This *Adjustment 1* retains the water RSC of 0.2 and a subchronic-to-chronic UF of 10. However, it would be more appropriate to use an UF of 1 due to the reasons stated above, yielding a value of 7,000 ng/L (column 5, *Adjustment 2*).

[14]https://www.epa.gov/bmds/what-benchmark-dose-software-bmds; BMDS 2.7 (rel. 2017-08-18)

[15]https://echa.europa.eu/registration-dossier/-/registered-dossier/2679/7/6/2/?documentUUID=d7c86805-db57-44b8-af3a-72f5c8bc3239

[16]An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice, Study No. WIL-189225, 2011

Table 2. Provisional Health Goal values that can be derived based on available data, with assumptions listed.

	Original NC Assessment (chronic)	Current NC Estimate (subchronic)	Current NC Estimate *Adjustment 1* (subchronic)	Current NC Estimate *Adjustment 2* (subchronic)	EPA Benchmark Dose Method (subchronic)	Lifetime Exposure Assessment (chronic)	Lifetime Exposure Assessment Adjustment 1 (chronic)
Provisional Health Goal (ng/L or ppt)	71,000	140	700	7,000	16,100	70,000	233,300
Population Assumed	Infant	Infant	Adult Lifetime Exposure	Adult Lifetime Exposure	Adult Lifetime Exposure	Adult Lifetime Exposure	Adult Lifetime Exposure
Key Study Used	2-year chronic rat	28-day subacute mouse	28-day subacute mouse	28-day subacute mouse	90-day and 70+ day subchronic mouse	2-year chronic rat	2-year chronic rat
Relative Source Contribution for Water	1.0	0.2	0.2	0.2	0.2	0.2	0.2
Study Duration UF *subchronic-chronic*	Not applicable*	10	10	1	1	Not applicable	Not applicable
Interspecies UF	10	10	10	10	10	10	3
Intraspecies UF	10	10	10	10	10	10	10

* Values using chronic study data (in green columns) do not require a subchronic-chronic UF

As discussed above, the EPA BMD method can provide a more statistically robust POD value compared to using the NOAEL when adequate data are available; data from the two longer-term (70-90 day, male only) mouse subchronic studies were combined for this EPA BMD method analysis. Using single-cell necrosis as the endpoint and a subchronic-to-chronic UF of 1 would yield an EPA Benchmark Dose method value of 16,100 ng/L (column 6). If subchronic study data were to be used instead of the more appropriate chronic study data, then this EPA Benchmark Dose method value of 16,100 ng/L would be the most scientifically defensible of those listed in Table 2 (in yellow columns). This value can be further adjusted using an interspecies UF of 3, based on the justification provided above, to yield a Provisional Health Goal value of 53,660 ng/L based on the EPA BMD method.

Returning to the chronic study -- the more appropriate for setting the Provisional Health Goal -- two additional values based on the 2-year chronic study are listed in green columns 7 and 8 in Table 2. The first value (70,000 ng/L) revises the original Provisional Health Goal to account for other possible sources of GenX (water RSC = 0.2) and to account for exposure over the entire lifespan of a human using exposure parameters relevant to an adult. Note that no subchronic-to-chronic UF is used here because the data rely on a chronic study already. The final value in column 8 (233,300 ng/L) further adjusts the Provisional Health Goal to use an interspecies UF of 3 rather than 10, based on the justification provided above.

Conclusion

In summary, I hope that the analysis presented above and possible Provisional Health Goal values listed in Tables 1 and 2 will be useful to the SAB and the State of North Carolina in setting a revised Provisional Health Goal for GenX in North Carolina waters. I would be pleased to provide further details of my analysis or any other information upon request.

Sincerely,

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