External Peer Review

Toxicological Review for 2,2,4-Trimethylpentane

Final Compilation of Reviewer Comments and Responses to Charge Questions

Prepared for
Integrated Risk Information System (IRIS) Program
Office of Research and Development
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U.S. Environmental Protection Agency

Prepared by
ORISE IRIS Technical Assistance Team
Oak Ridge Institute for Science and Education
Oak Ridge Associated Universities

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Toxicological Review for 2,2,4-Trimethylpentane

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EXTERNAL PEER REVIEWERS

Susan Borghoff, Ph.D., DABT, Panel Chair
Integrated Laboratory Systems, Inc.

Deborah Barsotti, Ph.D.
MACTEC Engineering and Consulting

Lawrence Lash, Ph.D.
Wayne State University

Brian Short, Ph.D., DVM, DACVP
Allergan, Inc.

The ORISE IRIS Technical Assistance Team has neither altered nor edited these comments for grammatical or other errors.

PEER REVIEW PROJECT

D. Charles Thompson, Project Manager
U. S. Environmental Protection Agency

Leslie Shapard, Peer Review Manager
Oak Ridge Institute for Science and Education

The U.S. EPA's National Center for Environmental Assessment (NCEA) has developed a draft Toxicological Review and IRIS Summary for 2,2,4 - trimethylpentane. These documents were subject to an EPA Agency review as well an Inter-Agency review for scientific accuracy and compliance with EPA risk assessment guidelines and procedures in 2006. The charge questions below specifically address the 2,2,4 - trimethylpentane assessment.

CHARGE QUESTION 1: KEY PUBLISHED STUDIES
Are there additional key published studies or publicly available scientific reports that are missing from the draft document that might be useful for the discussion of the hazards of 2,2,4-trimethylpentane?

Susan Borghoff, Ph.D., DABT, Panel Chair

All of the critical published papers associated with the hazards of 2,2,4-trimethylpentane appear to be cited in this review. There are some publications on trimethylpentane and its metabolite trimethylpentanol that have not been included in this report. In some cases these data may help to support some of the conclusions that have been made. See reference list at the end of these comments.

Deborah Barsotti, Ph.D.
While not an exhaustive literature review, I searched for current publications on the fate and transport, toxicokinetics and toxicology of 2,2,4-trimethylpentane and could not locate any relevant publications that have not be included in this draft review.

While not included in the general introduction section of these toxicological reviews, the Agency may wish to consider the summarizing the IRIS process for the compound that would include the IRIS history and the basis for the compounds selection for review. This is particularly important give the Agency's limited resources and priorities.

Lawrence Lash, Ph.D.
My search of the literature on 2,2,4-trimethylpentane (TMP) revealed two references that are, in my opinion, of some value to include in the database.

Paper #1: Standeven, A.M., and Goldsworthy, T.L. (1994) Identification of hepatic mitogenic and cytochrome P450-inducing fractions of unleaded gasoline in B6C3F1 mice. *J. Toxicol. Environ. Health* **43,** 213-224.
This paper examined the effects of unleaded gasoline (UG) and specific components of this complex mixture of over 300 hydrocarbons, on induction of tumors in female mouse liver. The liver tumors occur selectively in female mice. UG was divided into multiple fractions based on boiling points. The authors identified both a fraction containing TMP as well as purified TMP as producing the largest increase in bromodeoxyuridine labeling index, supporting the conclusion that TMP is mitogenic in hepatocytes from female mice.

Paper#2: Cuervo, A.M., Hildebrand, H., Bomhard, E.M., and Dice, J.F. (1999) Direct lysosomal uptake of alpha 2-microglobulin contributes to chemically induced nephropathy. *Kidney Int.* **55,** 529-545.

This paper characterized the uptake of alpha-2u in lysosomes isolated from untreated or TMP-treated rats. The results provide additional mechanistic information of how TMP and other compounds that produce the alpha-2u nephropathy act. The mechanism involves binding of alpha-2u to the heat shock cognate protein of 73 kDa (hsc73), binding of this complex to a membrane receptor on the lysosomal membrane, and uptake into the lysosomes. The authors show that TMP increases this uptake process.

It should be noted that neither paper significantly changes any of the present conclusions about the human health risk of TMP but should be included for completeness.

Brian Short, Ph.D., DVM, DACVP
Not that I am aware of.

CHARGE QUESTION 2: RfD RATIONALE AND JUSTIFICATION
No oral RfD has been derived in the current draft assessment. Has the rationale and justification for not deriving an RfD been transparently described? Is the rationale scientifically justified and appropriate?

Susan Borghoff, Ph.D., DABT, Panel Chair
I agree that the studies conducted with trimethylpentane were mainly short term oral administration with a focus on the kidney (rats) and liver (mice). Because these studies were designed to address specific mode of action questions, they are not sufficient for use in deriving an RfD. I felt that the discussion of this reasoning was justified and appropriate.

Deborah Barsotti, Ph.D.
Given the nature and extent of the database for 2,2,4-trimethylpentane, it is technically appropriate that an RfD was not developed. The document justifies this position adequately. To do other wise would put an RfD out to the pubic that would not reflect the intent of the agency for reference doses and may result in misunderstanding of the toxicological properties of the compound.

Lawrence Lash, Ph.D.
I agree with the conclusion of the draft report that there are no subchronic or chronic oral studies of TMP exposure that demonstrate a dose response effect that could be used to determine an RfD. Although there is some criticism that only effects associated with alpha-2u nephropathy were sought in most of the studies, in other studies (acute or short-term oral studies) in which potential liver effects were examined, either no effects or very modest effects were observed and no major histological changes in liver tissue were found. Thus, the status of searches for sufficient data to derive an RfD have been clearly described and the rationale for the decision to not be able to derive an RfD is scientifically justified and appropriate.

Brian Short, Ph.D., DVM, DACVP
Yes, the rationale has been transparently described and is scientifically justified and appropriate for not deriving an oral RfD.

CHARGE QUESTION 3: RfC RATIONALE AND JUSTIFICATION
No inhalation RfC has been derived in the current draft assessment. Has the rationale and justification for not deriving an RfC been transparently described? Is the rationale scientifically justified and appropriate?

Susan Borghoff, Ph.D., DABT, Panel Chair
Again, scientific justification for not deriving an RfC was appropriately described in the review.

Deborah Barsotti, Ph.D.
Given the nature and extent of the database for 2,2,4-trimethylpentane, it is technically appropriate that an RfD was not developed. The document justifies this position adequately. To do other wise would validate an RfD for public use that would not reflect the intent of the Agency for reference doses and may result in misunderstanding of the toxicological properties of the compound.

Lawrence Lash, Ph.D.
As for the RfD, I agree with the conclusion of the draft report that it is not possible to derive an RfC for TMP. No subchronic or chronic inhalation studies were identified that demonstrated a dose response effect that could be used for the derivation of an RfC. As noted for the oral exposure studies, the one subchronic study and the three short-term inhalation studies focused primarily on effects observed in male rat kidney related to the alpha-2u nephropathy. Thus, the status of searches for sufficient data to derive an RfC have been clearly described and the rationale for the decision to not be able to derive an RfC is scientifically justified and appropriate.

Brian Short, Ph.D., DVM, DACVP
Yes, the rationale has been transparently described and is scientifically justified and appropriate for not deriving an inhalation RfC.

CHARGE QUESTION 4: RELATIONSHIP OF α2u–GLOBULIN IN MALE RAT KIDNEY
Does the Toxicological Review provide sufficient information to support a conclusion that there is a causal relationship between accumulation of α2u-globulin and the pathology observed exclusively in the male rat kidney in response to 2,2,4-trimethylpentane exposure?

Susan Borghoff, Ph.D., DABT, Panel Chair
Some of the studies that are missing from this report that support the causal relationship been the accumulation of α2u and the pathology are associated with the confirmation of chemical binding to this protein both in vivo and in vitro (see abstracts attached). Chemical binding to this protein is one of the EPA's criteria to support that the chemical operates through this mode-of-action.

Deborah Barsotti, Ph.D.
The research and regulatory history associated with the α2μ–globulin mechanism of action and pathology is adequately summarized and documented in this toxicological review.

Lawrence Lash, Ph.D.
The EPA draft document carefully describes the toxicokinetics and hazard identification studies that are available for TMP. For the hazard identification studies, these are carefully divided up into three types: Studies in humans (of which there were none identified); subchronic and chronic studies in animals; and cancer bioassays. All the various studies that are available and the two additional studies that this reviewer identified (see response to Question 1), clearly show that the most prominent effect observed is the male rat-specific alpha-2u nephropathy. All the available data clearly demonstrate that TMP is a classic male rat-specific toxicant and that this nephropathy is unambiguously associated with hyaline droplet accumulation in the renal proximal tubules. In several studies, nephropathy was only observed in the male rat. There were two inhalation studies described in which mice or mice and guinea pigs exhibited lethality. However, these studies used extremely high doses of TMP (1,000-128,000 ppm and > 8,000 ppm, respectively), so that the results are really irrelevant to any real-life exposures. Overall, the only consistent finding from the best mechanistic toxicity data is that TMP produces the characteristic, alpha-2u nephropathy in male rats.

Brian Short, Ph.D., DVM, DACVP
Yes, the Tox Review provides sufficient information to support a causal relationship between α2u-globulin and the pathology observed.

CHARGE QUESTION 5: EFFECTS UNRELATED TO α2u–GLOBULIN-ASSOCIATED NEPHROPATHY
The majority of the studies available for 2,2,4-trimethylpentane were designed only to investigate various aspects of α2u-globulin-induced nephropathy. Thus, data and information on effects in target organ systems other than the kidney are limited in quantity and quality (e.g. liver). Has the available information on effects unrelated to α2u-globulin-associated nephropathy been adequately and appropriately described?

Susan Borghoff, Ph.D., DABT, Panel Chair
As stated there is limited information on the effects of trimethylpentane on organ systems other than liver. However, there are a few more references to that could be included associated with mouse liver effects. One such citation is Standeven and Goldsworthy, where they demonstrate that trimethypentane induces mitogenic activity in the mouse liver.

Deborah Barsotti, Ph.D.
Where possible, the draft document describes other information to an appropriate degree. However, I would urge a thorough review of the toxicokinetics section (Section 3) the original papers to assure that the document reflects the study results.

It should be made clear what was being recovered – radioactivity or radiolabelled 2,2,4-trimethylpentane parent compound.

In Section 3.2.1 – Is it correct to say that there is a "marked" distribution of 2,2,4-trimethylpentane in male rats when the differences between male and female liver were 244 and 177 nmol eq/g wet tissues for males versus 336 and 193 nmol eq/g wet tissues in females? While a marked difference is true for the kidneys there were no statistical differences between genders in peritoneal fat and livers.

There should be a global consistency in compound nomenclature, i.e, capitalization of "tri" or not.

The last sentence of Section 3.3.1 does not appear complete.

In the last section of 3.4.1, I suggest changing the "expired organic material" phrase to be more specific if appropriate.

Lawrence Lash, Ph.D.
While the statement is correct that most of the studies on TMP toxicity focused on the alpha-2u nephropathy response, findings in other potential target organs (e.g., the liver) were either not consistently observed or were not particularly prominent. The one study mentioned in response to Question 1 (paper #1) did demonstrate TMP was mitogenic in female mouse liver; this is the only relevant omission from the literature that describes effects of TMP in a target organ other than kidney. Overall, however, the draft TMP toxicology review adequately and appropriately presents the current state of the literature.

Brian Short, Ph.D., DVM, DACVP
Yes, effects unrelated to α2u-globulin-related nephropathy have been adequately and appropriately described.

CHARGE QUESTION 6: CANCER DESPCRIPTOR

Has the appropriate cancer descriptor been chosen? Has the rationale and justification for not deriving a quantitative cancer assessment been transparently described? Do you agree with EPA's rationale, justification and conclusion?

Susan Borghoff, Ph.D., DABT, Panel Chair
Yes

Deborah Barsotti, Ph.D.
Based on the current state of knowledge and regulatory status, the appropriate cancer descriptor has been chosen and adequately justified.

Lawrence Lash, Ph.D.
Unfortunately, no epidemiological studies in humans are available and no chronic bioassay studies in animals are available for TMP. The toxicology review document summarizes the few studies available on the ability of TMP to act as a tumor promoter, which showed TMP to be a tumor promoter in male, but not female, rats. Genotoxicity

tests were negative. The EPA document then describes studies and information based on hyaline droplet accumulation in the early stages of nephropathy, which are believed to lead to a sequence of events causing chronic proliferation of the renal tubular epithelium. Overall, however, there is a data gap concerning the carcinogenic potential of TMP. Thus, the analysis and the rationale to justify not deriving a quantitative cancer assessment for TMP are appropriate and justified.

Brian Short, Ph.D., DVM, DACVP

Yes, the appropriate cancer descriptor has been chosen, the rationale and justification for not driving a quantitative cancer assessment has been transparently described and I agree with the EPA's rationale, justification, and conclusion.

MISCELLANEOUS COMMENTS

Susan Borghoff, Ph.D., DABT, Panel Chair

Some editorial comments on the following pages:

Page 14, last sentence- GC/MS analysis did not show reversible binding between a metabolite of 2.2,4-trimethylpentaqne… and a2u-globulin. The low molecular weight protein fraction in male rat kidneys (from TMP-treated rats) was isolated and small molecules extracted from the protein, identified by GS/MS analysis to be its metabolite trimethyl-2-pentanol. Reversible binding was determine by dialysis with and without SDS.

Page 15, first paragraph
Hyaline droplets are present in control male rats, therefore with chemical treatment they accumulate or are exacerbated. Formation is not the right word to describe this syndrome.

It would be useful to the reader if all units of dose formulations were converted to either mg/kg or mmol/kg.

I would be cautious about the interpretation of the study with cycloheximide pretreatment suggested that the accumulation in the kidney was due in part to increased synthesis of the protein in the liver. If the authors did an extensive literature search on a2u I think they would find that this is not the case.

Page 18, 4.5.1

It is stated that the effects reported for trimethylpentane included altered function. I am not away that any of the trimethylpentane studies demonstrated altered kidney function so if this is the case it would be best to articulated what endpoints measured suggested altered kidney function.

Page 20, 4.7.1, second paragraph, second sentence

I suggest modifiying this sentence because chemicals bind to proteins and not the other way around- suggestion- The involvement of hyaline droplet accumulation in the early

stages of nephropathy is an important step in the sequence of events observed with classical renal carcinogens.

The sentence beginning with “The pathological changes …“needs to be modified. It is not clear what the authors are describing. Again, later in the paragraph I suggest changing to chemical-a2u complex.