Message

**From**: Bahadori, Tina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=7DA7967DCAFB4C5BBC39C666FEE31EC3-BAHADORI, TINA]
**Sent**: 6/18/2018 3:31:18 PM
**To**: White, Kimberly [Kimberly_White@americanchemistry.com]
**CC**: Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
**Subject**: RE: Letter Highlighting New Commentary Submitted on Behalf of the ACC Formaldehyde Panel

Dear Dr. Wise White,

Thank you for sending this commentary. We look forward to the opportunity to discuss our completed assessment with ACC's Formaldehyde Panel in a public forum.

Kind Regards,

Tina Bahadori, Sc.D.
Director, National Center for Environmental Assessment (EPA/ORD/NCEA)
National Program Director, Human Health Risk Assessment (EPA/ORD/HHRA)

**From:** White, Kimberly [mailto:Kimberly_White@americanchemistry.com]
**Sent:** Friday, June 15, 2018 1:03 PM
**To:** Bahadori, Tina <Bahadori.Tina@epa.gov>
**Cc:** Thayer, Kris <thayer.kris@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>
**Subject:** Letter Highlighting New Commentary Submitted on Behalf of the ACC Formaldehyde Panel

Dear Dr. Bahadori:

Please find attached a letter submitted on behalf of the American Chemistry Council's Formaldehyde Panel, highlighting a recently published commentary.

Kind Regards,

Kimberly Wise White, Ph.D. | American Chemistry Council
Senior Director, Chemical Products & Technology Division
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Message

**From**: White, Kimberly [Kimberly_White@americanchemistry.com]
**Sent**: 6/15/2018 5:03:04 PM
**To**: Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]
**CC**: Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
**Subject**: Letter Highlighting New Commentary Submitted on Behalf of the ACC Formaldehyde Panel
**Attachments**: ACC Formaldehyde Panel Letter to EPA on Thompson 2018 Commentary - 06 15 18.pdf; Attachment 1 - Thompson Commentary on Formaldehyde NTP Study - June 2018.pdf

Dear Dr. Bahadori:

Please find attached a letter submitted on behalf of the American Chemistry Council's Formaldehyde Panel, highlighting a recently published commentary.

Kind Regards,

Kimberly Wise White, Ph.D. | American Chemistry Council
Senior Director, Chemical Products & Technology Division
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June 15, 2018

Dr. Tina Bahadori
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**Re: 2018 Commentary on New Formaldehyde Studies in Trp53 Haploinsufficient Mice: Further Support for Nonlinear Risks From Inhaled Formaldehyde**

Dear Dr. Bahadori:

I am writing to call to your attention a June 2018 article by C. Thompson titled: "Commentary on New Formaldehyde Studies in Trp53 Haploinsufficient Mice: Further Support for Nonlinear Risks From Inhaled Formaldehyde." The article discusses the relevance of a 2017 final report by the U. S. National Toxicology Program (NTP) that explored the potential involvement of p53 mutation in formaldehyde-induced nasal tumors and lymphohematopoietic cancers. The NTP study demonstrated that inhalation of a maximum tolerated dose of formaldehyde did not cause nasal tumors, did not cause an increased prevalence of leukemia or lymphohematopoietic cancer, and did not cause any other type of cancer in $Trp53^{+/-}$ mice. It provides additional support for utilizing a non-linear threshold model for the dose-response analysis of formaldehyde.

The commentary reinforces that the mode of action of inhaled formaldehyde must be foundational for characterizing the hazard and dose-response assessment. The 2017 NTP report adds to the overall weight of the evidence illustrating that inhaled formaldehyde is not leukemogenic. The 2017 NTP report is consistent with results from available mode of action studies demonstrating that nasal tumors observed in rodent studies following inhalation exposure to formaldehyde are limited to the nasopharyngeal region and are only associated with exposure to high concentrations of formaldehyde. Moreover, the 2017 NTP report lends further support that formaldehyde-induced nasal tumors are highly unlikely to be caused via a mutagenic mode of action as is typically assumed in linear dose-response modeling for cancer assessments.

Consideration of mode of action information is critical in establishing the biological plausibility of carcinogenicity and understanding how inhalation of formaldehyde may impact normal physiological levels and processes. The 2011 NAS report[1] called for selecting outcomes on the basis of available evidence and an understanding of mode of action. The application and integration of this information is essential to reduce uncertainty in characterizing potential human health risk from formaldehyde exposures and its importance cannot be overstated. The

[1] National Academy of Sciences (NAS). National Research Council (NRC). 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Committee to Review EPA's Draft IRIS Assessment of Formaldehyde. Board of Environmental Studies and Toxicology. Division of Earth and Life Sciences.

Panel continues to urge the Agency to apply mode of action research as the foundation for a scientifically defensible hazard characterization and dose-response analysis for formaldehyde.

Feel free to contact me by phone Ex. 6 or email (Kimberly_White@americanchemistry.com) with any questions related to this letter. Additionally, a full copy of the commentary is attached for your reference.

Sincerely,

Kimberly Wise White, PhD
American Chemistry Council (ACC)
Senior Director
Chemical Products & Technology Division
On Behalf of the ACC Formaldehyde Panel

Cc:
Kris Thayer, Director of the Integrated Risk Information System Division
Richard Yamada, Deputy Assistant Administrator for the Office of Research and Development.
Jennifer Orme-Zavaleta, Principal Deputy Assistant Administrator for Science for the Office of Research and Development, and EPA Science Advisor

Attachment 1 – Thompson, C. M. (2018). Commentary on New Formaldehyde Studies in Trp53 Haploinsufficient Mice: Further Support for Nonlinear Risks From Inhaled Formaldehyde. Dose-Response, 16(2), 1559325818777931.

Dose-Response:
An International Journal
April-June 2018:1-2

DOI: 10.1177/1559325818777931
journals.sagepub.com/home/dos
SAGE

# Commentary on New Formaldehyde Studies in Trp53 Haploinsufficient Mice: Further Support for Nonlinear Risks From Inhaled Formaldehyde

**Chad M. Thompson**[1]

**Keywords**
dose–response, risk assessment, formaldehyde, mode of action

## Commentary

Formaldehyde is a widely used industrial chemical, a byproduct of combustion, and is generated endogenously. Although classified by many organizations as a carcinogen, the World Health Organization (WHO) has set an exposure guideline of 0.08 ppm based on irritant properties of formaldehyde.[1] The US Environmental Protection Agency has proposed far lower safety values based, in part, on controversial associations between formaldehyde exposure and increased risk of leukemia. Therefore, it is of interest that the National Toxicology Program (NTP), a division of the National Institute of Environmental Health Sciences, recently released an NTP Research Report that explored the potential involvement of p53 mutation in formaldehyde-induced nasal tumors as well as lymphohematopoietic cancers.[2] This study has not been published in the peer-review literature, nor is the report currently indexed in search engines like PubMed and Embase. Because the carcinogenicity of formaldehyde remains controversial and there are ongoing assessments of formaldehyde in the United States, the new NTP Research Report is an important addition to the database for informing the carcinogenicity of inhaled formaldehyde. This commentary highlights some important implications of this study for the risk assessment of formaldehyde.

In the new NTP Research Report, 2 mouse strains (note 1) haploinsufficient for TP53 were exposed to 7.5 and 15 ppm formaldehyde for 8 weeks and killed 32 weeks later at $\sim$50 weeks of age.[2] At termination, the NTP Research Report indicates that neither hematotoxicity nor lymphohematopoietic neoplasms were observed in either strain.[2] *Tp53*$^{+/-}$ mice were designed such that shortened cancer bioassays could be conducted due to their increased sensitivity to carcinogens—particularly genotoxic carcinogens.[3] These mouse strains are also reported to develop spontaneous lymphomas[3] and serve as models for lymphohematopoietic tumors in short-term studies.[2] These findings lend additional weight to the evidence that inhaled formaldehyde is not leukemogenic—including reanalysis of epidemiological studies[4] and animal studies that indicate that inhaled formaldehyde does not distribute beyond the nasal cavity or reach the blood or bone marrow.[5]

The new NTP Research Report also provides important insight into the mode of action (MOA) for nasal tumors in rodents. Formaldehyde-induced nasal tumor formation is well-documented in rats at $\geq$6 ppm,[6] and research indicates that tumors arise in nasal regions where there is cytotoxicity and regenerative hyperplasia. Research into the MOA for nasal tumors led to the development of one of the few biologically based dose–response (BBDR) models ever developed for use in risk assessment. The BBDR model and supporting research indicate that the tumor response in rats is most likely driven by increased cytotoxicity-induced regenerative hyperplasia with a negligible contribution from direct mutagenicity at noncytotoxic concentrations.[7] Subsequent in vivo genotoxicity studies have shown that exposure to up to 15 ppm for several weeks increases cell proliferation but not micronuclei or mutant frequency of *kras* or *Tp53* in the nasal cavity.[8,9] These data indicate a negligible contribution from direct mutagenicity at cytotoxic concentrations. The lack of nasal neoplasms in *Tp53*$^{+/-}$ mice considered well suited for detecting genotoxic carcinogens lends additional evidence that the MOA for

[1] ToxStrategies, Inc., Katy, TX, USA

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formaldehyde-induced nasal tumors is unlikely to be a mutagenic MOA, as typically defined for cancer risk assessment. Importantly, the NTP study authors state that, "The primary formaldehyde-related finding was squamous metaplasia of the respiratory epithelium of the nose..." indicating that "...formaldehyde caused significant injury to the nasal mucosa and cell proliferation..."[2(pvi)] These observations weaken any counterargument that the exposures were too low or too short to have potentially induced nasal tumors.

Some scientists have argued that formaldehyde induces nasal tumors via a mutagenic MOA, citing evidence for labeled DNA-protein cross-links and DNA adducts in nasal tissue following inhalation of isotope labeled formaldehyde, in vitro evidence of genotoxicity, and variable evidence for genotoxicity in exfoliated nasal and buccal cells as well as lymphocytes of humans occupationally exposed to formaldehyde. Additionally, recent studies demonstrate that *endogenous* formaldehyde is genotoxic in mice genetically engineered to be susceptible to formaldehyde due to increased production, decreased detoxification, compromised DNA repair, or some combination thereof.[10] However, as Speit et al[8] have noted, the absence of genotoxicity in nasal tissue of rats following inhalation exposure suggests that inhaled formaldehyde does not readily reach basal cells lining the nasal mucosa or that formaldehyde-induced DNA adducts and cross-links are readily repaired. The lack of nasal neoplasms in $Tp53^{+/-}$ mice seems consistent with this view.

In a vacuum, the new NTP Research Report does not exclude the possibility of a mutagenic MOA for nasal tumors. However, considered along with the broader in vivo data on formaldehyde, the weight of evidence supports the use of nonlinear approaches for estimating risks from exposure to environmental levels of formaldehyde. Indeed, the WHO argues that protection against the irritant effects of inhaled formaldehyde is protective against more severe effects such as cancer.[1] The new government-funded research in $Tp53^{+/-}$ mice further supports the argument that noncytotoxic concentrations of formaldehyde pose little/no carcinogenic risk. These important new findings should be considered by regulatory agencies currently assessing the carcinogenic risk of inhaled formaldehyde.

## Note

1. C3B6.129F1-Trp53$^{tm1Brd}$ and B6.129-Trp53$^{tm1Brd}$.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Consulting fees received for writing might be perceived by some as a conflict.

## Funding

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## References

1. World Health Organization. WHO guidelines for indoor air quality: selected pollutants. 2010. København, Denmark: WHO Regional Office for Europe.
2. Morgan DL, Dixon D, King DH, et al. NTP research report on absence of formaldehyde-induced neoplasia in trp53 haploinsufficient mice exposed by inhalation. *National Toxicology Program*. 2017;3:1-30.
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10. Pontel LB, Rosado IV, Burgos-Barragan G, et al. Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. *Mol Cell*. 2015;60(1):177-188.

Message

**From**: Thayer, Kris [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=3CE4AE3F107749C6815F243260DF98C3-THAYER, KRI]
**Sent**: 6/21/2018 8:43:21 AM
**To**: Conneely, Eileen [Eileen_Conneely@americanchemistry.com]
**CC**: Gibbons, Catherine [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2ac775a35a0945718edc7e02f50e6c12-Gibbons, Catherine]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Ohanian, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f119491e2ba8476381a39c57a456ac55-EOhanian]
**Subject**: RE: request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Eileen,

Thank you for sending this – very helpful to have the historical record of the genomics dataset.

We are currently trying to lock in a specific date and time for the public protocol meeting and will let you know as soon as one is identified. Again, we are open to providing the Cr(VI) research team multiple 10-minute slots so that the information you intended to present to us internally can be shared in a public forum.

Sincerely,

Kris

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Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS) Division
National Center for Environmental Assessment, NCEA
ORD, USEPA
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**From:** Conneely, Eileen [mailto:Eileen_Conneely@americanchemistry.com]
**Sent:** Wednesday, June 20, 2018 9:51 AM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina

<Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Dear Dr. Thayer,

Thank you for your response. It would be appreciated if the Cr(VI) research group would have adequate time to present at your protocol meeting.

Since the IRIS program has declined my request for a meeting, we are submitting the attached correspondence to the docket for the Cr(VI) IRIS assessment. The attached correspondence gives an overview of events relevant to the Cr(VI) genomics data, an explanation for the number of microarrays available through different online data repositories, and a general findings comparison between published analyses (i.e. genomics data analysis methods and standards have evolved over time and yet general findings have remained consistent despite varying approaches).

Please let me know if you have any questions.

Thank you,

Eileen Conneely

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**From:** Thayer, Kris [mailto:thayer.kris@epa.gov]
**Sent:** Monday, May 14, 2018 3:20 PM
**To:** Conneely, Eileen <Eileen_Conneely@americanchemistry.com>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Thank you Eileen for your note.

As you can imagine with all the focus on scientific transparency at the Agency we really prefer to have science meetings occur in a public forum. Perhaps it's possible that multiple 10-minute slots could be combined to give the research group more time to present the overview of recent MOA publications?

The approach for considering toxicogenomic and other mechanistic evidence would be described in the systematic review protocol. The protocol will also present the screening results of studies for consideration in the assessment (included, excluded, tagged as potentially relevant supplemental information). Our Cr(VI) team is certainly familiar with publications from your MOA research group but are not able to discuss our interpretation of the science until we have a draft assessment for public comment.

Thanks for understanding and we will try to solidify the date of the meeting shortly.

Sincerely,

Kris

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Kristina Thayer, Ph.D.
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**From:** Conneely, Eileen [mailto:Eileen_Conneely@americanchemistry.com]
**Sent:** Monday, April 30, 2018 3:31 PM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Dear Dr. Thayer,

I appreciate your response. You noted that EPA expects to release a protocol on hexavalent chromium (Cr(VI)) this June or July and that EPA plans to convene a public webinar where EPA staff would summarize the protocol content and listen to public comment on the protocol. We appreciate the opportunity to present comments during the planned EPA webinar, but ten minutes would not be adequate to present an overview of the most recent MOA research publications. We hope that additional time will be granted. Please also let us know as soon as possible when you have set the date for the webinar, as many interested parties may have planned to be away on summer vacations.

Additionally, we have questions on what full implementation of systematic review entails for the EPA IRIS program, especially since we have heard that the IRIS program has not fully considered the large toxicogenomics dataset funded by the Hexavalent Chromium Panel. The MOA researchers communicated with Lyle Burgoon, formerly at EPA, from 2014 – 2016 concerning the Cr(VI) genomics dataset, and we would like to present an overview of events relevant to the Cr(VI) genomics data, an explanation for the number of microarrays available through different online data repositories, and a general findings comparison between published analyses (i.e. genomics data analysis methods and standards have evolved over time and yet general findings have remained consistent despite varying approaches).

To ensure that all of this important genomics data is being considered in the assessment process, we would like to schedule a meeting as soon as possible with you and the IRIS staff working on the Cr(VI) assessment.

Sincerely,

Eileen Conneely

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**From:** Thayer, Kris [mailto:thayer.kris@epa.gov]
**Sent:** Thursday, March 29, 2018 5:08 AM
**To:** Conneely, Eileen <Eileen_Conneely@americanchemistry.com>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Eileen,

Thanks again for reaching out. We appreciate your sharing the list of recent publications.

We anticipate releasing a protocol on hexavalent chromium (Cr6) for public comment this June-July timeframe. The protocol will summarize the rationale for conducting the assessment, describe screening criteria to identify relevant literature, outline the approach for evaluating study quality, describe the process of evidence synthesis/integration, and dose-response methods. The protocol is a new document for the IRIS Program developed as part of its full implementation of systematic review (see presentation materials for the NAS Workshop "Review of Advances Made to the IRIS Process"). Obviously, the chromium assessment has been underway for some time now and release of this protocol should not be construed as a re-start. Rather, we are releasing the protocol so that the chromium assessment has similar public engagement steps as assessments started more recently. The protocol will include the list of studies currently included in the assessment.

Given the high level of interest in the IRIS hexavalent chromium assessment, we are planning to convene a public science webinar concurrent with the public comment period on the protocol. The format of this webinar would be a presentation by EPA staff summarizing the protocol content, followed by a public comment session where the Hexavalent Chromium Panel could present the overview suggested in your note below during a 10-minute public comment period. It may be possible to extend the length of the oral comment period depending on the number of registered speakers. EPA scientists would respond to questions of clarification but would not be discussing scientific interpretation of the evidence at this point in our process. Written materials could also be submitted as part of the public comment period.

We understand you may have questions on what full implementation of systematic review entails and how it integrates with the IRIS process. Although these topics are covered in the NAS presentation materials, we would be happy to schedule a meeting if you would like to discuss further.

Sincerely,

Kris

---

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**To:** Thayer, Kris <thayer.kris@epa.gov>
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**Subject:** Re: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Dear Dr. Thayer,

Please see the attached letter written on behalf of the Hexavalent Chromium Panel of the American Chemistry Council requesting a stakeholder meeting with IRIS staff to present an overview of the most recent publications by the MOA study researchers, and to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.

Thank you,

Eileen Conneely

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Message

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**CC**: Gibbons, Catherine [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2ac775a35a0945718edc7e02f50e6c12-Gibbons, Catherine]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Ohanian, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f119491e2ba8476381a39c57a456ac55-EOhanian]
**Subject**: RE: request to meet with IRIS staff concerning genomics dataset for Cr(VI)
**Attachments**: ACC EPA IRIS Correspondence re Cr6 Genomics Dataset 6 19 18.pdf

Dear Dr. Thayer,

Thank you for your response. It would be appreciated if the Cr(VI) research group would have adequate time to present at your protocol meeting.

Since the IRIS program has declined my request for a meeting, we are submitting the attached correspondence to the docket for the Cr(VI) IRIS assessment. The attached correspondence gives an overview of events relevant to the Cr(VI) genomics data, an explanation for the number of microarrays available through different online data repositories, and a general findings comparison between published analyses (i.e. genomics data analysis methods and standards have evolved over time and yet general findings have remained consistent despite varying approaches).

Please let me know if you have any questions.

Thank you,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
eileen_conneely@americanchemistry.com
700 2nd Street NE | Washington, DC 20002
T: Ex. 6 | F: (202) 330-5646 | C: Ex. 6 | www.americanchemistry.com

**From:** Thayer, Kris [mailto:thayer.kris@epa.gov]
**Sent:** Monday, May 14, 2018 3:20 PM
**To:** Conneely, Eileen <Eileen_Conneely@americanchemistry.com>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Thank you Eileen for your note.

As you can imagine with all the focus on scientific transparency at the Agency we really prefer to have science meetings occur in a public forum. Perhaps it's possible that multiple 10-minute slots could be combined to give the research group more time to present the overview of recent MOA publications?

The approach for considering toxicogenomic and other mechanistic evidence would be described in the systematic review protocol. The protocol will also present the screening results of studies for consideration in the assessment (included, excluded, tagged as potentially relevant supplemental information). Our Cr(VI) team is certainly familiar with publications from your MOA research group but are not able to discuss our interpretation of the science until we have a draft assessment for public comment.

Thanks for understanding and we will try to solidify the date of the meeting shortly.

Sincerely,

Kris

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Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS) Division
National Center for Environmental Assessment, NCEA
ORD, USEPA
Mail Code: B243-01
Building: Bldg B (Room B211I)
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(919) 541-0152 RTP
Skype: kristina.thayer
thayer.kris@epa.gov

**From:** Conneely, Eileen [mailto:Eileen_Conneely@americanchemistry.com]
**Sent:** Monday, April 30, 2018 3:31 PM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Dear Dr. Thayer,

I appreciate your response. You noted that EPA expects to release a protocol on hexavalent chromium (Cr(VI)) this June or July and that EPA plans to convene a public webinar where EPA staff would summarize the protocol content and listen to public comment on the protocol. We appreciate the opportunity to present comments during the planned EPA webinar, but ten minutes would not be adequate to present an overview of the most recent MOA research publications. We hope that additional time will be granted. Please also let us know as soon as possible when you have set the date for the webinar, as many interested parties may have planned to be away on summer vacations.

Additionally, we have questions on what full implementation of systematic review entails for the EPA IRIS program, especially since we have heard that the IRIS program has not fully considered the large toxicogenomics dataset funded by the Hexavalent Chromium Panel. The MOA researchers communicated with Lyle Burgoon, formerly at EPA, from 2014 – 2016 concerning the Cr(VI) genomics dataset, and we would like to present an overview of events relevant to the Cr(VI) genomics data, an explanation for the number of microarrays available through different online data repositories,

and a general findings comparison between published analyses (i.e. genomics data analysis methods and standards have evolved over time and yet general findings have remained consistent despite varying approaches).

To ensure that all of this important genomics data is being considered in the assessment process, we would like to schedule a meeting as soon as possible with you and the IRIS staff working on the Cr(VI) assessment.

Sincerely,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
eileen_conneely@americanchemistry.com
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**From:** Thayer, Kris [mailto:thayer.kris@epa.gov]
**Sent:** Thursday, March 29, 2018 5:08 AM
**To:** Conneely, Eileen <Eileen_Conneely@americanchemistry.com>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Eileen,

Thanks again for reaching out. We appreciate your sharing the list of recent publications.

We anticipate releasing a protocol on hexavalent chromium (Cr6) for public comment this June-July timeframe. The protocol will summarize the rationale for conducting the assessment, describe screening criteria to identify relevant literature, outline the approach for evaluating study quality, describe the process of evidence synthesis/integration, and dose-response methods. The protocol is a new document for the IRIS Program developed as part of its full implementation of systematic review (see presentation materials for the NAS Workshop "Review of Advances Made to the IRIS Process"). Obviously, the chromium assessment has been underway for some time now and release of this protocol should not be construed as a re-start. Rather, we are releasing the protocol so that the chromium assessment has similar public engagement steps as assessments started more recently. The protocol will include the list of studies currently included in the assessment.

Given the high level of interest in the IRIS hexavalent chromium assessment, we are planning to convene a public science webinar concurrent with the public comment period on the protocol. The format of this webinar would be a presentation by EPA staff summarizing the protocol content, followed by a public comment session where the Hexavalent Chromium Panel could present the overview suggested in your note below during a 10-minute public comment period. It may be possible to extend the length of the oral comment period depending on the number of registered speakers. EPA scientists would respond to questions of clarification but would not be discussing scientific interpretation of the evidence at this point in our process. Written materials could also be submitted as part of the public comment period.

We understand you may have questions on what full implementation of systematic review entails and how it integrates with the IRIS process. Although these topics are covered in the NAS presentation materials, we would be happy to schedule a meeting if you would like to discuss further.

Sincerely,

Kris

---

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS) Division
National Center for Environmental Assessment, NCEA
ORD, USEPA
Mail Code: B243-01
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thayer.kris@epa.gov

**From:** Conneely, Eileen [mailto:Eileen_Conneely@americanchemistry.com]
**Sent:** Thursday, March 22, 2018 8:24 PM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** Re: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Dear Dr. Thayer,

Please see the attached letter written on behalf of the Hexavalent Chromium Panel of the American Chemistry Council requesting a stakeholder meeting with IRIS staff to present an overview of the most recent publications by the MOA study researchers, and to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.

Thank you,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
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result of email transmission. American Chemistry Council, 700 – 2nd Street NE, Washington, DC 20002, www.americanchemistry.com


June 19, 2018

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS)
National Center for Environmental Assessment
USEPA Headquarters
Ariel Rios Building 1200 Pennsylvania Avenue, N.W.
***Mail Code:*** 8601P
Washington, DC 20460

By email (thayer.kris@EPA.gov) and submission to EPA Docket Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)
Docket ID Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

Dear Dr. Thayer,

Following up on my correspondence from March 21, 2018 and April 30, 2018, the American Chemistry Council's (ACC) Hexavalent Chromium Panel (Panel) has concerns about how the IRIS program will incorporate the large toxicogenomics dataset sponsored by the Panel into the assessment of hexavalent chromium [Cr(VI)]. The mode of action (MOA) researchers communicated with Dr. Lyle Burgoon, formerly at EPA, from 2014 – 2016 concerning this Cr(VI) toxicogenomics dataset, and in my last correspondence I requested a meeting with EPA to discuss the following concerning use of the dataset to inform the MOA for tumors observed in a 2-year rodent cancer bioassay:

1. A timeline of events relevant to the Cr(VI) genomics data;
2. An explanation for the varying number of microarrays available through different online data repositories; and
3. A general findings comparison between published analyses.

Since the IRIS program has declined my request for a meeting, we are submitting this information into the docket for the Cr(VI) IRIS assessment.

**Timeline of events relevant to Cr(VI) genomics data**

Table 1 summarizes the timeline of events surrounding the Cr(VI) genomics data and clarifies any potential discrepancies between data repositories over time and differences in analysis strategies used in different publications.

**Table 1. Timeline summarizing events involved in the Cr(VI) genomics dataset.**

| DATE | EVENT | RELEVANT WEBSITE[1] |
|---|---|---|
| **2012** | Two studies were published using:<br>1. Mouse duodenum and jejunum data (Kopec et al. 2012a)<br>2. Mouse and rat duodenum and jejunum data (Kopec et al. 2012b).<br>Data from manuscripts were deposited in dbZach, an internal data management system that is compliant with the Minimum Information About a Microarray Experiment (MIAME) standard (Burgoon et al. 2006). | dbZach |
| **2014** | Data were provided online through Cr6study.info. Data included:<br><u>1. qRT-PCR data used in the Kopec (2012a,b) publications</u><br><br><u>2. Raw genomics microarray data used in the Kopec (2012a,b) publications</u><br>• All raw microarray data (as .gpr files) for the mice and rat, oral palate, duodenum, and jejunum (day 8 and 91), alongside a metadata file<br><br><u>3. Genomics analysis code used in the Kopec (2012a,b) publications</u><br>• Note that this analysis included data from both array dyes (Cy3 and Cy5) and data were processed using in-house code and algorithms, which are provided in Cr6study.info, and include:<br>○ SAS code used to analyze microarray data (note: input files with appropriate tags referred to in the code were not originally provided – see 2018 update)<br>○ R code to calculate P1(t) statistics<br><br><u>4. Genomics analysis statistical results used in the Kopec (2012a,b) publications</u><br>• Excel worksheets containing all array probesets and their corresponding fold change values and P1(t) statistical results for the rat and mouse, duodenum and jejunum, at day 8 and 91 | Cr6study.info |
| **APRIL 2016** | A labeling error was identified in the raw genomics data for the rat within the files in Cr6study.info. | Cr6study.info |



| DATE | EVENT | RELEVANT WEBSITE[1] |
|---|---|---|
| | • Specifically, incorrect probeset identifiers were included in the rat raw array data files (.gpr files). These identifiers were corrected, and Dr. Burgoon was notified of this edit via email on January 8, 2016 and April 5, 2016 (see ATTACHMENT A)<br>• This labeling error had no effect on the analyses or interpretation reported in Kopec et al. (2012b) | |
| **AUGUST 2016** | ToxStrategies received a request from Stiven Foster at the US EPA to access data from the Cr6study.info website. Access was granted on August 10, 2016 (see ATTACHMENT A). | Cr6study.info |
| **SEPTEMBER 2016** | All raw and processed array files were uploaded into the NCBI's Gene Expression Omnibus (GEO) repository. These data included:<br>• All raw microarray files<br>• Processed array data, consisting of quantile normalized expression data derived from the Cy3 array signal. Note: these processed array data were updated in 2016 and differ from those used in the Kopec (2012a,b) publications. Updated results from these data were published (Thompson et al. 2016, Rager et al. 2017, described below)<br><br>Data were uploaded alongside a reference to the Thompson et al. (2016) publication (final publication on November 2016, below) | GEO |
| **NOVEMBER 2016** | A study was published using the mouse and rat palate data (Thompson et al. 2016), using the updated array processing strategy provided in GEO. | GEO |
| **MAY 2017** | A study was published using the mouse duodenum data (Rager et al. 2017), using the updated array processing strategy provided in GEO. | GEO |
| **FEBRUARY 2018** | The GEO posting was updated to include additional citation information, referencing Rager et al. (2017). | GEO |
| **FEBRUARY 2018** | The link to the raw microarray files was temporarily not working through Cr6study.info. This link was corrected. | Cr6study.info |
| **FEBRUARY 2018** | More data were provided to further support the analysis conducted in the Kopec (2012a,b) publications.<br>• To detail, the original SAS code used in the genomics statistical analysis was originally provided in the 2014 data upload. This update added the specific input files that were used to execute the SAS code. | Cr6study.info |

[1] website URLs are Cr6study.info (https://cr6study.info), dbZach (http://dbzach.fst.msu.edu/), GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE87262).

**Explanation for varying numbers of arrays on different websites**

The number of array samples included at Cr6study.info, representing the analysis published in the Kopec et al. (2012a,b) publications, differs slightly from the number of array samples included in the GEO repository, representing the analyses published in the recent publications (Rager et al. 2017, Thompson et al. 2016). Specifically, the arrays in **Table 2** were excluded from the GEO repository.

**Table 2. Arrays that are included in Cr6study.info but excluded in the GEO repositories.**

| GPR file | Species, Tissue, and Duration Group | Array Components[1] | Note |
|---|---|---|---|
| 4417.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3426, control<br>Cy5 signal: animal ID 3401, exposed at 4 mg/L dose | Duplicate |
| 4418.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3401, exposed at 4 mg/L dose<br>Cy5 signal: animal ID 3426, control | Duplicate |
| 4451.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3416, exposed at 14 mg/L dose<br>Cy5 signal: animal ID 3426, control | Technical replicate |
| 4452.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3421, exposed at 60 mg/L<br>Cy5 signal: animal ID 3426, control | Technical replicate |
| 4453.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3409, exposed at 170 mg/L<br>Cy5 signal: animal ID 3425, control | Technical replicate |
| 4454.gpr | Mouse duodenum day 91 | Cy3 signal: animal ID 3400, control<br>Cy5 signal: animal ID 3404, exposed at 520 mg/L | Technical replicate |

[1] Note that data from the Cy3 and Cy5 dye signals were used in the Kopec et al. (2012a,b) publications; while analyses only focused on data from the Cy3 dye signal in the Rager et al (2017) and Thompson et al. (2016) publications to minimize potential dye bias / interference issues.

These arrays in Table 2 were excluded from the GEO repository, reflective of the more recent Rager et al. (2017) publication, because these arrays represented duplicated data or technical replicates. Specifically, chip scan data in array IDs 4417 and 4418 were identified as duplicated data; thus both of these arrays were removed from the analysis. Array IDs 4451, 4452, 4453, and 4454 represented technical replicate arrays used for internal quality purposes. These arrays measured gene expression profiles of samples from animals that were already accounted for by

other arrays. Neither the Kopec et al. nor the Rager et al. analyses included technical replicate arrays.

The biological replicates were explained in the Rager et al. (2017) publication in the methods section:

> *"RNA samples were assessed in biological triplicate in all dose groups (0, 0.03, 4, 14, 60, 170, and 520 mg/l SDD at day 8 and day 91) except for the 4 mg/l SDD exposure group from day 91, which was analyzed in biological duplicate due to potential microarray quality issues."*

The arrays that were removed due to duplicate issues or represented technical replicates were excluded from the data in the GEO repository, to be consistent with the Rager et al. (2017) publication. The number of biological replicates used in this analysis is detailed in Table 3. All array data, including duplicate data/technical replicates, are included in Cr6study.info.

**Table 3. Biological replicate numbers in the GEO database, reflective of the array analysis in Rager et al. (2017).**

| Duration Group | Species, Tissue | Dose (mg/L sodium dichromate dihydrate) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.3 | 4 | 14 | 60 | 170 | 520 | Total |
| 8 days | Mouse, duodenum | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 21 |
| 91 days | Mouse, duodenum | 3 | 3 | 2[1] | 3 | 3 | 3 | 3 | 20 |

[1] Note that the analyses conducted by Kopec et al. (2012a,b) contained one more biological replicate in this dose group.

## General comparison between publication findings

Genomics data analysis methods and standards have evolved over time. Thus, the data processing and statistical methods differed between the original publications using the Cr(VI) genomics data in the mouse duodenum (Kopec et al. 2012a,b) and more recent, updated publication (Rager et al. 2017); however, overall general findings remained consistent despite these varying approaches. For example, Kopec et al. 2012a, which focused on the mouse intestinal responses, identified genes involved in oxidative stress and cytotoxicity with altered expression, representing early key events. Other pathways noted as enriched included those involved in cell cycle, lipid metabolism, and immune response. Pathways involved in DNA repair were also noted in the day 8 results, including the nucleotide excision repair pathway, which did not show enrichment at day 91 (Kopec et al. 2012a). Similarly, Kopec et al. (2012b) noted pathways relevant to oxidative stress, immune response, protein synthesis, cell cycle/cell growth and proliferation, and DNA damage and repair in the mouse and rat duodenum and jejunum.

Similar results were apparent in the Rager et al. (2017) analysis focusing on the mouse duodenum, with pathways involved in cell stress and injury, cell death, and cell growth, proliferation and

development involved in early key events that were also sustained in the day 91 results. The nucleotide excision repair pathway was also identified as enriched in the day 8 results, but not the day 91 results; and it was noted that this pathway included eight genes showing increased expression associated with Cr(VI), all of which were involved in general DNA transcription and cell cycle signaling. Pathways involved in metabolism and immune response were also identified as enriched, similar to Kopec et al. (2012a). **In summary, analysis strategies have evolved over time surrounding the use of the Cr(VI) genomics data; still, general findings have remained consistent despite varying approaches.**

In conclusion, the raw and processed Cr(VI) genomics data are provided online, and the methods used to analyze these data have evolved over time to parallel ongoing advances in genomics-based assessments. This robust data set provides important information that can be used to further understand the MOA of Cr(VI). We have been fully transparent about the research conducted, including making the raw genomics data as well as other raw data available to EPA and other scientists.

If you have any questions, please contact me at eileen_conneely@americanchemistry.com or at 202-249-6711.

Sincerely,

*Eileen Conneely*

Eileen Conneely, M.P.H., J.D.
Director, Chemical Products & Technology Division
American Chemistry Council

Attachment A: Email communication on updates to Cr6study.info genomics data

cc: C. Gibbons, gibbons.catherine@epa.gov
A. Sasso, sasso.alan@epa.gov
R. Yamada, yamada.richard@epa.gov
J. Orme-Zavaleta, orme-zavaleta.jennifer@epa.gov
T. Bahadori, bahadori.tina@epa.gov
E. Ohanian, ohanian.edward@epa.gov

ATTACHMENT A

# Email communication on updates to Cr6study.info genomics data

**Subject:** Cr(VI) raw microarray data
**Date:** Thursday, March 13, 2014 at 12:37:11 PM Central Daylight Time
**From:** Apple-Mail=_405249D0-D0F5-4909-96A9-35F59E23F6BE Mark Harris boundary=
**To:** Burgoon.Lyle@epa.gov
**CC:** Rusty Thomas, Tim Zacharewski, Gibbons.Catherine@epa.gov, Chad Thompson, Deb Proctor

Lyle: as I mentioned in the meeting on Monday, the data will be posted to our new Cr(VI) MOA website before SOT. Once the site is live I will send you all a link to it. You will be able to download the genomics data at that time.

The approach and methods that were used to analyze the data are adequately described in the Kopec papers. Our use of the genomics data to support the Mode of Action can be found in our Critical Reviews in Toxicology paper. Links to these Open Access papers can be found at the web page below:

http://www.toxstrategies.com/publications/CRVI_MOA_study.htm

mark

**From:** "Burgoon, Lyle" <Burgoon.Lyle@epa.gov>
**Date:** March 12, 2014 at 8:43:16 AM CDT
**To:** "cthompson@toxstrategies.com" <cthompson@toxstrategies.com>
**Cc:** "tzachare@msu.edu" <tzachare@msu.edu>, "Gibbons, Catherine" <Gibbons.Catherine@epa.gov>
**Subject: Cr(VI) raw microarray data**

Dr. Thompson,

It was nice seeing you and Dr. Harris (via video) at Monday¹s meeting @ NCEA HQ. I was quite pleased to hear Ms. Mason state that ACC expected the researchers would share their data and results with NCEA.

I¹m following up on our discussion from Monday and am requesting access to all of your raw microarray data, as well as your analyzed data that supports the conclusions in your papers. In addition, it would be helpful if you could also supply us with the analysis code that was used, any protocols used for the analyses, and any other supporting documentation that may help us understand how the assays and analyses were performed.

For clarity, I am using the MIAME definition of ³raw data², and my request for the additional information is in line and keeping with the MIAME standard, which can be found here: http://www.mged.org/Workgroups/MIAME/miame.html.

To facilitate data transfer, I can set-up an EPA-based FTP site where you can upload the data.

Thanks again for presenting your latest results to us, and I look forward to receiving the data.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
National Center for Environmental Assessment
Chief, Hazardous Pollutant Assessment Group (Acting)
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: Ex. 6



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**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

Direct Dial: Ex. 6
Houston Office: (281) 712-2062 Ext: Ex. 6
Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Cr(VI) MOA Study Website
**Date:** Monday, March 24, 2014 at 8:34:03 AM Central Daylight Time
**From:** Apple-Mail=_B33E4A44-0724-416B-A519-C1B4A7F9AE29 Mark Harris boundary=
**To:** Burgoon.Lyle@epa.gov
**CC:** Tim Zacharewski, Gibbons.Catherine@epa.gov, Chad Thompson, Rusty Thomas

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

~~

**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

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Direct Dial: Ex. 6
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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Re: Cr(VI) MOA Study Website
**Date:** Thursday, April 3, 2014 at 3:07:45 PM Central Daylight Time
**From:** Mark Harris
**To:** Burgoon, Lyle
**CC:** Gibbons, Catherine, Vandenberg, John, Flowers, Lynn

I will have to talk to our legal folks about this.

Back in touch shortly.

mark

**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

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Website: http://www.toxstrategies.com



On Apr 3, 2014, at 2:59 PM, Burgoon, Lyle <Burgoon.Lyle@epa.gov> wrote:

> Hi Mark,
>
> We cannot access the data at this time due to our inability to agree to the Terms and Conditions associated with data access found here: http://cr6study.info/study-data/terms-and-conditions/
>
> Specifically, we request that ToxStrategies delete the indemnification clause in the Terms and Conditions. As written, the Agency cannot agree to the terms as it would be a violation of the Anti-Deficiency Act (i.e., making a commitment to an unknown future cost is a direct violation of the Anti-Deficiency Act).
>
> Please let me know what action you are willing to take as soon as possible. As you are aware,

time is of the essence with respect to the Cr(VI) IRIS Assessment. Your prompt attention to this matter is appreciated.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
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National Center for Environmental Assessment
US Environmental Protection Agency
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Cell: Ex. 6



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**From:** Mark Harris [mailto:mharris@toxstrategies.com]
**Sent:** Monday, March 24, 2014 9:34 AM
**To:** Burgoon, Lyle
**Cc:** Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Thomas, Russell
**Subject:** Cr(VI) MOA Study Website

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

Direct Dial: Ex. 6
Houston Office: (281) 712-2062 Ext: Ex. 6

Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Terms and Conditions Modified
**Date:** Monday, April 7, 2014 at 12:23:08 PM Central Daylight Time
**From:** Apple-Mail=_54682036-6E73-4A57-B537-DA70B702C164 Mark Harris boundary=
**To:** Burgoon, Lyle

We modified the terms and conditions for the US Government for our website http://cr6study.info. The additional text is: If you are a United States Government entity, the indemnification provision of the Terms and Conditions is hereby waived. Your liability for any breach of the Terms and Conditions, or any claim arising from the Terms and Conditions, shall be determined under the Federal Tort Claims Act, or other governing authority.

mark

--

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Website
**Date:** Monday, April 7, 2014 at 3:43:02 PM Central Daylight Time
**From:** Apple-Mail=_5CAFA6EE-E253-474E-8737-E357CC0E69C7 Mark Harris boundary=
**To:** Burgoon, Lyle

We have also posted the genomics analysis code to the website today. Only thing remaining is the QRT-PCR info which is still being assembled.

mark

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** T&C - how does this
**Date:** Wednesday, April 23, 2014 at 2:09:01 PM Central Daylight Time
**From:** Apple-Mail=_1704D469-54A7-4A17-9C82-0F0AADD0DE20 Mark Harris boundary=
**To:** Burgoon, Lyle
**Attachments:** Revised Cr6 Website Terms.docx

Lyle: I got some revised T&C language from the attorneys today. See attached in Tracked Changes. If this works let me know and I will get the website updated.

Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

thanks

mark

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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Re: T&C - how does this
**Date:** Tuesday, April 29, 2014 at 7:28:32 AM Central Daylight Time
**From:** Apple-Mail=_3480B4DA-5FF4-4C62-AE8C-422044ED03FF Mark Harris boundary=
**To:** Burgoon, Lyle

Lyle: the website has been updated with this revised language. Also, we have added the QRT-PCR data that we received from Tim.

mark

--

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Website: http://www.toxstrategies.com



On Apr 28, 2014, at 10:08 AM, Burgoon, Lyle <Burgoon.Lyle@epa.gov> wrote:

> Hi Mark,
>
> Thanks for working with us that language meets our needs. Sorry for the delay was out sick last week.
>
> Cheers,
>
> Lyle
>
> Lyle D. Burgoon, Ph.D
> Chief, Hazardous Pollutant Assessment Group (Acting)
> National Center for Environmental Assessment
> US Environmental Protection Agency
> Phone: 919.541.7808
> Fax: 919.685.3473

Cell: Ex. 6

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**Sent:** Wednesday, April 23, 2014 3:09 PM
**To:** Burgoon, Lyle
**Subject:** T&C - how does this

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Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** cr6study.info
**Date:** Friday, September 18, 2015 at 12:51:30 PM Central Daylight Time
**From:** Mark Harris
**To:** lyle.d.burgoon@usace.army.mil

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
--

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Website: http://www.toxstrategies.com
Linkedin: http://www.linkedin.com/in/toxstrategiesharris

**Subject:** Re: [EXTERNAL] cr6study.info
**Date:** Friday, September 18, 2015 at 3:40:14 PM Central Daylight Time
**From:** Mark Harris
**To:** Burgoon, Lyle D ERD-MS

Lyle: that is good to know about being able to collaborate on future projects. You never know when a client will ask for such analyses.

One question – is it is relatively straightforward to engage you all for a project? Meaning, if something came up, could the paperwork being done relatively quickly to get going?

mark

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**From:** "Burgoon, Lyle D ERD-MS"
**Date:** Friday, September 18, 2015 at 3:35 PM
**To:** Mark Harris
**Subject:** Re: [EXTERNAL] cr6study.info

Hey Mark,

Thanks. The Army has no interest in chromium (thankfully). However, EPA is in the process of contracting with the Army to reanalyze the data. So, in preparation for that, we requested the data.

On a side note, in my new federal job I'm soft money, and we operate like a consulting company. So if in the future you need CompTox or toxicogenomic support, let me know. We can accept funds from companies for collaborative work.

If you want to chat sometime, my number is **Ex. 6**

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

---

**From:** Mark Harris
**Sent:** Friday, September 18, 2015 1:51 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** [EXTERNAL] cr6study.info

---

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~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

**Subject:** Re: [EXTERNAL] cr6study.info
**Date:** Friday, January 8, 2016 at 5:04:08 PM Central Standard Time
**From:** Burgoon, Lyle D ERD-MS
**To:** Mark Harris

Thanks for the heads up, Mark.

Have a nice weekend.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

**From:** Mark Harris
**Sent:** Friday, January 8, 2016 6:02 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** Re: [EXTERNAL] cr6study.info

Lyle: we were doing some work on the Cr(VI) genomics data and noticed an error in the rat data (some probe ID's were mislabeled). The actual data is correct - just the labels are wrong. This doesn't impact our publications but does impact the data available for download from our Cr(VI) study website. MSU is fixing and I will alert you when we update the files.

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LinkedIn: Blockedhttp://www.linkedin.com/in/toxstrategiesharris

**From:** "Burgoon, Lyle D ERD-MS" <Lyle.D.Burgoon@usace.army.mil>
**Date:** Friday, September 18, 2015 at 3:50 PM
**To:** Mark Harris <mharris@toxstrategies.com>
**Subject:** Re: [EXTERNAL] cr6study.info

Army moves paperwork pretty quick. If I know it's coming, I can grease the skids. If you have a tight turnaround on paperwork approval needed my lab director can expedite and maybe 1-2weeks we should be able to turn it around. Unless it's after July 31, then it might take longer bc of the backlog at contracts.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

**From:** Mark Harris
**Sent:** Friday, September 18, 2015 4:40 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** Re: [EXTERNAL] cr6study.info

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One question – is it is relatively straightforward to engage you all for a project? Meaning, if something came up, could the paperwork being done relatively quickly to get going?

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If you want to chat sometime, my number is **Ex. 6**

Cheers,

Lyle

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**To:** Burgoon, Lyle D ERD-MS
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Website: BlockedBlockedhttp://www.toxstrategies.com
LinkedIn: BlockedBlockedhttp://www.linkedin.com/in/toxstrategiesharris

**Subject:** Re: [EXTERNAL] cr6study.info
**Date:** Tuesday, April 5, 2016 at 2:42:13 PM Central Daylight Time
**From:** Burgoon, Lyle D ERD-MS
**To:** Mark Harris

Hey Mark,

Not started yet, but thanks for the update. Once I get cranking if I have questions I'll let ya know.

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

---

**From:** Mark Harris
**Sent:** Tuesday, April 5, 2016 2:09 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** Re: [EXTERNAL] cr6study.info

---

Lyle: just touching base. Have you done anything with the data – the labels are all fixed in the raw data.

Trust all is well.

mark

--

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---

**From:** "Burgoon, Lyle D ERD-MS" <Lyle.D.Burgoon@usace.army.mil>
**Date:** Friday, January 8, 2016 at 5:04 PM
**To:** Mark Harris <mharris@toxstrategies.com>
**Subject:** Re: [EXTERNAL] cr6study.info

Thanks for the heads up, Mark.

Have a nice weekend.

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US Army Engineer Research and Development Center
Research Triangle Park, NC

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LinkedIn: BlockedBlockedBlockedhttp://www.linkedin.com/in/toxstrategiesharris

Ex. 6

**Subject:** Re: Cr(VI) raw microarray data
**Date:** Thursday, March 13, 2014 at 12:39:33 PM Central Daylight Time
**From:** Burgoon, Lyle
**To:** Mark Harris

Thanks, Mark; looking forward to the data. If you're going to SOT, safe travels.

Cheers,

Lyle

---

**From:** Mark Harris <mharris@toxstrategies.com>
**Sent:** Thursday, March 13, 2014 1:37:10 PM
**To:** Burgoon, Lyle
**Cc:** Thomas, Russell; Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Deborah Proctor
**Subject:** Cr(VI) raw microarray data

Lyle: as I mentioned in the meeting on Monday, the data will be posted to our new Cr(VI) MOA website before SOT. Once the site is live I will send you all a link to it. You will be able to download the genomics data at that time.

The approach and methods that were used to analyze the data are adequately described in the Kopec papers. Our use of the genomics data to support the Mode of Action can be found in our Critical Reviews in Toxicology paper. Links to these Open Access papers can be found at the web page below:

http://www.toxstrategies.com/publications/CRVI_MOA_study.htm

mark

**From:** "Burgoon, Lyle" <Burgoon.Lyle@epa.gov>
**Date:** March 12, 2014 at 8:43:16 AM CDT
**To:** "cthompson@toxstrategies.com" <cthompson@toxstrategies.com>
**Cc:** "tzachare@msu.edu" <tzachare@msu.edu>, "Gibbons, Catherine" <Gibbons.Catherine@epa.gov>
**Subject: Cr(VI) raw microarray data**

Dr. Thompson,

It was nice seeing you and Dr. Harris (via video) at Monday's meeting @ NCEA HQ. I was quite pleased to hear Ms. Mason state that ACC expected the researchers would share their data and results with NCEA.

I'm following up on our discussion from Monday and am requesting access to all of your raw microarray data, as well as your analyzed data that supports the conclusions in your papers. In addition, it would be helpful if you could also supply us with the analysis code that was used,

any protocols used for the analyses, and any other supporting documentation that may help us understand how the assays and analyses were performed.

For clarity, I am using the MIAME definition of "raw data", and my request for the additional information is in line and keeping with the MIAME standard, which can be found here: http://www.mged.org/Workgroups/MIAME/miame.html.

To facilitate data transfer, I can set-up an EPA-based FTP site where you can upload the data.

Thanks again for presenting your latest results to us, and I look forward to receiving the data.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
National Center for Environmental Assessment
Chief, Hazardous Pollutant Assessment Group (Acting)
US Environmental Protection Agency
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Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Re: Cr(VI) raw microarray data
**Date:** Monday, March 17, 2014 at 9:40:37 AM Central Daylight Time
**From:** Burgoon, Lyle
**To:** mharris@toxstrategies.com
**CC:** tzachare@msu.edu, Gibbons, Catherine, cthompson@toxstrategies.com

Hi Mark,

I have read the Kopec, et al papers again, at your suggestion. Reading them reaffirms that we absolutely need to have the analysis code in order to understand what exactly was done. Per the MIAME standard (Minimum Information About a Microarray Experiment: http://www.mged.org/Workgroups/MIAME/miame_2.0.html):

> The essential laboratory and data processing protocols are usually described in the journal methods section. It is sufficient to simply reference the standard experimental or data processing protocols, such as MAS5 or RMA. However, if a protocol depends on parameters that can be varied, their values should be provided. If novel or non-standard data processing protocols are used, these should be described in sufficient detail to allow the user to understand what exactly has been done in the experiment and how the data have been analysed to reach the conclusions of the study.

In the case of the Kopec, et al papers, the analysis protocols used are non-standard data processing methods. As far as I can tell, these methods are commonly used in Dr. Zacharewski's laboratory; however, they are not commonly used elsewhere. The Eckel, et al 2005 and Eckel, et al 2004 papers describe analysis methods used in the Kopec, et al papers that require parameterization or changes to the code in order to work properly. Thus, it is critical for us to see the code in order to understand how the data were treated. Without the code we will be unable to understand how you came to draw the hypotheses you did in the Kopec, et al and subsequent papers. Without this understanding, we cannot interpret your results.

As MIAME is a community standard, first adopted several years ago by many prominent journals, you can see that this request is not unusual.

Likewise, we need to have the rawest form of the data possible for the QRT-PCR analyses, which Kopec, et al have used to support their microarray data. At a minimum, this would include any spreadsheets or text files of the data from the ABI machine, detailing all of the mathematical manipulations of the data from prior to standardization, through normalization, ratio calculation, and statistical analysis. Note: I couldn't find any details on how the statistical analysis of the QRT-PCR was performed, nor can I find a reference for how the standard errors of a ratio were calculated.

We may have additional data requirements and needs as we dig into the toxicogenomics data further, and we look forward to working with you in obtaining the information we need to better understand these studies.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
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Dr. Thompson,

It was nice seeing you and Dr. Harris (via video) at Monday's meeting @ NCEA HQ. I was quite pleased to hear Ms. Mason state that ACC expected the researchers would share their data and results with NCEA.

I'm following up on our discussion from Monday and am requesting access to all of your raw microarray data, as well as your analyzed data that supports the conclusions in your papers. In addition, it would be helpful if you could also supply us with the analysis code that was used, any protocols used for the analyses, and any other supporting documentation that may help us understand how the assays and analyses were performed.

For clarity, I am using the MIAME definition of "raw data", and my request for the additional information is in line and keeping with the MIAME standard, which can be found here: http://www.mged.org/Workgroups/MIAME/miame.html.

To facilitate data transfer, I can set-up an EPA-based FTP site where you can upload the data.

Thanks again for presenting your latest results to us, and I look forward to receiving the data.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
National Center for Environmental Assessment
Chief, Hazardous Pollutant Assessment Group (Acting)
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: Ex. 6



--

**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

Direct Dial: Ex. 6

Houston Office: (281) 712-2062 Ex. 6
Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** RE: Cr(VI) MOA Study Website
**Date:** Thursday, April 3, 2014 at 2:59:52 PM Central Daylight Time
**From:** Burgoon, Lyle
**To:** Mark Harris
**CC:** Gibbons, Catherine, Vandenberg, John, Flowers, Lynn

Hi Mark,

We cannot access the data at this time due to our inability to agree to the Terms and Conditions associated with data access found here: http://cr6study.info/study-data/terms-and-conditions/

Specifically, we request that ToxStrategies delete the indemnification clause in the Terms and Conditions. As written, the Agency cannot agree to the terms as it would be a violation of the Anti-Deficiency Act (i.e., making a commitment to an unknown future cost is a direct violation of the Anti-Deficiency Act).

Please let me know what action you are willing to take as soon as possible. As you are aware, time is of the essence with respect to the Cr(VI) IRIS Assessment. Your prompt attention to this matter is appreciated.

Thanks,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: Ex. 6
Fax: 919.685.3473
Cell: Ex. 6



---

**From:** Mark Harris [mailto:mharris@toxstrategies.com]
**Sent:** Monday, March 24, 2014 9:34 AM
**To:** Burgoon, Lyle
**Cc:** Tim Zacharewski; Gibbons, Catherine; Chad Thompson; Thomas, Russell
**Subject:** Cr(VI) MOA Study Website

Lyle: our Cr(VI) MOA study website is live. You can access is at www.cr6study.info

Under the Study Data tab you can register to download data. Once registered, you will receive a password to access the data download portion of the site.

Not all of the genomics data you requested has been posted. Things that are not posted yet are:

1. Analysis Code
2. QRT-PCR data

These items will be added in the next few weeks.

mark

--

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**Mark Harris, Ph.D.**
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

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Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** RE: T&C - how does this
**Date:** Monday, April 28, 2014 at 10:08:36 AM Central Daylight Time
**From:** Burgoon, Lyle
**To:** Mark Harris

Hi Mark,

Thanks for working with us – that language meets our needs. Sorry for the delay – was out sick last week.

Cheers,

Lyle

Lyle D. Burgoon, Ph.D
Chief, Hazardous Pollutant Assessment Group (Acting)
National Center for Environmental Assessment
US Environmental Protection Agency
Phone: 919.541.7808
Fax: 919.685.3473
Cell: Ex. 6



---

**From:** Mark Harris [mailto:mharris@toxstrategies.com]
**Sent:** Wednesday, April 23, 2014 3:09 PM
**To:** Burgoon, Lyle
**Subject:** T&C - how does this

Lyle: I got some revised T&C language from the attorneys today. See attached in Tracked Changes. If this works let me know and I will get the website updated.

Also, if these words don't work, can you ask the attorney reviewing this to suggest some words that would work for the US government?

thanks

mark

--

**Note New Address**
**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

23123 Cinco Ranch Blvd, Suite 220

Katy, TX 77494

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Houston Office: (281) 712-2062 Ext 2001
Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com

**Subject:** Re: [EXTERNAL] cr6study.info
**Date:** Friday, September 18, 2015 at 3:35:41 PM Central Daylight Time
**From:** Burgoon, Lyle D ERD-MS
**To:** Mark Harris

Hey Mark,

Thanks. The Army has no interest in chromium (thankfully). However, EPA is in the process of contracting with the Army to reanalyze the data. So, in preparation for that, we requested the data.

On a side note, in my new federal job I'm soft money, and we operate like a consulting company. So if in the future you need CompTox or toxicogenomic support, let me know. We can accept funds from companies for collaborative work.

If you want to chat sometime, my number is Ex. 6

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

---

**From:** Mark Harris
**Sent:** Friday, September 18, 2015 1:51 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** [EXTERNAL] cr6study.info

---

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
--

**ToxStrategies, Inc.**
Innovative Solutions > Sound Science

**Mark Harris, Ph.D.**
Principal Health Scientist

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fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: Blockedhttp://www.toxstrategies.com
LinkedIn: Blockedhttp://www.linkedin.com/in/toxstrategiesharris

**Subject:** Re: [EXTERNAL] cr6study.info
**Date:** Friday, September 18, 2015 at 3:50:25 PM Central Daylight Time
**From:** Burgoon, Lyle D ERD-MS
**To:** Mark Harris

Army moves paperwork pretty quick. If I know it's coming, I can grease the skids. If you have a tight turnaround on paperwork approval needed my lab director can expedite and maybe 1-2weeks we should be able to turn it around. Unless it's after July 31, then it might take longer bc of the backlog at contracts.

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center
Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

---

**From:** Mark Harris
**Sent:** Friday, September 18, 2015 4:40 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** Re: [EXTERNAL] cr6study.info

---

Lyle: that is good to know about being able to collaborate on future projects. You never know when a client will ask for such analyses.

One question – is it is relatively straightforward to engage you all for a project? Meaning, if something came up, could the paperwork being done relatively quickly to get going?

mark

---

**From:** "Burgoon, Lyle D ERD-MS"
**Date:** Friday, September 18, 2015 at 3:35 PM
**To:** Mark Harris
**Subject:** Re: [EXTERNAL] cr6study.info

Hey Mark,

Thanks. The Army has no interest in chromium (thankfully). However, EPA is in the process of contracting with the Army to reanalyze the data. So, in preparation for that, we requested the data.

On a side note, in my new federal job I'm soft money, and we operate like a consulting company. So if in the future you need CompTox or toxicogenomic support, let me know. We can accept funds from companies for collaborative work.

If you want to chat sometime, my number is Ex. 6

Cheers,

Lyle

Lyle D. Burgoon, PhD
Environmental Laboratory
US Army Engineer Research and Development Center

Research Triangle Park, NC

Sent from my BlackBerry 10 smartphone on the Verizon Wireless 4G LTE network.

**From:** Mark Harris
**Sent:** Friday, September 18, 2015 1:51 PM
**To:** Burgoon, Lyle D ERD-MS
**Subject:** [EXTERNAL] cr6study.info

Lyle: you should have received an approval for access to the data via email. If you have any issues downloading data just let me know. I did not realize you had changed jobs. Good luck with the new position. Why is the Army interested in Cr(VI)?

mark
--

**ToxStrategies, Inc.**
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**Mark Harris, Ph.D.**
Principal Health Scientist

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Direct Dial: Ex. 6
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Email: mharris@toxstrategies.com
Website: Blockedhttp://www.toxstrategies.com
Linkedin: Blockedhttp://www.linkedin.com/in/toxstrategiesharris

**Subject:** New user registration for Cr(VI) MOA Study
**Date:** Wednesday, August 10, 2016 at 1:16:17 PM Central Daylight Time
**From:** CrVI MOA Study
**To:** mharris@toxstrategies.com

The following user registered for Cr(VI) MOA Study (and is pending approval)

username: sfoster
email: foster.stiven@epa.gov

First Name: Stiven
Last Name: Foster
Company: USEPA
Position: Toxicologist
Address 1: 1200 Pennsylvania Ave, NW
Address 2: MC-5103T
City: Washington
State: DC
Zip: 20460
Country: USA
Day Phone: 202-566-1911
Plans for Data Use: Evaluation of finding for consideration in EPA efforts.
TOS: agree

This user registered here:
http://cr6study.info/register/

**Ex. 6**

---
This is an automated message from Cr(VI) MOA Study
Please do not reply to this address

**Subject:** (none)
**Date:** Wednesday, August 10, 2016 at 1:19:59 PM Central Daylight Time
**From:** Mark Harris
**To:** foster.stiven@epa.gov

I have activated your access to the raw data. If you have any problems accessing the data let me know.

Mark

--
ToxStrategies, Inc.
Innovative Solutions > Sound Science

Mark Harris, Ph.D.
Principal Health Scientist
---

23123 Cinco Ranch Blvd, Suite 220
Katy, TX 77494

Direct Dial: Ex. 6
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Cell: Ex. 6
fax: (832) 218-2756
Email: mharris@toxstrategies.com
Website: http://www.toxstrategies.com
Linkedin: http://www.linkedin.com/in/toxstrategiesharris

Message

**From**: Conneely, Eileen [Eileen_Conneely@americanchemistry.com]
**Sent**: 4/30/2018 7:30:57 PM
**To**: Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]
**CC**: Gibbons, Catherine [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2ac775a35a0945718edc7e02f50e6c12-Gibbons, Catherine]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Ohanian, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f119491e2ba8476381a39c57a456ac55-EOhanian]
**Subject**: request to meet with IRIS staff concerning genomics dataset for Cr(VI)

Dear Dr. Thayer,

I appreciate your response. You noted that EPA expects to release a protocol on hexavalent chromium (Cr(VI)) this June or July and that EPA plans to convene a public webinar where EPA staff would summarize the protocol content and listen to public comment on the protocol. We appreciate the opportunity to present comments during the planned EPA webinar, but ten minutes would not be adequate to present an overview of the most recent MOA research publications. We hope that additional time will be granted. Please also let us know as soon as possible when you have set the date for the webinar, as many interested parties may have planned to be away on summer vacations.

Additionally, we have questions on what full implementation of systematic review entails for the EPA IRIS program, especially since we have heard that the IRIS program has not fully considered the large toxicogenomics dataset funded by the Hexavalent Chromium Panel. The MOA researchers communicated with Lyle Burgoon, formerly at EPA, from 2014 – 2016 concerning the Cr(VI) genomics dataset, and we would like to present an overview of events relevant to the Cr(VI) genomics data, an explanation for the number of microarrays available through different online data repositories, and a general findings comparison between published analyses (i.e. genomics data analysis methods and standards have evolved over time and yet general findings have remained consistent despite varying approaches).

To ensure that all of this important genomics data is being considered in the assessment process, we would like to schedule a meeting as soon as possible with you and the IRIS staff working on the Cr(VI) assessment.

Sincerely,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
eileen_conneely@americanchemistry.com
700 2nd Street NE | Washington, DC 20002
T: Ex. 6 | F: (202) 330-5646 | C: Ex. 6 www.americanchemistry.com

**From:** Thayer, Kris [mailto:thayer.kris@epa.gov]
**Sent:** Thursday, March 29, 2018 5:08 AM

**To:** Conneely, Eileen <Eileen_Conneely@americanchemistry.com>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Eileen,

Thanks again for reaching out. We appreciate your sharing the list of recent publications.

We anticipate releasing a protocol on hexavalent chromium (Cr6) for public comment this June-July timeframe. The protocol will summarize the rationale for conducting the assessment, describe screening criteria to identify relevant literature, outline the approach for evaluating study quality, describe the process of evidence synthesis/integration, and dose-response methods. The protocol is a new document for the IRIS Program developed as part of its full implementation of systematic review (see presentation materials for the NAS Workshop "Review of Advances Made to the IRIS Process"). Obviously, the chromium assessment has been underway for some time now and release of this protocol should not be construed as a re-start. Rather, we are releasing the protocol so that the chromium assessment has similar public engagement steps as assessments started more recently. The protocol will include the list of studies currently included in the assessment.

Given the high level of interest in the IRIS hexavalent chromium assessment, we are planning to convene a public science webinar concurrent with the public comment period on the protocol. The format of this webinar would be a presentation by EPA staff summarizing the protocol content, followed by a public comment session where the Hexavalent Chromium Panel could present the overview suggested in your note below during a 10-minute public comment period. It may be possible to extend the length of the oral comment period depending on the number of registered speakers. EPA scientists would respond to questions of clarification but would not be discussing scientific interpretation of the evidence at this point in our process. Written materials could also be submitted as part of the public comment period.

We understand you may have questions on what full implementation of systematic review entails and how it integrates with the IRIS process. Although these topics are covered in the NAS presentation materials, we would be happy to schedule a meeting if you would like to discuss further.

Sincerely,

Kris

---

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS) Division
National Center for Environmental Assessment, NCEA
ORD, USEPA
Mail Code: B243-01
Building: Bldg B (Room B211I)
Research Triangle Park, NC 27711
(919) 541-0152 RTP
(202) 564-1771 Ronald Reagan Building (room 51203)
Skype: kristina.thayer
thayer.kris@epa.gov

**From:** Conneely, Eileen [mailto:Eileen_Conneely@americanchemistry.com]
**Sent:** Thursday, March 22, 2018 8:24 PM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** Re: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Dear Dr. Thayer,

Please see the attached letter written on behalf of the Hexavalent Chromium Panel of the American Chemistry Council requesting a stakeholder meeting with IRIS staff to present an overview of the most recent publications by the MOA study researchers, and to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.

Thank you,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
eileen_conneely@americanchemistry.com
700 2nd Street NE | Washington, DC 20002
T: Ex. 6 | F: (202) 330-5646 | C: Ex. 6 www.americanchemistry.com


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Message

**From**: Thayer, Kris [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=3CE4AE3F107749C6815F243260DF98C3-THAYER, KRI]
**Sent**: 3/23/2018 2:19:42 PM
**To**: Conneely, Eileen [Eileen_Conneely@americanchemistry.com]
**CC**: Gibbons, Catherine [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2ac775a35a0945718edc7e02f50e6c12-Gibbons, Catherine]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Ohanian, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f119491e2ba8476381a39c57a456ac55-EOhanian]
**Subject**: RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Thank you Eileen for the letter.

We will get back to you soon on the best way to proceed given the high level of interest in this assessment and timing.

Sincerely,

Kris

---

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS) Division
National Center for Environmental Assessment, NCEA
ORD, USEPA
Mail Code: B243-01
Building: Bldg B (Room B211I)
Research Triangle Park, NC 27711
(919) 541-0152 RTP
(202) 564-1771 Ronald Reagan Building (room 51203)
Skype: kristina.thayer
thayer.kris@epa.gov

---

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**Sent:** Thursday, March 22, 2018 8:24 PM
**To:** Thayer, Kris <thayer.kris@epa.gov>
**Cc:** Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>; Yamada, Richard (Yujiro) <yamada.richard@epa.gov>; Orme-Zavaleta, Jennifer <Orme-Zavaleta.Jennifer@epa.gov>; Bahadori, Tina <Bahadori.Tina@epa.gov>; Ohanian, Edward <Ohanian.Edward@epa.gov>
**Subject:** Re: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)

Dear Dr. Thayer,

Please see the attached letter written on behalf of the Hexavalent Chromium Panel of the American Chemistry Council requesting a stakeholder meeting with IRIS staff to present an overview of the most recent publications by the MOA study researchers, and to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.

Thank you,

Eileen Conneely

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Director, Chemical Products & Technology Division
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700 2nd Street NE | Washington, DC 20002
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Message

**From**: Conneely, Eileen [Eileen_Conneely@americanchemistry.com]
**Sent**: 3/23/2018 12:24:13 AM
**To**: Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]
**CC**: Gibbons, Catherine [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2ac775a35a0945718edc7e02f50e6c12-Gibbons, Catherine]; Sasso, Alan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8cb867519abc4dcea88149d12ef3e8e9-Sasso, Alan]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; Bahadori, Tina [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=7da7967dcafb4c5bbc39c666fee31ec3-Bahadori, Tina]; Ohanian, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f119491e2ba8476381a39c57a456ac55-EOhanian]
**Subject**: Re: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)
**Attachments**: 2018 March 21 ACC IRIS Correspondence re Cr6 Studies.pdf

Dear Dr. Thayer,

Please see the attached letter written on behalf of the Hexavalent Chromium Panel of the American Chemistry Council requesting a stakeholder meeting with IRIS staff to present an overview of the most recent publications by the MOA study researchers, and to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.

Thank you,

Eileen Conneely

*Eileen Conneely, M.P.H., J.D.* | American Chemistry Council
Director, Chemical Products & Technology Division
eileen_conneely@americanchemistry.com
700 2nd Street NE | Washington, DC 20002
T: Ex. 6 | F: (202) 330-5646 | C: Ex. 6 | www.americanchemistry.com

March 21, 2018

By email (thayer.kris@EPA.gov) and submission to EPA Docket Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

Kristina Thayer, Ph.D.
Director, Integrated Risk Information System (IRIS)
National Center for Environmental Assessment
USEPA Headquarters
Ariel Rios Building 1200 Pennsylvania Avenue, N.W.
***Mail Code:*** 8601P
Washington, DC 20460

RE: Draft IRIS Assessment of Hexavalent Chromium (Chromium VI)
Docket ID Nos. EPA-HQ-ORD-2014-0313 and EPA-HQ-ORD-2010-0540

Dear Dr. Thayer,

We recently learned from the EPA Office of Water that a draft IRIS assessment of hexavalent chromium (Cr(VI)) is expected to be publicly released by the end of 2018. To that end, we want to ensure that the IRIS office has been briefed on the most recent findings and publications by the Cr(VI) mode of action (MOA) study researchers, is familiar with all aspects of the MOA research, and considers the findings of this research, including the Cr(VI) genomics dataset, during the development of the toxicological review for oral exposure to Cr(VI). Thus, on behalf of the Hexavalent Chromium Panel of the American Chemistry Council (ACC), I write to request a stakeholder meeting as soon as possible to present an overview of the most recent publications by the MOA study researchers, including

- an integration of mechanistic and pharmacokinetic information to derive an oral reference dose and margin-of-exposure values for Cr(VI),
- an analysis of Eastmond's ten factors for considering the mode of action of Cr(VI)-induced gastrointestinal tumors in rodents, and
- a recovery study comparing the duodenal histopathology in mice following exposure to Cr(VI), captan, and folpet.

Additionally, we wish to review important information relevant to the Cr(VI) genomics dataset that was communicated to Dr. Lyle Burgoon prior to his departure from EPA.[1]

[1] This letter follows our most recent correspondence to EPA's IRIS program on October 16, 2017 and April 27, 2017, our February 4, 2016, correspondence to Vincent Cogliano, and an August 10, 2016 meeting between ACC, the MOA study researchers, and EPA's IRIS staff working on the Cr(VI) assessment.

I will be following up to schedule a meeting as soon as possible with you and other appropriate staff to discuss this information in detail. If you have any questions, please contact me at eileen_conneely@americanchemistry.com or at Ex. 6

Sincerely,

*Eileen Conneely*

Eileen Conneely, M.P.H., J.D.
Director, Chemical Products & Technology Division
American Chemistry Council

Attachment 1: Hexavalent Chromium Research MOA Study Published Papers
as of March 20, 2018.

cc:
C. Gibbons, gibbons.catherine@epa.gov
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## Attachment 1. Cr(VI) MOA Study Published Papers (updated March 20, 2018)

### Overview

The Cr(VI) Mode of Action (MOA) Research Study was designed to understand how hexavalent chromium [Cr(VI)] in drinking water is associated with carcinogenesis in rats and mice. The project involved investigators from multiple institutions and conducted two 90-day drinking water studies, using the same mouse (B6C3F1) and rat (Fisher 344) strains used in the NTP study. The in-life portions of the study (i.e., the exposure, macro- and microscopic examinations, and some biochemical analyses) were conducted at the same research facility, Southern Research, that conducted the NTP study to further minimize inter-study variability. Histological lesions, biochemical analyses, toxicogenomic analyses, pharmacokinetic analyses, and mutational analyses were examined in the target tissues of interest, i.e., the small intestine and oral mucosa, of the mice and rats. In addition, in vitro cell culture studies were conducted to further inform the Cr(VI) MOA. The Cr(VI) MOA Research Study used the same concentrations of Cr(VI) in drinking water as the NTP study and also included lower Cr(VI) concentrations, which are more indicative of possible environmental exposures, such as U.S. drinking water.

See http://cr6study.info/ for more information on the MOA research.
Technical Contacts: ToxStrategies, Dr. Mark Harris (281-394-1567) or Dr. Chad Thompson (281-769-2195).

### Publications (all open access)

1) Thompson, C.M., L.C. Haws, M.A. Harris, N.M. Gatto, and D.M. Proctor. 2011. Application of the U.S. EPA Mode of Action Framework for Purposes of Guiding Future Research: A Case Study Involving the Oral Carcinogenicity of Hexavalent Chromium. *Toxicological Sciences*. 119(1): 20-40. Manuscript: http://toxsci.oxfordjournals.org/content/119/1/20.full.pdf+html?sid=896b4c7e-b535-4884-aa79-261ecc512de8; Supplemental data: http://toxsci.oxfordjournals.org/content/119/1/20/suppl/DC1

2) Thompson, C.M., D.M. Proctor, L.C. Haws, C.D. Hébert, S.D. Grimes, H.G. Shertzer, A.K. Kopec, J.G. Hixon, T.R. Zacharewski, and M.A. Harris. 2011. Investigation of the Mode of Action Underlying the Tumorigenic Response Induced in B6C3F1 Mice Exposed Orally to Hexavalent Chromium. *Toxicological Sciences*. 123(1): 58-70. Manuscript: http://toxsci.oxfordjournals.org/content/123/1/58.full.pdf+html?sid=d185e9e8-71d0-415b-9e2d-97ca5844207c; Supplemental data: http://toxsci.oxfordjournals.org/content/123/1/58/suppl/DC1

3) Thompson, C.M., D.M. Proctor, M. Suh, L.C. Haws, C.D. Hébert, J.F. Mann, H.G. Shertzer, J.G. Hixon, and M.A. Harris. 2011. Comparison of the Effects on Hexavalent Chromium in the Alimentary Canal of F344 rats and B6C3F1 Mice Following Exposure in Drinking Water: Implications for Carcinogenic Modes of Action. *Toxicological Sciences.* 125(1): 79-90. Manuscript: http://toxsci.oxfordjournals.org/content/125/1/79.full.pdf+html?sid=d7c79f9a-4b38-46d1-953e-81449fd02eae; Supplemental data: http://toxsci.oxfordjournals.org/content/125/1/79/suppl/DC1

4) Thompson, C.M., D.M. Proctor, and M.A. Harris. 2012. Duodenal GSH/GSSG Ratios in Mice Following Oral Exposure to Cr(VI). *Toxicological Sciences.* 126(1): 287-288. Letter to Editor: http://toxsci.oxfordjournals.org/content/126/1/287.full.pdf+html

5) Kopec, A.K., S. Kim, A.L. Forgacs, T.R. Zacharewski, D.M. Proctor, M.A. Harris, L.C. Haws, and C.M. Thompson. 2012. Genome-wide gene expression effects in B6C3F1 mouse intestinal epithelia following 7 and 90 days of exposure to hexavalent chromium in drinking water. *Toxicology and Applied Pharmacology.* 259(1): 13-26. Manuscript: http://www.sciencedirect.com/science/article/pii/S0041008X11004480;

6) Kopec, A.K., C.M. Thompson, S. Kim, A.L. Forgacs, and T.R. Zacharewski. 2012. Comparative toxicogenomic analysis of oral Cr(VI) exposure effects in rat and mouse small intestinal epithelia. *Toxicology and Applied Pharmacology*. 262(2): 124-138. http://www.sciencedirect.com/science/article/pii/S0041008X12001731

7) Thompson, C.M., J.G. Hixon, D.M. Proctor, L.C. Haws, M. Suh, J.D. Urban, and M.A. Harris. 2012. Assessment of Genotoxic Potential of Cr(VI) in the Mouse Duodenum: An In Silico Comparison with Mutagenic and Nonmutagenic Carcinogens Across Tissues. *Regulatory Toxicology and Pharmacology*. 64(1): 68-76. http://www.sciencedirect.com/science/article/pii/S0273230012001134?v=s5

8) Proctor, D., M. Suh, L.L. Aylward, C.R. Kirman, M.A. Harris, C.M. Thompson, H. Gürleyük, R. Gerads, L.C. Haws, and S.M. Hays. 2012. Hexavalent Chromium Reduction Kinetics in Rodent Stomach Contents. *Chemosphere*. 89 (5): 487-493. http://www.sciencedirect.com/science/article/pii/S0045653512005978.

9) Thompson, C.M., Y. Federov, D.D. Brown, M. Suh, D.M. Proctor, L. Kuriakose, L.C. Haws, and M.A. Harris. 2012. Assessment of Cr(VI)-Induced Cytotoxicity and Genotoxicity Using High Content Analysis. *PLoS ONE*. 7(8): e42720. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0042720

10) Kirman, C.R., S.M. Hays, L.L. Aylward, M. Suh, M.A. Harris, C.M. Thompson, L.C. Haws, and D.M. Proctor. 2012. Physiologically Based Pharmacokinetic Model for Rats and Mice Orally Exposed to Chromium. *Chemico-Biological Interactions*. 200(1): 45-64. http://www.sciencedirect.com/science/article/pii/S0009279712001548?v=s5

11) Thompson, C.M., D.M. Proctor, M. Suh, L.C. Haws, C.R. Kirman, and M.A. Harris. 2013. Assessment of the Mode of Action Underlying Development of Rodent Small Intestinal Tumors Following Oral Exposure to Hexavalent Chromium and Relevance to Humans. *Critical Reviews in Toxicology*. 43(3): 244-274. http://informahealthcare.com/doi/abs/10.3109/10408444.2013.768596

12) Kirman, C.R., L.L. Aylward, M. Suh, M.A. Harris, C.M. Thompson, L.C. Haws, D.M. Proctor, W. Parker, and S.M. Hays. 2013. Physiologically Based Pharmacokinetic Model for Humans Orally Exposed to Chromium. *Chemico-Biological Interactions.* 204(1): 13-27. http://www.sciencedirect.com/science/article/pii/S0009279713000823

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*Applied Toxicology.* 34: 525-536. http://onlinelibrary.wiley.com/doi/10.1002/jat.2907/pdf; Supplemental Information: http://onlinelibrary.wiley.com/store/10.1002/jat.2907/asset/supinfo/jat_2907_Supplemental%20Material_Appendices.docx?v=1&s=64762ef5f64f32afac1581e845f27ebcc9eeed1e

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responses to oral Cr(VI) exposure. *Toxicological Sciences.* 158(1): 199-212. https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfx085

24) Kirman, C.R., M. Suh, D.M. Proctor, and S. Hays. 2017. Improved physiologically based pharmacokinetic model for oral exposures to chromium in mice, rats, and humans to address temporal variation and sensitive populations. *Toxicology and Applied Pharmacology.* 325: 9-17. http://www.sciencedirect.com/science/article/pii/S0041008X17301370?via%3Dihub

25) Thompson, C.M., M. Suh, D.M. Proctor, L.C. Haws, and M.A. Harris. 2017. Ten factors for considering the mode of action of Cr(VI)-induced gastrointestinal tumors in rodents. *Mutation Research.* 823: 45-57. https://www.sciencedirect.com/science/article/pii/S1383571817301365

26) Thompson, C.M., J. Wolf, A. McCoy, M. Suh, D.M. Proctor, C. Kirman, L.C. Haws, and M.A. Harris. 2017. Comparison of toxicity and recovery in the duodenum of B6C3F1 mice following treatment with intestinal carcinogens captan, folpet, and hexavalent chromium. *Toxicologic Pathology.* 45(8): 1091-1101. http://journals.sagepub.com/doi/10.1177/0192623317742324

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**Papers on Cr6 Supported by sponsors other than ACC**

28) Proctor, D.M., M. Suh, S.L. Campleman, and C.M. Thompson. 2014. Assessment of the mode of action for hexavalent chromium-induced lung cancer following inhalation exposures. *Toxicology*. 325: 160-179. http://www.sciencedirect.com/science/article/pii/S0300483X14001681

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Message

**From**: Bromberg, Kevin L. [kevin.bromberg@sba.gov]
**Sent**: 8/31/2017 6:20:56 PM
**To**: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Zarba, Christopher [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=74cc5a9e87dc483b9ee0a78a06a06827-Zarba, Christopher]
**CC**: Nancy_Beck@americanchemistry.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=2922d8f69dd84e5386dac0de980c51e5-Nancy_Beck@americanchemistry.com]
**Subject**: FW: House Hearing on IRIS

FYI

Just read about this in BNA.

https://science.house.gov/legislation/hearings/joint-subcommittee-environment-and-subcommittee-oversight-hearing-examining

# Joint Subcommittee on Environment and Subcommittee on Oversight Hearing - Examining the Scientific and Operational Integrity of EPA's IRIS Program

Date:
Wednesday, September 6, 2017 - 10:00am
Location:
2318 Rayburn House Office Building
Subcommittees:

- Subcommittee on Environment (115th Congress)
- Subcommittee on Oversight (115th Congress)

**Examining the Scientific and Operational Integrity of EPA's IRIS Program**

**Witnesses:**

- **Dr. Kenneth Mundt**, principal, Ramboll Environ
- **Dr. James Bus**, senior managing scientist, Exponent
- **Dr. Thomas Burke,** Johns Hopkins University

115th Congress

Kevin Bromberg
Assistant Chief Counsel for Environmental Policy

SBA // Office of Advocacy
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Message

**From**: Bahadori, Tina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=7DA7967DCAFB4C5BBC39C666FEE31EC3-BAHADORI, TINA]
**Sent**: 10/7/2017 4:02:52 PM
**To**: White, Kimberly [Kimberly_White@americanchemistry.com]
**CC**: Thayer, Kris [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3ce4ae3f107749c6815f243260df98c3-Thayer, Kri]; morgand@niehs.nih.gov; Kavlock, Robert [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=eebac67f01094409a7fdaa955a837884-Kavlock, Robert]; Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]; Orme-Zavaleta, Jennifer [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=3c5a111dc377411595e5b24b5d96146b-Orme-Zavaleta, Jennifer]; D'Amico, Louis [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=78a91f83c4414910be286efe02004dbc-D'Amico, Louis J.]
**Subject**: Response to Letter on Behalf of the ACC Formaldehyde Panel
**Attachments**: ACC FA Response_Oct 2017_LHS.pdf

Dear Dr. Wise White,

Please find attached a response to your letter on behalf of the ACC Formaldehyde Panel.

Thank you again for reaching out to EPA/NCEA.

Tina

**Tina Bahadori, Sc.D.**
**Director, National Center for Environmental Assessment (EPA/ORD/NCEA)**
**National Program Director, Human Health Risk Assessment (EPA/ORD/HHRA)**
1200 Pennsylvania Avenue, NW, MC 8601P, Washington, DC 20460
Office: PYS-11622; Phone: 703-347-8600; Email: Bahadori.Tina@epa.gov

**From:** White, Kimberly [mailto:Kimberly_White@americanchemistry.com]
**Sent:** Wednesday, September 13, 2017 4:35 PM
**To:** Bahadori, Tina <Bahadori.Tina@epa.gov>
**Cc:** Thayer, Kris <thayer.kris@epa.gov>; morgand@niehs.nih.gov; Kavlock, Robert <Kavlock.Robert@epa.gov>; Yamanda.richard@Epa.gov
**Subject:** Submission of Letter on Behalf of the ACC Formaldehyde Panel

Dear Dr. Bahadori:

Please find attached a letter on behalf of the American Chemistry Council Formaldehyde Panel regarding a recent study report by NTP on formaldehyde.

Kind Regards,

Kimberly Wise White, Ph.D. | American Chemistry Council
Senior Director, Chemical Products & Technology Division
Kimberly_White@americanchemistry.com
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Center for Environmental Assessment
Washington, DC 20460

OFFICE OF
RESEARCH AND DEVELOPMENT

October 06, 2017

Kimberly Wise White, Ph.D.
Senior Director
American Chemistry Council
Chemical Products and Technology Division
On Behalf of the ACC Formaldehyde Panel
700 Second St., NE
Washington, DC 20002

Dear Dr. Wise White,

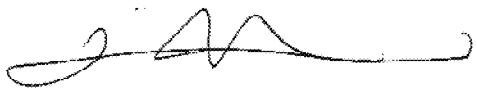
Thank you for your letter of September 13, 2017 reiterating the American Chemistry Council (ACC) Formaldehyde Panel's interest in the EPA's formaldehyde IRIS assessment. I forwarded a copy of your letter and the accompanying National Toxicology Program (NTP) report to the assessment team. The assessment team is aware of this report and will be including consideration of its findings in the public comment draft of the formaldehyde assessment.

I would like to reassure you and the Panel again that we are very aware of the importance of this assessment and are mindful of your concerns. This is why we hope to complete the draft of this assessment as expeditiously as possible and make it available for public comment and peer review by the National Academy of Sciences (NAS). We are also aware that the Panel has been committed to conducting research to address the recommendations of the NAS and engaging scientists on approaches to integrate the scientific evidence for formaldehyde. As you indicated, EPA scientists will participate in the ACC-sponsored October workshop.

In your letter, you also raised a number of questions about the draft assessment which we addressed separately below. But truthfully, the only way to demonstrate our commitment to a scientifically robust and transparent formaldehyde assessment is to present the document for public comment and rigorous peer review by the NAS.

Again, thank you for your letter. Should you have further questions, you may contact me by phone (703-347-8600), or email (bahadori.tina@epa.gov).

Sincerely,



Tina Bahadori, Sc.D.
Director, National Center for Environmental Assessment
National Program Director, Human Health Risk Assessment
U.S. EPA, Office of Research and Development

CC:
Robert Kavlock
Richard Yamada
Kris Thayer
Dan Morgan

**Responses to ACC Questions on the IRIS Toxicological Review of Formaldehyde (October 2017)**

*1. How is EPA considering new scientific information, like the NTP study, for incorporation into the weight of evidence for the formaldehyde IRIS assessment?*

EPA is carefully reviewing and considering new, peer-reviewed science as it becomes available, for inclusion in the revised draft formaldehyde assessment. We are fully incorporating the NTP study into the current draft assessment.

*2. When did EPA last conduct a search of the formaldehyde literature for science to incorporate into the IRIS assessment and how frequently does EPA monitor the formaldehyde literature to identify potential studies that should be incorporated into the assessment?*

The last formal literature search was completed in October, 2016, and the next formal literature search is currently underway. In addition, the assessment managers and team of scientists working on the assessment continually monitor the scientific literature for awareness and consideration of the latest available research. Our partners and stakeholders who have great interest in this assessment have remained vigilant in ensuring that all pertinent studies are brought to our attention and confirming that our formal and informal searches are complete. The NTP study is just one example of that very situation, where a document released after the last formal literature search has already been incorporated into the draft assessment, as appropriate.

*3. What guidance documents or procedures will EPA utilize to evaluate study quality for studies relied upon to reach conclusions in the formaldehyde IRIS assessment? Please provide specific references if available.*

EPA will be using Agency risk assessment guidelines as a framework for evaluating study quality and to reach conclusions in the draft formaldehyde assessment. Public guidance documents can easily be accessed at https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance. In addition, as you know, EPA has been incorporating principles of systematic review into the assessment development process, in response to the recommendations from the 2011 and 2014 NAS reports. The draft assessment which we hope to share with the public soon will transparently explain the procedures utilized in development of the assessment.

*4. When will EPA release a weight of evidence framework illustrating how various data streams (i.e. mechanistic, toxicology and epidemiology studies) are evaluated for quality and then integrated to reach conclusions about formaldehyde?*

EPA is using existing Agency guidance to weigh, synthesize, and integrate evidence to evaluate formaldehyde toxicity. The criteria used for identifying studies, evaluating quality, and integrating evidence streams, will be clearly and transparently described in the formaldehyde assessment, as was recommended by the NAS.

*5. How has EPA addressed all the 2011 NAS recommendations for formaldehyde?*

EPA has addressed all the 2011 NAS recommendations for formaldehyde in the revised draft assessment. A section in the appendix will clearly describe how the Agency addressed the recommendations.

*6. How will EPA seek public input and peer review on the formaldehyde IRIS assessment and what types of public meetings or workshops will be held to receive input?*

The revised draft formaldehyde assessment EPA will follow the established IRIS process. Following agency and interagency review, the draft assessment will be released for public comment, and an accompanying public science meeting. Following the public comment draft, EPA will make any necessary revisions, and a peer review draft will be released for independent peer review by the NAS. The NAS peer review will also include an opportunity for public comment.

Message

**From**: DuVall, Amy [Amy_Duvall@americanchemistry.com]
**Sent**: 7/17/2017 6:14:37 PM
**To**: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
**Subject**: Do you have 30 seconds to call me?

Just left you a VM re: same – won't take more than a minute, promise. Hope you are doing well!

**Amy L. DuVall** | American Chemistry Council
Senior Director, Federal Affairs
amy_duvall@americanchemistry.com
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Message

**From:** White, Kimberly [Kimberly_White@americanchemistry.com]
**Sent:** 9/13/2017 8:56:17 PM
**To:** Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
**Subject:** FW: Submission of Letter on Behalf of the ACC Formaldehyde Panel
**Attachments:** Letter to EPA on NTP 2017 Formaldehyde Study Report - Final.pdf; Attachment 1- NTP Research Report on Absence of Formaldehyde Induced Neoplasia - August 2017.pdf

Dear Mr. Yamada,

Please see attached letter submission on behalf of the ACC Formaldehyde Panel. I initially received an email failure notice when using the email address indicated on the EPA's Employee Directory (e.g. Yamanda.richard@Epa.gov).

Kind Regards,

Kimberly Wise White, Ph.D. | American Chemistry Council
Senior Director, Chemical Products & Technology Division
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**Cc:** 'Thayer, Kris'; morgand@niehs.nih.gov; 'Kavlock.robert@Epa.gov'; 'Yamanda.richard@Epa.gov'
**Subject:** Submission of Letter on Behalf of the ACC Formaldehyde Panel

Dear Dr. Bahadori:

Please find attached a letter on behalf of the American Chemistry Council Formaldehyde Panel regarding a recent study report by NTP on formaldehyde.

Kind Regards,

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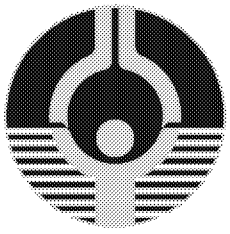

NTP
National Toxicology Program
U.S. Department of Health and Human Services

# NTP Research Report on

# Absence of Formaldehyde-Induced Neoplasia in Trp53 Haploinsufficient Mice Exposed by Inhalation

NTP RR 3

AUGUST 2017

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## Peer Review

The draft research report on the study of formaldehyde exposure to T53 haploinsufficient mice was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies was appropriate and ensured that this NTP Research Report presented the experimental results and conclusions fully and clearly.

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# Abstract

Formaldehyde inhalation is linked to nasal cancer and leukemia in humans. Formaldehyde-induced DNA-protein crosslinks and enhanced cell proliferation are important in the pathogenesis of nasal cancer and, potentially, leukemia. Mutations in the tumor suppressor gene Trp53 have been associated with formaldehyde-induced nasal tumors and might be a key mechanistic event in formaldehyde-induced leukemia. The objective of this study was to evaluate the potential role of the Trp53 gene in formaldehyde-induced nasal carcinogenicity, leukemia or lymphohematopoietic cancer, and potentially other neoplasms in genetically susceptible mice. Male, Trp53 haploinsufficient ($Trp53^{\pm}$) mouse strains (B6.129-*Trp53*$^{tm1Brd}$ and C3B6.129F1-*Trp53*$^{tm1Brd}$) were exposed to 0-, 7.5- or 15-ppm formaldehyde (25/group) 6 h/d, 5 d/wk for 8 wk, and then held for 32 wk. Blood was collected for hematology, and major tissues and gross lesions were collected for histopathology. The primary formaldehyde-related finding was squamous metaplasia of the respiratory epithelium of the nose. Inhalation of a maximum tolerated dose of formaldehyde caused significant injury to the nasal mucosa and cell proliferation, but did not cause nasal tumors or an increased prevalence of leukemia or lymphohematopoietic cancer in $Trp53^{\pm}$ mice. All observed neoplasms were considered background lesions for these mouse strains. The results of this short-term carcinogenicity study do not support a role for Trp53 in formaldehyde-induced neoplasia.

Key Words: Formaldehyde, Trp53 mutations, $Trp53^{\pm}$ mice, nasal cavity, neoplasia, inhalation

## Introduction

Formaldehyde is recognized as a nasal carcinogen in humans [1] and laboratory rats [2-4]. Exposure of rats to formaldehyde results in development of squamous cell carcinoma (SCC) in the anterior and posterior lateral meatus of the nasal cavity [2]. DNA-protein crosslinks (DPC) and cell proliferation are prominent at sites of SCC formation in the rat nasal cavity [4-6], suggesting that incomplete repair of DPC results in mutations within the proliferating cell population and might lead to neoplasia. Considerable evidence shows that formaldehyde-induced mutations in the tumor suppressor gene *Trp53* are important in the pathogenesis of nasal cancer [7; 8]. Mutations in *Trp53* were identified in formaldehyde-induced nasal SCC in rats [9], and abnormal Trp53 protein was shown to accumulate in the nasal tissue of formaldehyde-exposed rats [10]. Mutations in *Trp53* result in loss of tumor suppression functions including RNA repair, cell cycle arrest, senescence, and apoptosis [11], resulting in a loss of normal growth control and clonal expansion of mutated cells [12-14].

Formaldehyde also has been reported to cause myeloid leukemia in humans[15], although the mechanism is unknown. Mutations in the Trp53 gene could be important in the pathogenesis of leukemia or lymphohematopoietic cancer. Zhang et al. [16] proposed that hematopoietic stem cells in the nasal epithelium or in circulation undergo formaldehyde-induced mutations that result in loss of *Trp53* and acquisition of the capacity for self-renewal. The *Trp53* gene is involved in regulating the self-renewal of hematopoietic stem cells [17-20]. In addition, disruption of the *Trp53* pathway has been shown to enhance production of induced pluripotent stem cells capable of self-renewal [21]. Acquisition of self-renewal capacity is one of the initial steps in cancer development [22]. Formaldehyde-induced loss of *Trp53* would be rare, however, and indeed an increased incidence of lymphohematopoietic cancer has not been observed in formaldehyde-exposed wild type rats and mice [3].

Currently no good animal model is available for investigating the mechanism(s) by which formaldehyde causes leukemia. Because considerable evidence suggests a role of *Trp53* in formaldehyde-induced nasal SCC, we evaluated two *Trp53*-haploinsufficient ($Trp53^{\pm}$) mouse strains in this study. We hypothesized that formaldehyde-induced loss of Trp53 would be increased in $Trp53^{\pm}$ mice, resulting in an increased incidence of SCC of the nose and leukemia or lymphohematopoietic cancer, and potentially neoplasms at other sites.

## Materials and Methods

### *Animals*

Two mouse strains heterozygous for the null and wildtype *Trp53* allele were used to evaluate the potential role of Trp53 in formaldehyde-induced lymphohematopoietic cancer. The inbred B6.129-*Trp53*$^{tm1Brd}$ (Model P53N12-M) mouse strain was selected for this study because of its previous use as a model for lymphohematopoietic tumors in short-term cancer bioassays [23-29]. The C3B6.129F1-*Trp53*$^{tm1Brd}$ strain also was selected based on the observation of chemical exposure-dependent lymphohematopoietic tumors of either lymphoid- or myeloid- committed stem cell origin [30; 31]. This strain is an outcross between C3H/HeNTac female (Model C3H-F) x B6.129-*Trp53*$^{tm1Brd}$ (Model P53N12-M) homozygous Trp53 null allele male to produce the haploinsufficient F1 progeny. Heterozygotes were selected because they have a lower background incidence of sporadic tumors than the homozygotes, and the latency for sporadically

occurring tumors is longer than in the homozygote [32; 33]. Although rats appear to be more susceptible than mice to formaldehyde-induced SCC of the nose, a genetically modified rat strain was not available at the time of this study.

Male C3B6.129F1-*Trp53*$^{tm1Brd}$ mice were 9 weeks old on arrival and male B6.129-*Trp53*$^{tm1Brd}$ haploinsufficient mice (Taconic, Hudson, NY) were 8–10 weeks old on arrival. After a 3- (B6.129-*Trp53*$^{tm1Brd}$) to 4-week (C3B6.129F1-*Trp53*$^{tm1Brd}$) quarantine period, mice were weighed and randomly assigned to three exposure groups (Table 1). Five mice per strain were assigned to sentinel groups. All mice were acclimated to the Hazleton 2000 exposure chambers for 3 days prior to exposure. Food (NIH-31) was removed during the 6-hour exposure periods; and water was always available. Body weights were recorded weekly.

### *Formaldehyde Generation and Monitoring*

Nominal chamber concentrations of 7.5-ppm and 15-ppm formaldehyde vapor were generated by heating paraformaldehyde. These concentrations were selected based on reports in the literature and on the results of a 2-week range-finding study in mice. Because cell proliferation plays a key role in formaldehyde-induced neoplasia, exposure concentrations were selected that caused significant injury and cell regeneration in the nasal cavity in the range-finding study. Two 1-liter Woulff bottles containing 200 grams of prilled paraformaldehyde (Sigma Aldrich, St. Louis, MO) were heated on hot plates to 95°C (7.5 ppm) and 120°C (15 ppm). A constant volume (1 liter per minute [LPM]) of breathing-quality air (dried, filtered, and carbon-scrubbed) was delivered to the Woulff bottles. A metered volume of the formaldehyde-enriched headspace was mixed with the chamber supply air stream (500-LPM humidified, filtered, and carbon-scrubbed air). The Hazleton 2000 exposure chamber concentrations were monitored using a Model Z-300XP Formaldehyde Meter (Environmental Sensors, Boca Raton, FL).

### *Animal Exposure*

Mice were individually housed in Hazleton 2000 chambers and exposed to either conditioned air (charcoal and HEPA filtered, temperature and humidity controlled), or to 7.5- or 15-ppm formaldehyde in conditioned air 6 hours/day, 5 days/week, for 8 weeks. This treatment regimen represents a standard 5-day workweek, used for studying chemicals for which exposure is primarily occupational. Exposures of the two strains were staggered by 1 week because of the large numbers of mice to be necropsied. The exposure of the B6.129-*Trp53*$^{tm1Brd}$ mice began 1 week after beginning the exposure of C3B6.129F1-*Trp53*$^{tm1Brd}$ mice. Following the 8-week inhalation exposures, mice were transferred to individual polycarbonate cages and monitored for ~32 weeks (until 51–53 weeks of age). Mice were weighed and examined for gross lesions weekly. Mice with large or ulcerated tumors were euthanized and a necropsy conducted.

### *Hematology*

On the day of the scheduled necropsy, whole blood samples were collected under isoflurane anesthesia by means of the retro-orbital venous sinus into tubes containing $K_2$EDTA as anticoagulant. The whole blood was analyzed for a complete blood count (CBC) evaluation. CBC end points, including absolute leukocyte (total and differential), erythrocyte (red blood cell [RBC]), reticulocyte (% and absolute [Retics]) and platelet (Plat) counts, hemoglobin (HgB) concentrations and hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) values, were

obtained using the ProCyte Dx hematology analyzer (IDEXX Laboratories, Westbrook, ME). A packed cell volume (spun hematocrit) was performed using an Autocrit Ultra 3 micro-hematocrit centrifuge (Becton, Dickinson and Company, Franklin Lakes, NJ). Microscopic evaluation of Romanowsky-stained blood smears was performed to assess leukocyte differential distribution percentages and RBC, WBC, and Plt morphology.

### *Necropsy*

Mice found moribund during the study and those that survived to the end of the study were euthanized by $CO_2$ asphyxiation and exsanguination. At necropsy, major organs were weighed and the following tissues, including gross lesions, were collected for pathology evaluations. The distal 1–2 cm of the left femur was sectioned through the frontal plane to include the articular cartilage and articular surface, the femoral condyles with epiphyseal plate, and diaphysis with bone marrow. The left femur was fixed in 10% neutral-buffered formalin (NBF). The nasal cavities were fixed by retrograde infusion of NBF, and then immersed in NBF. The formalin-fixed nasal cavities were decalcified (RDO Rapid Decalcifier; Apex Engineering Products, Aurora, IL) for 12 hours. After decalcification, three separate sections of the nasal cavity were taken at (1) the level of the incisor teeth (Level 1), (2) midway between incisors and first molar (Level 2), and (3) middle of second molar (olfactory region) (Level 3). The remainder of the nasal cavity and turbinates were carefully examined for gross lesions. One-half of the trachea was left attached to the lung. After weighing the lung, the trachea was used to inflate the lungs with fixative. The lungs were trimmed to allow the largest cross-section surface area possible. A transverse section of the larynx was taken at the base of the epiglottis just anterior to the laryngeal saccule. Two sections of liver including transverse sections through the left and median lobes were taken midway along the greatest dimension. A section of gallbladder was included in the section of median lobe. A mid-longitudinal section (left kidney) and a cross section (right kidney) through the entire cortex, pelvis, and medulla of each kidney were collected. Mesenteric, mandibular, mediastinal, and bronchial lymph nodes were collected and placed in cassettes. After formalin fixation, tissues were trimmed and processed, embedded in paraffin wax, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). A pathologist evaluated the slides, and then the tumor slides were reviewed by four additional pathologists during a Pathology Peer Review.

### *Immunohistochemistry Staining*

Immunohistochemistry was used to better characterize sarcomas. Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through graded alcohols. Heat-induced antigen retrieval in citrate buffer (pH 6.0, Biocare Medical, Concord, CA) was done in a Decloaker® pressure chamber for 5 minutes at 110°C, followed by 3% hydrogen peroxide for 15 minutes to quench endogenous peroxidase activity. Nonspecific sites were blocked by incubating slides for 20 minutes with 2.5% normal horse serum (Vector, Burlingame, CA). The sections then were then incubated with rabbit monoclonal antimyogenin antibody (Cat# ab124800, Lot# GR155521-1, Abcam, Cambridge, MA) at 1:500 dilution, or a rabbit monoclonal anti-vimentin antibody (Cat# ab92547, lot# GR145336-12, Abcam) at 1:1000 dilution for 1 hour at room temperature. For negative control tissue sections, normal rabbit IgG (Calbiochem®, San Diego, CA), diluted to match the protein concentration of the myogenin or vimentin antibodies was utilized. The antigen-antibody complex was detected using ImmPRESS HRP anti-rabbit IgG (Vector) and 3,3-diaminobenzidine (Dako, Carpinteria, CA). Slides were

then counterstained with hematoxylin, dehydrated, and cover-slipped. The myogenin slides were stained manually, whereas, the vimentin slides were stained via a Biocare Intellipath™ FLX autostainer (Biocare Medical).

### *Statistical Analyses*

Cochran-Armitage trend tests were used to test for dose-related trends in incidences of neoplasms. Fisher's exact tests (one-sided) were used to compare incidences of neoplasms between each dose group and the control group. Hematology data were analyzed using nonparametric multiple comparison methods [34; 35].

## Results

### *Survival*

Nine C3B6F1.129-*Trp53*$^{tm1Brd}$ animals died prior to the scheduled sacrifice (Table 1). Sixty-five animals of this strain survived to study termination. Sixteen B6.129-*Trp53*$^{tm1Brd}$ animals died prior to the time of scheduled sacrifice (Table 1). Most of these animals were euthanized early due to the presence of large, grossly visible tumors. Seventy-five B6.129-*Trp53*$^{tm1Brd}$ animals survived to study termination.

### *Body and Organ Weights*

Body weights of C3B6F1.129-*Trp53*$^{tm1Brd}$ mice exposed to 7.5-ppm formaldehyde were significantly less than controls ($p<0.05$) from week 1 to week 11, and at weeks 37 and 38. Body weights of mice exposed to 15-ppm formaldehyde were significantly less than controls ($p<0.05$) from week 1 to week 24, and at weeks 37 and 38 (Figure 1). Absolute and relative liver weights were significantly decreased 11% and 8%, respectively, in mice exposed to 15-ppm formaldehyde. Lung, kidney, spleen, and thymus weights of formaldehyde-exposed mice were not significantly different from controls (data not shown).

Body weights of B6.129-*Trp53*$^{tm1Brd}$ mice exposed to formaldehyde were not significantly different from controls at any time point (Figure 1). Absolute and relative liver, lung, kidney, spleen, and thymus weights of formaldehyde-exposed B6.129-*Trp53*$^{tm1Brd}$ mice were not significantly different from controls (data not shown).

### *Hematology*

Hematological parameters for formaldehyde-exposed C3B6F1.129-*Trp53*$^{tm1Brd}$ (Table 2) and B6.129-*Trp53*$^{tm1Brd}$ (Table 3) mice were not significantly different from their respective controls. Neither strain showed any indication of treatment-related hematotoxicity, leukemia, or lymphoma.

### *Histopathology*

#### *Nasal Cavity*

Formaldehyde exposure-related non-neoplastic lesions were observed primarily in the nasal cavity of C3B6F1.129-*Trp53*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ mice. These nasal lesions were quantitatively and qualitatively similar in both strains. Nasal cavity lesions considered related to

formaldehyde exposure are summarized in Table 4. The number of animals examined per group includes the early death animals.

The only nasal cavity lesion observed in the control C3B6F1.129-*Trp53*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ animals was minimal hyaline degeneration of the respiratory epithelium, consisting of one or more small foci of epithelial cells containing intra-cytoplasmic accumulation of brightly stained eosinophilic material. This minimal lesion, observed in many of the control animals, is considered a spontaneous background finding.

Squamous metaplasia of the respiratory epithelium was observed in the nasal cavity of many C3B6F1.129-*Trp53*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ mice exposed to 7.5- and 15-ppm formaldehyde. The incidence and average severity (minimal to mild) of squamous metaplasia were greater in the 15-ppm group animals as compared with the 7.5-ppm animals (Table 4). Squamous metaplasia was observed consistently in Level 1, presumably because this level of the nose was exposed to the highest concentration of formaldehyde, and occasionally in Level 2, especially in the 15-ppm exposure groups (Figure 2). This lesion was not observed in Level 3. Squamous metaplasia was observed most commonly on the medial surface of the maxilloturbinates, facing the nasal septum. Other affected sites included the lateral wall, tips of the nasoturbinates, and, occasionally, the dorsal surface of the dorsal meatus. Squamous metaplasia was characterized microscopically by areas of replacement of the normal respiratory epithelium by a thin to occasionally moderately thick layer of stratified squamous epithelium. In some cases, the squamous epithelium was quite thin and was recognized as squamous epithelium by the presence of a layer of thin, laterally flattened cells on the epithelial surface typical of squamous epithelium. Often, a thin to moderately thick layer of keratin, sometimes containing cell debris, was present on the epithelial surface of turbinates in exposed (Figure 3B) but not control (Figure 3A) mice. In a few noses, accumulated keratin was present in the space behind a turbinate scroll, and in some cases filled the space behind the scroll (Figure 3C).

Osteogenesis, proliferation of new bone, was observed in the turbinate bone in a few C3B6.129F1-*Trp53*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ animals exposed to formaldehyde (Table 4). Microscopically, the osteogenesis had a similar appearance in the two strains and in each animal consisted of a small focus of proliferating bone within the lamina propria of the affected turbinate characterized by eosinophilic osteoid containing numerous large plump osteoblast nuclei (Figure 3D).

### *Neoplasms in C3B6.129F1-Trp53$^{tm1Brd}$ Mice*

Several neoplasms were observed in control and formaldehyde-exposed C3B6.129F1-*Trp53*$^{tm1Brd}$ mice, and none was considered caused by formaldehyde exposure (Table 5). Three lymphomas were found in the early death C3B6.129F1-*Trp53*$^{tm1Brd}$ animals: one in the 7.5-ppm group involving the thymus and two lymph nodes, and the remaining two in the 15-ppm group involving only the thymus. No lymphomas were observed in the control group. The incidences of lymphoma in the formaldehyde-treated groups were not statistically significant ($p>0.05$) relative to controls. The lymphomas all had a typical and similar morphologic appearance, and consisted of diffuse sheets of relatively large lymphocytes with large, moderately pleomorphic basophilic, granular nuclei with one or more prominent nucleoli and a scant amount of eosinophilic cytoplasm (Supplemental Figure 1).

Five osteosarcomas were found in bone and skeletal muscle of C3B6.129F1-*Trp53*$^{tm1Brd}$ mice (Table 5). Osteosarcomas were present in two scheduled sacrifice animals (one control and one 7.5-ppm animal), and both had metastasized to the lung. The three remaining osteosarcomas occurred in the early death control animals. Microscopically, the osteosarcomas were discrete masses composed of irregular intersecting trabeculae of eosinophilic osteoid, the matrix material for bone, admixed with variable numbers of highly pleomorphic neoplastic spindle-shaped cells (Figure 4A). Although osteosarcomas are considered to originate from bone, three osteosarcomas (from the early death control animals) had skeletal muscle attached to them but no bone. We assumed they had originated in bone and had invaded adjacent skeletal muscle, but that the bony origins of these neoplasms were not included in the microscopic sections. Osteosarcomas stained positively for vimentin, an intermediate filament typically present in cytoplasm of non-epithelial cells (Figure 4B), and stained negatively for smooth muscle actin, myogenin, and F4/80.

In addition to osteosarcomas, an undifferentiated sarcoma was also observed in the skeletal muscle of a 7.5-ppm C3B6.129F1-*Trp53*$^{tm1Brd}$ animal. This neoplasm was a large, discrete mass consisting of numerous large, round to ovoid to elongated cells with abundant eosinophilic cytoplasm and small basophilic nuclei. Hepatocellular adenoma and carcinoma, histiocytic sarcoma, and hemangiosarcoma also were observed in some animals, but the incidences were not significantly increased or concentration related (Table 5).

Osteosarcomas in two control mice and a hemangiosarcoma in one 7.5-ppm animal were in contact with or had enveloped the microchip transponders used for animal identification. Sarcomas, osteosarcomas, and histiocytic sarcoma in eight C3B6.129F1-*Trp53*$^{tm1Brd}$ animals were located remotely from the microchips.

### *Neoplasms in B6.129-Trp53$^{tm1Brd}$ Mice*

A variety of observed neoplasms in formaldehyde-exposed B6.129-*Trp53*$^{tm1Brd}$ mice were not considered chemical related (Table 6). Lymphomas were present in two formaldehyde-exposed B6.129-*Trp53*$^{tm1Brd}$ animals, one in the 7.5-ppm group and one in the 15-ppm group. The lymphoma in the 7.5-ppm animal, which appeared to have originated in the thymus, had spread widely, being present in nearly every tissue examined. In contrast, the lymphoma in the 15-ppm animal appeared to have originated in the mesenteric lymph node with a small amount of involvement of the bronchial lymph node. The lymphomas appeared morphologically similar to one another and to the lymphomas observed in the C3B6.129F1-*Trp53*$^{tm1Brd}$ animals (Supplemental Figure 1).

Skeletal muscle rhabdomyosarcomas were observed in five formaldehyde-exposed B6.129-*Trp53*$^{tm1Brd}$ animals, one in the 7.5-ppm group and four in the 15-ppm group (includes early death animals) (Table 6). Rhabdomyosarcomas were not observed in B6.129-*Trp53*$^{tm1Brd}$ control animals. Although a statistically significant trend ($p=0.042$) was found, the incidence of rhabdomyosarcomas in the 15-ppm group was not statistically significant ($p=0.133$) relative to controls. Microscopically, the neoplasms were large masses composed of solid foci and interlacing bands of neoplastic cells generally admixed with varying amounts of dense fibrous tissue stroma (Figure 4C). The neoplasms were composed primarily of variably sized pleomorphic polygonal to fusiform cells, with large ovoid to fusiform, moderately basophilic to vesicular nuclei with one to several prominent nucleoli and small to moderate and occasionally large amounts of eosinophilic cytoplasm with indistinct borders. Few to numerous mitotic

figures were present, and varying numbers of large multinucleated cells resembling rhabdomyoblasts were observed. Cross striations were not observed; however, the tumors stained positively for vimentin, a marker for cells of mesenchymal origin including skeletal muscle, and myogenin, a nuclear transcription factor essential for skeletal muscle development and repair, and a specific marker for skeletal muscle (Figure 4D).

Various other neoplasms occurred at low incidences in control and formaldehyde-exposed B6.129-*Trp53*$^{tm1Brd}$ animals, all of which were considered background lesions unrelated to formaldehyde exposure (Table 6). Microchip transponders were found in contact with, or enveloped by, tumors in five B6.129-*Trp53*$^{tm1Brd}$ animals exposed to formaldehyde. Sarcomas in 10 B6.129-*Trp53*$^{tm1Brd}$ animals were not associated with microchip transponders.

## Discussion

Formaldehyde-induced injury to the nasal epithelium, regenerative proliferation, and mutations in these proliferating cells are key steps in the pathogenesis of formaldehyde-induced nasal SCC [4; 6]. Mutations in the tumor suppressor gene Trp53 have been detected in formaldehyde-induced nasal tumors and in preneoplastic hyperkeratotic plaques, indicating that Trp53 mutations are involved in the pathogenesis of SCC [9; 10]. Based on these data, Trp53$^{\pm}$ mice were expected to be highly susceptible to formaldehyde-induced nasal SCC, and possibly leukemia and other neoplasms. Under the conditions of this short-term study, however, formaldehyde exposure did not result in SCC of the nasal cavity or in an increased incidence of leukemia or lymphohematopoietic neoplasms in two genetically susceptible Trp53$^{\pm}$ mouse strains. Formaldehyde-induced loss heterozygosity (LOH) in Trp53 and loss of tumor suppressor function may have been insufficient to induce neoplasia. Although mutations to Trp53 can result in a dysfunctional protein, some mutations to Trp53 can result in a gain of function (GOF) that promotes tumor development [36; 37]. Formaldehyde-induced mutations of Trp53 that result in GOF may be necessary for induction of neoplasia.

Although rats are more susceptible than mice to formaldehyde-induced nasal tumors [3], a Trp53$^{\pm}$ rat strain was not available at the time of this study. Mice are reportedly less susceptible than rats because of their greater ability to reduce their minute ventilation upon repeated formaldehyde exposures [38; 39], thereby reducing tissue damage and cell turnover in the nasal mucosa. Spontaneous SCC of the nasal cavity is extremely rare in rats and mice. Tumors of the nasal cavity have not been observed in NTP historical controls for B6C3F1/N or B6.129-*Trp53*$^{tm1Brd}$ mice. Background nasal tumor incidences for the C3B6.129F1-*Trp53*$^{tm1Brd}$ were not available. Induction of SCC in wild type mice by formaldehyde is also extremely rare. Exposure of C57BL/6 x C3HF1 mice to 14.3-ppm formaldehyde for 2 years followed by a 6-month holding period resulted in only two mice with nasal tumors [3].

In this study, mice were exposed to concentrations of formaldehyde that caused significant cell injury, inflammation, and regeneration in the nasal mucosa, which are recognized as key events in nasal SCC development [5]. Although significant regenerative cell proliferation and squamous metaplasia were observed in exposed mice, a higher formaldehyde concentration, a longer exposure duration, or a longer holding period might be necessary for accumulation of genetic alterations in Trp53 and development of SCC in mice. The severity of nasal lesions in both mouse strains, the significantly reduced body weights of C3B6.129F1-*Trp53*$^{tm1Brd}$ mice, and the lack of body weight gain in both strains, however, indicated that exposure to 15 ppm for 8 weeks

represented a maximum tolerated dose. An 8-week exposure duration was considered sufficient because the doubling time for HSPCs is reported to be between 2 and 4 weeks with the entire HSPC pool turning over every 8 weeks [40; 41]. Although exposed mice could have been held longer, 50 weeks of age was selected because, at approximately 70 weeks of age, about one-half of Trp53$^{\pm}$ mice reportedly develop background tumors [33].

Formaldehyde exposure did not significantly increase the incidence of leukemia or lymphohematopoietic neoplasms in either B6.129-*Trp53*$^{tm1Brd}$ or C3B6.129F1-*Trp53*$^{tm1Brd}$ mice. The formaldehyde exposure concentrations were observed to cause disruption of the nasal epithelium, potentially allowing exposure of the underlying nasal cavity stem cells and circulating hematopoietic stem cells in the nasal vasculature. Maximal exposure of stem cells would be expected to occur following disruption (ulceration, necrosis) of the epithelium. As noted in this study, however, the accessibility of the nasal stem cells to inhaled formaldehyde could decrease as the damaged nasal epithelium is rapidly replaced by the more resistant squamous epithelium. In addition, the squamous epithelium produced multiple layers of keratin that might also protect underlying cells from inhaled formaldehyde. Whether a similar phenomenon occurs in exposed rats is unclear. Lymphoma was observed in several formaldehyde-exposed mice and was absent in control groups for both mouse strains. The low incidence of lymphoma in formaldehyde-exposed mice, however, was not statistically significant. Lymphoma was not considered exposure related because of the low incidence, the lack of statistical significance, and because lymphoma is one of the most common spontaneous tumors reported in Trp53$^{\pm}$ mice [32; 42].

Sarcomas were the most prevalent tumor observed in B6.129-*Trp53*$^{tm1Brd}$ mice and were the most common cause of early deaths due to the rapid growth of these tumors. The use of subcutaneous microchip transponders for animal identification might have influenced the incidence of sarcomas in B6.129-*Trp53*$^{tm1Brd}$. Sarcomas can occur in B6.129-*Trp53*$^{tm1Brd}$ mice in association with subcutaneous microchips but can also occur spontaneously and in reaction to other treatments [43]. Although the mechanism for a synergistic or additive effect of subcutaneous microchip implantation and formaldehyde exposure on skeletal muscle sarcoma development in B6.129-*Trp53*$^{tm1Brd}$ is not clear, evidence is insufficient to rule out such an effect.

Osteosarcoma was a common spontaneous lesion in C3B6.129F1-*Trp53*$^{tm1Brd}$ mice, occurring in about 15% of controls and in only one animal exposed to formaldehyde (7.5 ppm). Osteosarcomas are generally uncommon neoplasms in mice but appear to be more common in Trp53$^{\pm}$ mice. Microchips were found within or in contact with tumors in two of the four control mice with osteosarcoma, and in one mouse in the 7.5-ppm group, the microchip was in contact with a hemangiosarcoma. As noted with the skeletal muscle sarcomas in B6.129-*Trp53*$^{tm1Brd}$ mice, immunostaining of osteosarcomas associated with, or remote from, microchips showed no differences.

## Conclusion

Under the conditions of this study, inhalation exposure to a maximum tolerated dose of formaldehyde did not cause an increased incidence of nasal SCC, leukemia, or lymphohematopoietic tumors in Trp53$^{\pm}$ mice. Both mouse strains developed a variety of neoplasms, but all were considered spontaneous background lesions and not a result of

formaldehyde exposure. The results of this short-term carcinogenicity study do not support a role for Trp53 in formaldehyde-induced neoplasia.

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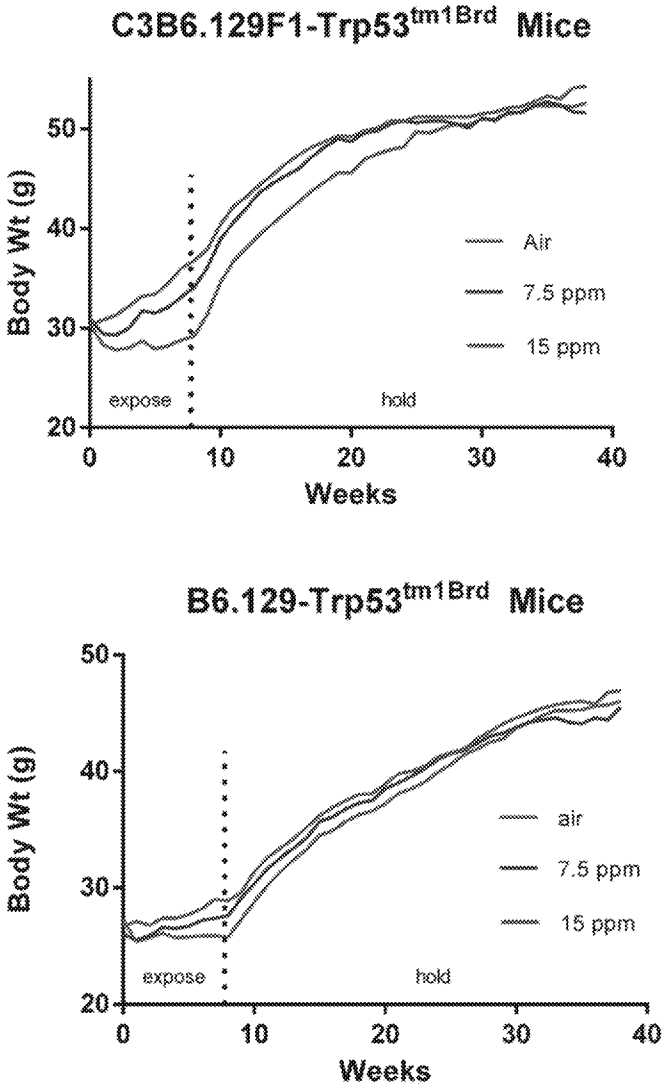



**Figure 1. Body Weights of Trp53$^{\pm}$ Mice Exposed to Formaldehyde**

C3B6.129F1-*Trp53*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ mice were exposed to formaldehyde at 0 (air), 7.5, or 15 ppm for 8 weeks, and then held for 32 weeks without exposure. (A) Body weights of C3B6F1.129-*Trp53*$^{tm1Brd}$ mice exposed to 7.5-ppm formaldehyde were significantly less than controls ($p<0.05$) from week 1 to week 11 and at weeks 37 and 38. Body weights of mice exposed to 15-ppm formaldehyde were significantly less than controls ($p<0.05$) from week 1 to week 24 and at weeks 37 and 38. (B) Body weights of B6.129-*Trp53*$^{tm1Brd}$ mice exposed to formaldehyde were not significantly different from controls at any time point.

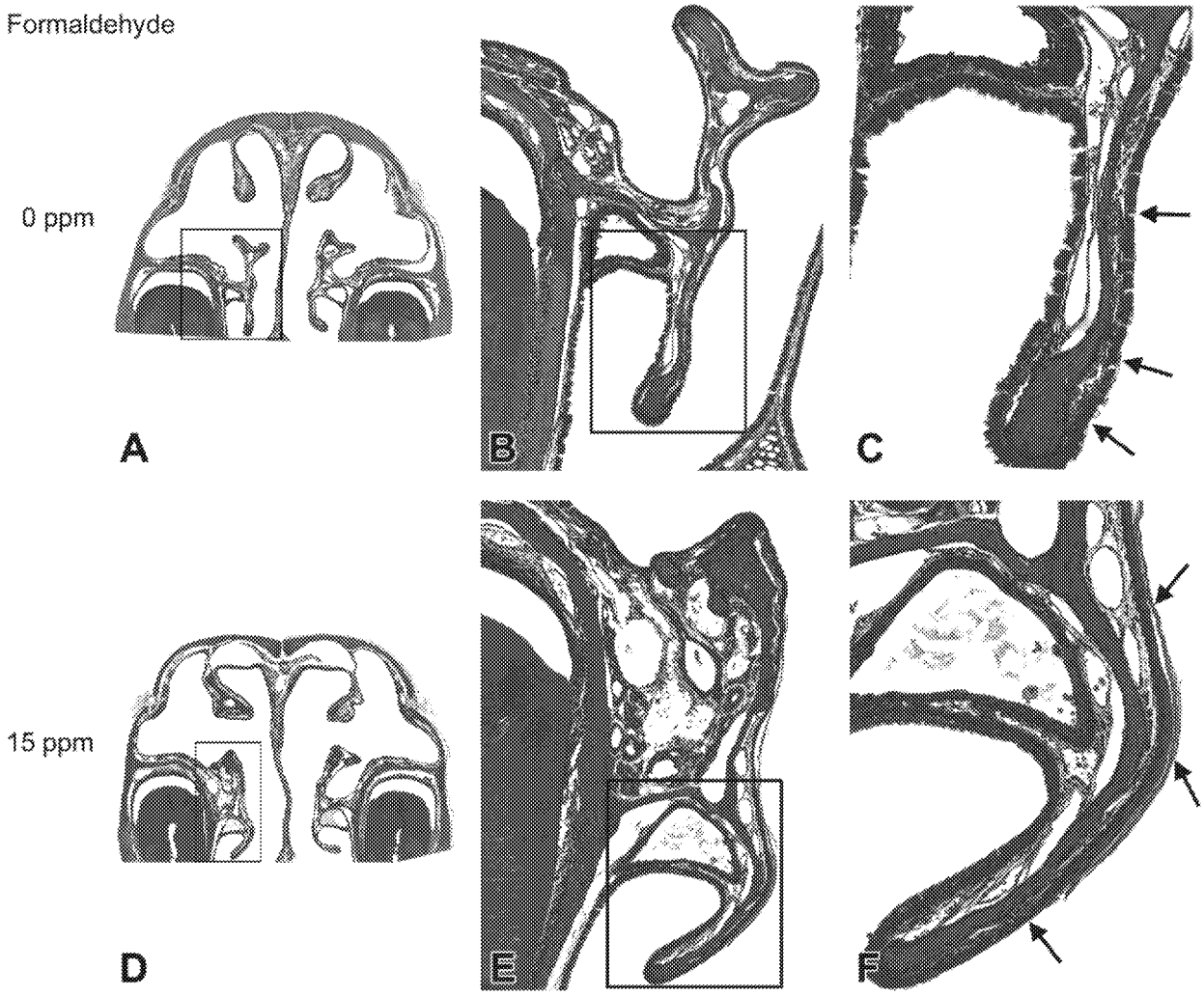



**Figure 2. Squamous Metaplasia of the Nasal Cavity in Mice Exposed to Formaldehyde**

(A) A low magnification showing the location of the maxilloturbinates (within the box) and the nasoturbinates located dorsally and attached to the roof of the nasal cavity on either side of the nasal septum, Level 1. (B and C) Higher magnifications of the maxilloturbinate (within the box) showing the normal respiratory epithelium covering the turbinate bones. The normal respiratory epithelium in this area consists of a single layer of ciliated, columnar, and non-ciliated cuboidal cells (arrows in C). (D, E, and F) Level I section of the nasal cavity from a high-dose (15-ppm) animal. As in A, panel D is a low magnification showing the location of the maxilloturbinates (within the box) and the nasoturbinates dorsally. E and F are higher magnifications of the affected turbinate epithelium (within the box). The normal respiratory epithelium has been replaced by keratinized, stratified squamous epithelium (arrows in panel F).

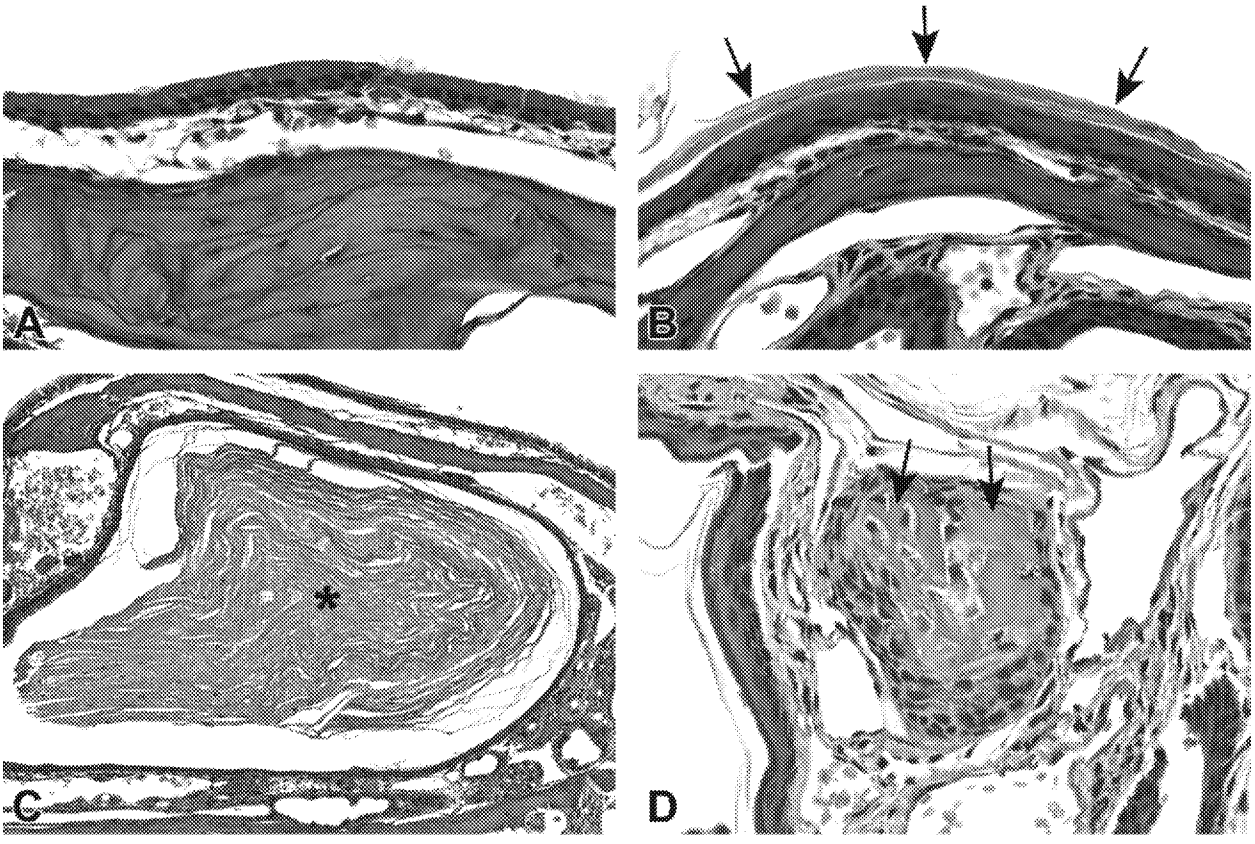



**Figure 3. Keratin Accumulation and Osteogenesis in Nasal Cavity of Mice Exposed to Formaldehyde**

C3B6.129F1-*Trp5*$^{tm1Brd}$ and B6.129-*Trp53*$^{tm1Brd}$ mice were exposed to formaldehyde at 0 (air), 7.5, or 15 ppm for 8 weeks, and then held for 32 weeks without exposure. Significant lesions were still present in the nasal cavity of both mouse strains when examined 32 weeks after formaldehyde exposure. (A) Normal appearing respiratory epithelium on the nasal turbinate of an air-exposed control C3B6.129F1-*Trp53*$^{tm1Brd}$ mouse. H&E, 60x. (B) Squamous metaplasia of respiratory epithelium on the nasal turbinate of a C3B6.129F1-*Trp53*$^{tm1Brd}$ mouse exposed to 15-ppm formaldehyde. Note multiple layers of keratin (arrows). H&E, 60x. (C) In some animals exposed to 15-ppm formaldehyde, accumulated keratin was present in the space behind a turbinate scroll and, in some cases, filled the space behind the scroll (asterisk). H&E, 20x. (D) Minimal osteogenesis of the nasoturbinate was present in a few mice exposed to 15-ppm formaldehyde. The lesion consisted of a small focus within the lamina propria consisting of eosinophilic osteoid containing numerous large plump osteoblast nuclei (arrows). H&E, 60x.

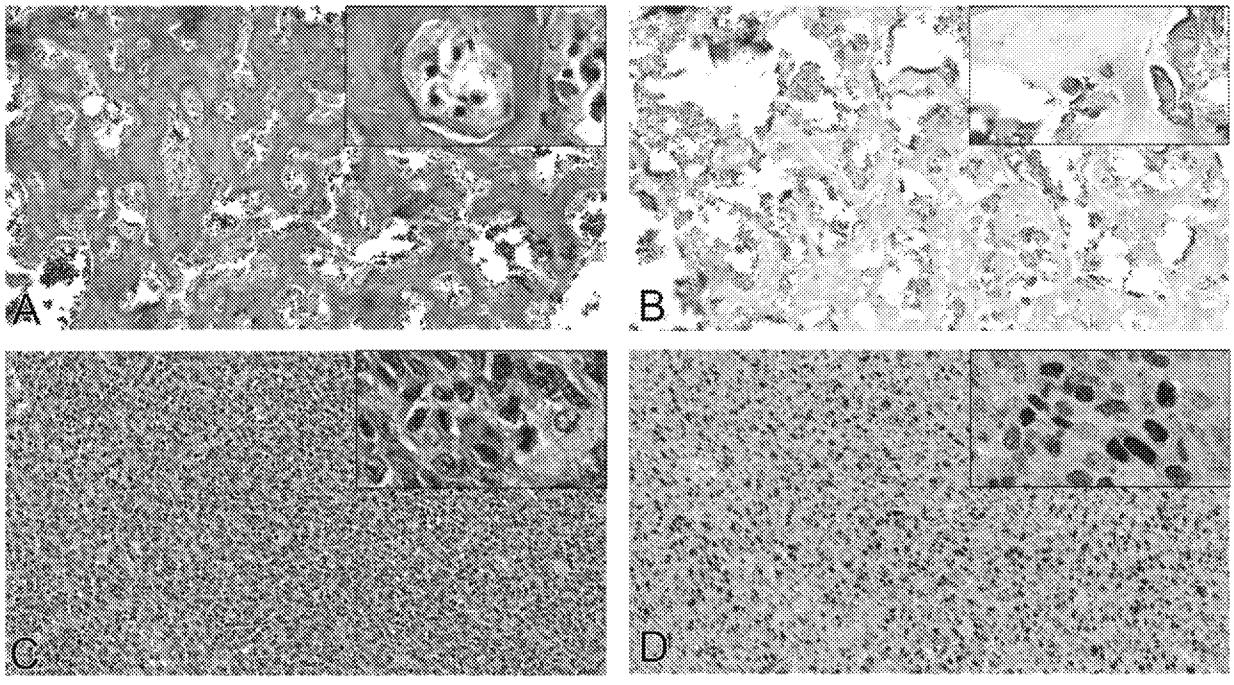



**Figure 4. Neoplastic Lesions in Trp53$^{\pm}$ Mice Exposed to Formaldehyde**

Histological appearance (A, C); hematoxylin and eosin, and immunohistochemical staining with vimentin (B) and myogenin (D) of tumors in C3B6.129F1-*Trp53*$^{tm1Brd}$ mice and B6.129-*Trp53*$^{tm1Brd}$ mice. (A) Osteosarcoma in a C3B6.129F1-*Trp53*$^{tm1Brd}$ mouse, characterized by formation of irregular bone trabeculae and adjacent highly pleomorphic neoplastic spindle-shaped cells. Inset: Higher magnification of pleomorphic neoplastic cell types adjacent to bone trabeculae. (B) Vimentin-stained section of osteosarcoma shown in (A). Note pleomorphic neoplastic cells with cytoplasmic expression of vimentin, an intermediate filament typically present in non-epithelial cells. Inset: Higher magnification of pleomorphic neoplastic cell types with positively staining cytoplasm. (C) Rhabdomyosarcoma in a B6.129-*Trp53*$^{tm1Brd}$ mouse, characterized by pleomorphic cells with varying amounts of cytoplasm and oval-to-elongated nuclei. Inset: Higher magnification of tumor cells showing oval-to-elongated nuclei, abundant cytoplasm, and mitotic activity. (D) Myogenin-stained section of rhabdomyosarcoma shown in (C). Note variably sized pleomorphic tumor cells with nuclear expression of myogenin, a transcription factor essential for skeletal muscle development and repair. Inset: Higher magnification of pleomorphic tumor cells showing positively staining oval and elongated nuclei.

**Table 1. Mortality in Trp53$^{\pm}$ Mice Exposed to Formaldehyde**

| Formaldehyde Concentration | 0 ppm | 7.5 ppm | 15 ppm |
|---|---|---|---|
| | **C3B6.129F1-*Trp53*$^{tm1Brd}$ Mice** | | |
| **Animals/group:** | 25 | 24 | 25 |
| **Early Deaths:** | 4 (16%) | 3 (11%) | 2 (8%) |
| **Cause of Death:** | | | |
| Lymphoma | 0 | 1 | 2 |
| Osteosarcoma | 3 | 0 | 0 |
| Sarcoma | 0 | 1 | 0 |
| Carcinoma NOS[a] metastatic[b] | 0 | 1 | 0 |
| Undetermined | 1 | 0 | 0 |
| | **B6.129-*Trp53*$^{tm1Brd}$ Mice** | | |
| **Animals/group:** | 25 | 31 | 35 |
| **Early Deaths:** | 3 (12%) | 4 (13%) | 9 (26%) |
| **Cause of Death:** | | | |
| Rhabdomyosarcoma | 0 | 1 | 3 |
| Sarcoma | 1 | 2 | 1 |
| Osteosarcoma | 1 | 0 | 0 |
| Leiomyosarcoma | 0 | 0 | 1 |
| Carcinosarcoma | 0 | 0 | 1 |
| Histiocytic sarcoma | 0 | 0 | 1 |
| Ulcerative dermatitis | 0 | 0 | 1 |
| Nephropathy | 0 | 0 | 1 |
| Undetermined | 1 | 1 | 0 |

Formaldehyde exposure had no statistically significant effect on survival of either mouse strain. Nine *C3B6.129F1-Trp53*$^{tm1Brd}$ mice died or were euthanized early primarily due to the presence of large or ulcerated osteosarcomas. Most early deaths of B6.129-*Trp53*$^{tm1Brd}$ mice (16) were due to the presence of large sarcomas and rhabdomyosarcomas.

[a] NOS - not otherwise specified.

[b] Carcinoma in lung, primary site unknown.

**Table 2. Hematological Parameters of C3B6.129F1-*Trp53*$^{tm1Brd}$ Mice Exposed to Formaldehyde**

| | | | Erythron | | | | |
|---|---|---|---|---|---|---|---|
| **Formaldehyde Concentration** | **RBC $10^6$/ml** | **HgB g/dl** | **Hct %** | **MCV fL** | **MCH pg** | **MCHC g/dl** | **Retics $10^6$/µl** |
| 0 ppm | 9.45 ± 0.52 | 14.7 ± 0.7 | 46.7 ± 2.2 | 49.5 ± 0.7 | 15.6 ± 0.3 | 31.5 ± 0.4 | 0.29 ± 0.05 |
| 7.5 ppm | 9.29 ± 1.08 | 14.3 ± 1.5 | 45.4 ± 4.6 | 49.0 ± 1.5 | 15.4 ± 0.5 | 31.5 ± 0.4 | 0.32 ± 0.12 |
| 15 ppm | 9.44 ± 0.51 | 14.7 ± 0.8 | 46.2 ± 2.2 | 49.0 ± 0.8 | 15.5 ± 0.2 | 31.7 ± 0.5 | 0.31 ± 0.07 |
| | | | **Leukon** | | | | |
| **Formaldehyde Concentration** | **WBC $10^3$/µl** | **Neut $10^3$/µl** | **Lymph $10^3$/µl** | **Mono $10^3$/µl** | **Eos $10^3$/µl** | **Plats $10^3$/µl** | |
| 0 ppm | 5.07 ± 1.40 | 1.05 ± 1.09 | 3.67 ± 0.86 | 0.20 ± 0.11 | 0.13 ± 0.10 | 1312 ± 214 | |
| 7.5 ppm | 4.93 ± 1.70 | 1.13 ± 0.94 | 3.45 ± 0.79 | 0.21 ± 0.14 | 0.12 ± 0.09 | 1283 ± 200 | |
| 15 ppm | 4.37 ± 0.79 | 0.80 ± 0.26 | 3.23 ± 0.57 | 0.22 ± 0.11 | 0.12 ± 0.04 | 1209 ± 121 | |

Formaldehyde exposure had no significant effect on any of the hematological parameters of C3B6.129F1-*Trp53*$^{tm1Brd}$ mice.

**Table 3. Hematological Parameters of B6.129-*Trp53*$^{tm1Brd}$ Mice Exposed to Formaldehyde**

| | | | Erythron | | | | |
|---|---|---|---|---|---|---|---|
| **Formaldehyde Concentration** | **RBC $10^6$/ml** | **HgB g/dl** | **Hct %** | **MCV fL** | **MCH pg** | **MCHC g/dl** | **Retics $10^6$/µl** |
| 0 ppm | 9.62 ± 0.33 | 14.0 ± 0.5 | 46.4 ± 1.5 | 48.3 ± 0.9 | 14.6 ± 0.3 | 30.2 ± 0.4 | 0.31 ± 0.03 |
| 7.5 ppm | 9.34 ± 1.09 | 13.6 ± 1.9 | 45.4 ± 4.6 | 48.8 ± 2.2 | 14.5 ± 0.8 | 29.7 ± 2.1 | 0.39 ± 0.31 |
| 15 ppm | 9.38 ± 0.62 | 13.7 ± 0.8 | 45.3 ± 2.8 | 48.3 ± 0.9 | 14.6 ± 0.3 | 30.3 ± 0.6 | 0.30 ± 0.03 |
| | | | **Leukon** | | | | |
| **Formaldehyde Concentration** | **WBC $10^3$/µl** | **Neut $10^3$/µl** | **Lymph $10^3$/µl** | **Mono $10^3$/µl** | **Eos $10^3$/µl** | **Plats $10^3$/µl** | |
| 0 ppm | 5.4 ± 1.8 | 0.60 ± .150 | 4.44 ± 0.77 | 0.192 ± 0.07 | 0.127 ± 0.03 | 1409 ± 182 | |
| 7.5 ppm | 5.9 ± 1.9 | 0.97 ± 1.49 | 4.54 ± 0.92 | 0.220 ± 0.08 | 0.125 ± 0.04 | 1350 ± 292 | |
| 15 ppm | 5.3 ± 1.3 | 0.62 ± 0.29 | 4.37 ± 1.12 | 0.172 ± 0.07 | 0.141 ± 0.06 | 1344 ± 283 | |

Formaldehyde exposure had no significant effect on any of the hematological parameters of B6.129-*Trp53*$^{tm1Brd}$ mice.

**Table 4. Nasal Lesions in Trp53$^{\pm}$ Mice Exposed to Formaldehyde**

| Formaldehyde Concentration | 0 ppm | 7.5 ppm | 15 ppm |
|---|---|---|---|
| | **C3B6.129F1-*Trp53*$^{tm1Brd}$ Mice** | | |
| Respiratory Epithelium | | | |
| Squamous metaplasia | 0/21[a] | 14/21 (1.2)[b] | 22/23 (1.5) |
| Hyperplasia | 0/21 | 0/21 | 1/23 (1.0) |
| Turbinate | | | |
| Osteogenesis | 0/21 | 0/21 | 3/23 (3.0) |
| | **B6.129-*Trp53*$^{tm1Brd}$ Mice** | | |
| Respiratory Epithelium | | | |
| Squamous metaplasia | 0/22 | 13/27 (1.0) | 17/26 (1.5) |
| Turbinate | | | |
| Osteogenesis | 0/22 | 1/27 (1.0) | 1/26 (1.0) |

Mice were exposed to conditioned air, 7.5 or 15 ppm formaldehyde 6hr/d, 5d/week for 8 weeks and then held without further exposure for 32 weeks.

[a] Number of mice with lesion/number examined.

[b] Average severity score based upon 1=minimal, 2=mild, 3=moderate, 4=marked.

**Table 5. Neoplasms in C3B6.129F1-*Trp53*$^{tm1Brd}$ Mice Exposed to Formaldehyde**

| Formaldehyde Concentration | 0 ppm | 7.5 ppm | 15 ppm |
|---|---|---|---|
| **Number examined:** | **25** | **24** | **25** |
| Osteosarcoma | 4[a] (16%)[b] | 1 (4.2%) | 0 |
| Sarcoma, NOS[c], stomach/rib cage | 0 | 1 (4.2%) | 0 |
| Sarcoma, NOS, subcutis | 1 (4%) | 1 (4.2%)[d] | 0 |
| Sarcoma, NOS[e] | 0 | 0 | 1 (4%) |
| Sarcoma, NOS, Harderian gland | 0 | 1 (4.2%) | 0 |
| Hepatocellular adenoma | 5 (20%) | 4 (16.7%) | 1 (4%) |
| Hepatocellular carcinoma | 0 | 2 (8.3%) | 0 |
| Alveolar/bronchiolar carcinoma | 1 (4%) | 0 | 0 |
| Hemangiosarcoma, subcutis | 0 | 1 (4.2%)[d] | 0 |
| Lymphoma | 0 | 1 (4.2%) | 2 (8%) |

Mice were exposed to conditioned air, 7.5 or 15 ppm formaldehyde 6hr/d, 5d/week for 8 weeks and then held without further exposure for 32 weeks.

[a] Number of mice with lesion (includes animals that died early).

[b] Percentage of animals with lesion.

[c] Not otherwise specified.

[d] Sarcoma and hemangiosarcoma present in same animal.

[e] Tissue undetermined.

**Table 6. Neoplasms in B6.129-*Trp53*$^{tm1Brd}$ Mice Exposed to Formaldehyde**

| Formaldehyde Concentration | 0 ppm | 7.5 ppm | 15 ppm |
|---|---|---|---|
| **Number Examined:** | **25** | **31** | **35** |
| Osteosarcoma | 1[a] (4.0%)[b] | 0 | 0 |
| Hepatocellular adenoma | 2 (8.0%) | 0 | 0 |
| Alveolar/bronchiolar adenoma | 1 (4.0%) | 0 | 0 |
| Histiocytic sarcoma | 0 | 1 (3.2%) | 2 (5.7%) |
| Lymphoma | 0 | 1 (3.2%) | 1 (2.9%) |
| Rhabdomyosarcoma | 0* | 1 (3.2%) | 4 (11.4%) |
| Leiomyosarcoma, subcutis | 0 | 0 | 1 (2.9%) |
| Carcinosarcoma, subcutis | 0 | 0 | 1 (2.9%) |
| Sarcoma, NOS[c], subcutis | 1 (4.0%) | 3 (9.7%) | 1 (2.9%) |

Mice were exposed to conditioned air, 7.5 or 15 ppm formaldehyde 6hr/d, 5d/week for 8 weeks and then held without further exposure for 32 weeks.

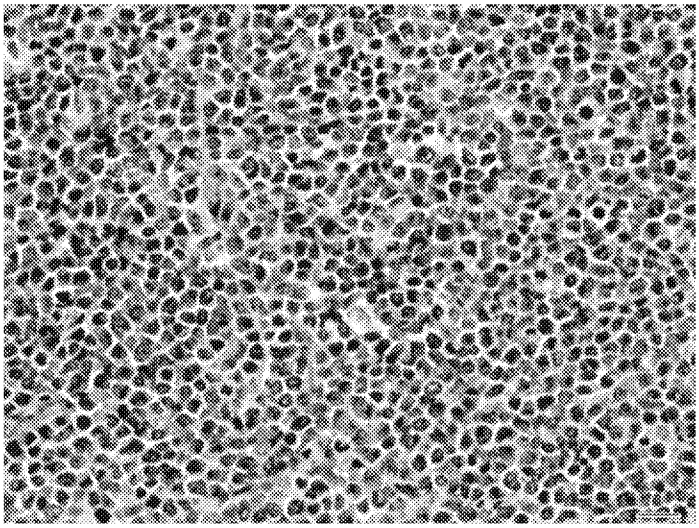
[a] Number of mice with lesion (includes animals that died early).

[b] Percentage of animals with lesion.

[c] Not otherwise specified.

* Significant trend ($p<0.05$).

## Supplemental Materials



**Supplemental Figure 1. Thymus, Cortex, Lymphoma of C3B6.129F1-*Trp53*$^{tm1Brd}$ Mice Exposed to Formaldehyde**

The lymphomas appeared as diffuse sheets of relatively large lymphocytes with large, moderately pleomorphic basophilic, granular nuclei with one or more prominent nucleoli and a scant amount of eosinophilic cytoplasm. H&E, 40X.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| Title | Passed | Document title is showing in title bar |
| Bookmarks | Passed | Bookmarks are present in large documents |
| Color contrast | Passed manually | Document has appropriate color contrast |

## Page Content

| Rule Name | Status | Description |
|---|---|---|
| Tagged content | Passed | All page content is tagged |
| Tagged annotations | Passed | All annotations are tagged |
| Tab order | Passed | Tab order is consistent with structure order |
| Character encoding | Passed | Reliable character encoding is provided |
| Tagged multimedia | Passed | All multimedia objects are tagged |
| Screen flicker | Passed | Page will not cause screen flicker |
| Scripts | Passed | No inaccessible scripts |
| Timed responses | Passed | Page does not require timed responses |
| Navigation links | Passed | Navigation links are not repetitive |

## Forms

| Rule Name | Status | Description |
|---|---|---|
| Tagged form fields | Passed | All form fields are tagged |
| Field descriptions | Passed | All form fields have description |

## Alternate Text

| Rule Name | Status | Description |
|---|---|---|
| Figures alternate text | Passed | Figures require alternate text |
| Nested alternate text | Passed | Alternate text that will never be read |
| Associated with content | Passed | Alternate text must be associated with some content |
| Hides annotation | Passed | Alternate text should not hide annotation |
| Other elements alternate text | Passed | Other elements that require alternate text |

## Tables

| Rule Name | Status | Description |
|---|---|---|
| Rows | Passed | TR must be a child of Table, THead, TBody, or TFoot |
| TH and TD | Passed | TH and TD must be children of TR |

| | | |
|---|---|---|
| Headers | Passed | Tables should have headers |
| Regularity | Passed | Tables must contain the same number of columns in each row and rows in each column |
| Summary | Skipped | Tables must have a summary |

## Lists

| Rule Name | Status | Description |
|---|---|---|
| List items | Passed | LI must be a child of L |
| Lbl and LBody | Passed | Lbl and LBody must be children of LI |

## Headings

| Rule Name | Status | Description |
|---|---|---|
| Appropriate nesting | Passed | Appropriate nesting |

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September 13, 2017

Dr. Tina Bahadori
Director, NCEA
USEPA Headquarters
Ariel Rios Building
1200 Pennsylvania Avenue, N. W.
Mail Code: 8601P
Washington, DC 20460

**Re: NTP Mode of Action Research Relevant to the Formaldehyde IRIS Assessment**

Dear Dr. Bahadori:

In October 2015, the American Chemistry Council Formaldehyde Panel (the Panel) submitted a letter to the previous NCEA Director, Dr. Ken Olden, calling attention to formaldehyde research conducted by the National Toxicology Program (NTP) National Institute of Environmental Health Sciences (NIEHS) which was presented at the 2014 and 2015 Society of Toxicology (SOT) meetings. The NTP research explored a hypothesized mode of action for leukemia in humans using two genetically predisposed strains of mice exposed to formaldehyde and found that formaldehyde inhalation did not cause leukemia or lymphohematopoietic neoplasia. In October 2016, we submitted a follow-up letter to EPA communicating our formal request to NTP to publish the research in a peer-reviewed scientific journal or, at a minimum, for NTP to issue a public technical report. I am pleased to report that in August 2017, the NTP released a research report titled: Absence of Formaldehyde-Induced Neoplasia in Trp53 Haploinsufficient Mice Exposed by Inhalation,[1] which provides the full details of the research summarily presented at SOT.

The objective of the NTP study was to evaluate the potential role of the Trp53 gene in nasal carcinogenicity, leukemia or lymphohematopoietic cancer, and potentially other neoplasms in genetically susceptible mice. Male Trp53 haploinsufficient (Trp53+) mouse strains (B6.129-Trp53tm1Brd and C3B6.129F1-Trp53tm1Brd) were exposed via inhalation to 0 ppm, 7.5 ppm or 15 ppm formaldehyde for 8 weeks. Because evidence suggests a possible role of the Trp53 gene in formaldehyde-induced nasal squamous cell carcinomas, the authors hypothesized that formaldehyde-induced loss of Trp53 would result in an increase in susceptibility to formaldehyde-induced nasal squamous cell carcinoma, and possibly leukemia and other neoplasms. However, the study found that inhalation of a maximum tolerated dose of formaldehyde did not cause nasal tumors, an increased prevalence of leukemia or lymphohematopoietic cancer, or any other type of cancer in Trp53+ mice. The results from this study increase the weight of evidence that formaldehyde exposure is not causally associated with leukemia. EPA's IRIS Stopping Rules[2] allow for the inclusion of new research information until a few months before an assessment is released for review. This study report provides important information related to postulated modes of action for formaldehyde and should be evaluated and integrated into the formaldehyde weight of evidence framework. We also strongly encourage EPA to reach out to the NTP for additional insight and information on this study.

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[1] NTP Research Report on Absence of Formaldehyde-Induced Neoplasia in Trp53 Haploinsufficient Mice Exposed by Inhalation Research Report 3, National Toxicology Program, August 2017. The full report can be found at: https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/formaldehyde_508.pdf

[2] EPA IRIS Stopping Rules - https://www.epa.gov/sites/production/files/2014-06/documents/iris_stoppingrules.pdf

EPA has previously indicated that it is committed to ensuring that the revised draft formaldehyde assessment reflects a transparent, rigorous, systematic review of available formaldehyde evidence which is consistent with the 2011 National Academy of Sciences (NAS) recommendations. The Panel has been committed to conducting research to address the recommendations of the NAS and engaging scientists on approaches to integrate the scientific evidence for formaldehyde. As we have previously communicated to EPA, in support of efforts to engage scientific experts on the formaldehyde science and methodologies for integrating the evidence, an invited scientific expert workshop has been scheduled for October 2017. This workshop will provide valuable insight on integrating the formaldehyde science which can inform the EPA's formaldehyde IRIS assessment. We are pleased that EPA IRIS staff accepted an invitation to participate in this workshop and look forward to the discussion.

The methods and approaches that EPA utilizes to systematically review and integrate the science to draw conclusions regarding potential human health risk will be a cornerstone in any future formaldehyde assessment. To help improve our understanding of the processes that will be applied to the formaldehyde assessment, we request that you provide responses to the following questions.

1. How is EPA considering new scientific information, like the NTP study, for incorporation into the weight of evidence for the formaldehyde IRIS assessment?
2. When did EPA last conduct a search of the formaldehyde literature for science to incorporate into the IRIS assessment and how frequently does EPA monitor the formaldehyde literature to identify potential studies that should be incorporated into the assessment?
3. What guidance documents or procedures will EPA utilize to evaluate study quality for studies relied upon to reach conclusions in the formaldehyde IRIS assessment? Please provide specific references if available.
4. When will EPA release a weight of evidence framework illustrating how various data streams (i.e. mechanistic, toxicology and epidemiology studies) are evaluated for quality and then integrated to reach conclusions about formaldehyde?
5. How has EPA addressed all the 2011 NAS recommendations for formaldehyde?
6. How will EPA seek public input and peer review on the formaldehyde IRIS assessment and what types of public meetings or workshops will be held to receive input?

Feel free to contact me by phone Ex. 6 or email (Kimberly_White@americanchemistry.com) with any questions related to this letter. Additionally, a full copy of the study report is attached for your reference.

Sincerely,

Kimberly Wise White, PhD
American Chemistry Council (ACC)
Senior Director
Chemical Products & Technology Division
On Behalf of the ACC Formaldehyde Panel

Cc:
Robert Kavlock
Dan Morgan
Kris Thayer
Richard Yamada

Attachment 1 – NTP Research Report on Absences of Formaldehyde-Induced Neoplasia in TRP53 Haploinsufficient Mice Exposed by Inhalation, August 2017



Message

**From**: Fischer, David [David_Fischer@americanchemistry.com]
**Sent**: 7/6/2017 5:01:03 PM
**To**: Yamada, Richard (Yujiro) [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=4c34a1e0345e4d26b361b5031430639d-Yamada, Yuj]
**Subject**: following up

Richard, hope all is well and that you are smoothly transitioning into your new role. On behalf of ACC, congrats again on your appointment! I, along with a few of my colleagues, would like to meet with you to touch base on a number of items. Might you have availability over the next few weeks? Looking forward to meeting with you. Thanks.

David

David Fischer, M.P.H., J.D. | American Chemistry Council
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Chemical Products & Technology Division
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O: **Ex. 6**
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