

Comments on U.S. EPA's
Human Health Toxicity Values for
Hexafluoropropylene Oxide (HFPO) Dimer Acid and
Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3)
Also Known as "GenX Chemicals"

EPA-823-P-18-001 (Public Comment Draft)

Docket EPA-HQ-OW-2018-0614

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1. Necrosis versus apoptosis.

In developing its "human health toxicity values" for GenX, U.S. EPA (2018) assumes that study-reports of hepatocellular "single cell necrosis" or "individual cell necrosis," as seen in some but not all of the various mouse and rat bioassays, are:

- (i) all compound-related;
- (ii) all adverse, as opposed to some or all such responses being adaptive; and
- (iii) directly relevant for purposes of human health risk assessment.

As explained below, the second and third of these assumptions do not appear to be reliable. Moreover, we would note that these liver cell changes were not observed in either the initial rat 14-day developmental study nor in the rat 90-day study.

It is important to note that all of rat and mouse bioassays of GenX *preceded* the publication (Elmore et al., 2016) of consensus diagnostic criteria, developed by expert pathologists, for distinguishing between adaptive apoptosis and potentially pathological necrosis.

Diagnostic criteria:

The diagnostic criteria for distinguishing apoptosis from necrosis are provided in:

- Elmore et al., 2016 (Recommendation of INHAND Apoptosis/Necrosis Working Group) <https://www.ncbi.nlm.nih.gov/pubmed/26879688>
- NTP (undated) at <https://ntp.niehs.nih.gov/nl/hepatobiliary/liver/hapop/index.htm> and <https://ntp.niehs.nih.gov/nl/hepatobiliary/liver/necrosis/index.htm>. These are consistent with Elmore et al., 2016.

The following observations were made during all available rodent toxicity tests.

Mouse observations:

28-day study:

No diagnostic criteria were specified in the study design or results. Hepatocellular “single cell necrosis,” characterized as “minimal” in every case, was reversible after a 28-day recovery period. All animals with hepatocellular “single cell necrosis” also had liver hypertrophy.

90-day study:

“... single cell hepatocellular necrosis. The latter change was characterized by isolated eosinophilic bodies with occasional pyknotic nuclear fragments and unaccompanied by inflammation, and thus was consistent with apoptosis” (page 33). This is consistent with the Elmore et al. (2016) criteria for apoptosis. All cases were consistent with “minimal”. All 10 animals with hepatocellular “single cell necrosis” also had liver hypertrophy.

Reproductive/developmental study:

No diagnostic criteria were specified in the study design or results. Hepatocellular “single cell necrosis” was characterized as minimal except in the highest dose groups (5 mg/kg/day) where there were 4, 18, 3 characterized as minimal, mild, or moderate respectively in males,¹ and 17, 4, 0 respectively in females. This study was the largest of the three on mice, so the background prevalence of hepatocellular “single cell necrosis” was observable. In males at doses of (0, 0.1, 0.5, 5) mg/kg-day, (0/1,0/1,3/5,25/25) mice² with hepatocellular “single cell necrosis” also had liver hypertrophy. In females at the same doses, (0/1,0/3,2/2,21/21) mice with hepatocellular “single cell necrosis” also had liver hypertrophy.

Rat observations:

14-day developmental study:

No diagnostic criteria were specified in the study design, and no animals were characterized with hepatocellular “single cell necrosis” or “individual cell necrosis” in the results.

14-day developmental follow-up study:

No diagnostic criteria were specified in the study design or results. All three cases of hepatocellular “single cell necrosis” were characterized as “minimal”. Of these, only the background case (zero dose) did not also have liver hypertrophy.

¹ The 25th animal at 5 mg/kg-day died at 55 days (versus 84 or 85 for the scheduled sacrifices), and is not included in the summary data used in dose-response analyses. It is included in this summary (as the 18th mild case).

² See footnote 1.

28-day follow-up study:

No diagnostic criteria were specified in the study design or results. The single case (in males, at the highest dose of 30 mg/kg-day) of hepatocellular “single cell necrosis” was characterized as “minimal”, and also had liver hypertrophy.

90-day study

No diagnostic criteria were specified in the study design, and no animals were characterized with hepatocellular “single cell necrosis” or “individual cell necrosis” in the results.

2-year study:

“Individual cell necrosis was characterized by the presence of scattered single hepatocytes with features characteristic of apoptosis.” (page 41 and 1114 of 4037). All three cases (in females, at the highest dose of 500 mg/kg-day) were characterized as “minimal” or “mild” (page 1297 of 4037), and all three cases also had liver hypertrophy.

Thus, these reports of (hepatocellular) “single cell necrosis” or “individual cell necrosis” should be interpreted to reflect apoptosis. The prevalence is increased above background levels only during exposures that cause liver cell hypertrophy, with no increase above background in the absence of such hypertrophy. Such an effect should thus be considered adaptive and/or a consequence of hypertrophy, and not an adverse event in the absence of hypertrophy. As is well known, hypertrophy would not be expected in human livers at potential human exposure levels (which are, of course, vastly smaller than levels to which the test-rodents were exposed), since for chemicals such as GenX, liver cell hypertrophy is a consequence of PPAR α activation to which humans are much less sensitive than are rats and mice (but perhaps not less sensitive than such effects in other rodent-species, such as the guinea pig). This rat and mouse-based response is thus irrelevant for purposes of assessing risks to human health from exposures to GenX.

If despite the foregoing analysis, U.S. EPA nonetheless wishes to assess risks to humans based on these mouse-specific liver-responses, then the Agency might wish to consider the following points as it prepares its next estimate of a reference dose (RfD) for GenX.

2. Omission of relevant data

In devising its draft RfD for GenX, EPA relied on the hepatocellular single cell necrosis dose-response data for male mice in a reproductive/developmental study in which dosing was for 84 or 85 days. As it happens, the 90-day male mouse study was carried out at exactly the same dose levels, and used the same species, same strain, and same mouse supplier company. The results from these two bioassays *do not differ significantly at any of the individual doses or overall*. As tabulated below, the overall difference between these two studies is not significant ($p=0.15$, two-sided, taken as double the exact one-sided result).

	Dose level (mg/kg-d)			
	0	0.1	0.5	5
Study	Single cell necrosis results			
Repro/devel	1/25	1/24	5/24	24/24
90-day	0/10	0/10	0/10	10/10
	Fisher exact, 1-sided, p-values for differences			
Individual doses	0.714	0.706	0.153	1.00
Overall	0.077			

Thus, these two bioassays should be considered of equivalent status, and treated as if they represent a single experiment. The resulting BMDLo is 0.2141 mg/kg-day, and is a more precise estimate, compared with the estimate of 0.151 mg/kg-d, which EPA obtained from the reproductive/developmental study alone. This results in an increase in the estimated RfD of a factor of 1.42.

3. Extrapolating from Mice to Men: Allometric vs. AUC scaling

EPA has used allometric scaling from mouse to human, based on the Agency's default approach. In this case, however, the default approach is clearly inaccurate, in that it yields predictions that are contradicted by actual measurements.

The default approach is designed to approximate (at least) the *toxicokinetic* portion of the interspecies extrapolations (e.g. see EPA, 2002, Section 4.4.3.4): that is, for chronic studies, an approximation for extrapolation of the area under the concentration-time curve (the AUC) for the active chemical between and among species. However, for GenX there are *direct measurements* of AUC for three mammalian species — mouse, rat, and monkey — thus allowing comparison of the allometric scaling with scaling of AUC.³

³ AUC/dose is 1/clearance. These values are averages of maximum likelihood estimates of AUC/dose obtained for the 6 individual monkeys (3 male, 3 female), the 18 individual rats per sex tested at all time points, and the 3 different mice per sex per time point, including all dose-levels tested and both GenX acid and salt. Fitting was of tri-exponential curves for monkeys and rats, bi-exponential for mice, since tri-exponential curves were a statistically significant improvement over bi-exponential for monkeys and rats. Log(plasma concentration) was assumed to follow a different curve with a homoscedastic normal uncertainty for each animal for the individual monkeys and rats followed over time, and an average curve with normal uncertainty allowed to be linearly proportional to (the negative of) predicted log(concentration) for the 3 different mice tested at each time point (the heteroscedasticity allowed for differences between mice).

Species/sex	AUC/dose in kg-hr/L
Monkey ⁴ m & f	37.2
Rat m	26.1
Rat f	5.0
Mouse m	92.5
Mouse f	43.9

The body weights of the tested animals and the default standard human body weight are as follows (the mouse male weight is that chosen for use by EPA, the others are chosen to mimic this choice as closely as possible in the respective studies):

Species/sex	Body weight (kg)	Source (mean values)
Mouse m	0.0372	Control group on the last day of the reproduction study
Rat m	0.538	Control group on last day of 90-day study
Monkey m	2.443	Three male monkeys used in cross-species study
Human	80	EPA standard default

Using these results allows comparison of allometric ($BW^{2/3}$) scaling and AUC/dose scaling between male mice, male rats, and monkeys for equal average plasma concentrations from long-term dosing, as follows:

	Allometric	AUC/dose
	Multiply external dose rate by:	
Mouse m to Rat m	0.513	3.541
Rat m to Monkey	0.685	0.701
Monkey to Mouse m	2.847	0.403
Monkey to human	0.418	No data

As can be seen, allometric scaling from male mouse to male rat or monkey does not agree with the AUC scaling, indicating a substantial divergence from the default assumption of approximate equivalence for toxicokinetic scaling. Interestingly, allometric scaling between male rat and monkey does correspond well with AUC/dose scaling.

Since the mouse does not appear to conform to equivalence between AUC/dose and allometric scaling, a more defensible, evidence-based approach than default allometric scaling from male mouse to human would be to (i) use the measured AUC/dose scaling from mouse to monkey,

⁴ AUC/dose did not differ significantly between male and female monkeys

and then (ii) assume allometric scaling from monkey to human. This approach results in an increase in the estimated RfD by a factor of 7.07.

4. Extrapolation from subchronic to chronic exposure

In developing its draft RfD, EPA used a factor 3 to extrapolate from its chosen subchronic study to a lifetime (or chronic) exposure. However, there is no evidence for such a factor for this particular end-point, and good evidence against it. The 28-day studies in mice show that the end-point appears within 28 days in mice at the same level as seen in the 90-day study. There is no demonstrable difference in the dose-response curves at 28 days and at 84-90 days in the sense that the empirical observations for the 28-day study interleave with those of the 90 day plus reproductive/developmental study, so that there exists a dose-response curve that is consistent with both⁵.

In addition, the 28-day studies with 28-day recovery show that the end-point is eliminated after the recovery period, strongly suggesting that this end-point is a continuing steady-state process rather than a cumulative one.

Finally, there is no cumulative effect seen in any of the other studies (in female mice, or male or female rats). For example, in female rats, “single cell necrosis” is seen at indistinguishable rates in the 14-day maternal/developmental study at 1,000 mg/kg-day and in the 2-year study at 500 mg/kg-day.

Overall, if this end-point is to be used, then this factor should be replaced by unity, leading to an increase in the estimated RfD of a factor of 3.

5. Summary

The overall effect of the factors discussed above is a factor of $30.1 = (1.42 \times 7.07 \times 3)$ increase in the RfD estimate, giving a value for the chronic, oral, RfD for GenX of $2.5e-3$ mg/kg-day.

To assist the Agency, we have provided the accompanying and attached⁶ spreadsheet. We would, of course, be pleased to assist in any other way. Please feel free to write to us at: Crouch@GreenToxicology.com and Green@GreenToxicology.com.

⁵ Given the shape of the dose-response curve, it would be possible to evaluate statistically whether the observations are consistent with a single curve. However, the shape of the dose-response curve for this end-point is not known *a priori*; the observations of interleaving of the responses means that such a single dose-response curve exists. For example, a suitable multistage model is consistent with both the 28-day data and the 84–85 day data of the reproductive/developmental study combined with the 90-day study. A statistical test was available above, in our Section 2, because the dose-groups were identical in the two studies.

⁶ Embedded as an attachment in this PDF file.

6. Acknowledgement

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7. References

Elmore SA, Dixon D, Hailey JR, Harada T, Herbert RA, Maronpot RR, Nolte T, Rehg JE, Rittinghausen S, Rosol TJ, Satoh H. Recommendations from the INHAND apoptosis/necrosis working group. *Toxicologic Pathology*. 2016 Feb;44(2):173–188.

EPA, 2002. *A review of the reference dose and reference concentration processes*, Prepared for the Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC. EPA/630/P-02/002F, December 2002.