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March 23, 2001

The Honorable Christine Todd Whitman  
Administrator  
U.S. Environmental Protection Agency  
Ariel Rios Building  
Room 3000, #1101-A  
1200 Pennsylvania Ave., N.W.  
Washington, DC 20460

Subject: Comments on HPV Test Plan and Robust Summaries for C5 Noncyclics Category

Dear Administrator Whitman:

The following comments on the American Chemistry Council's (ACC's) test plan for the C5 noncyclics category are submitted on behalf of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, The Humane Society of the United States, The Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than nine million Americans.

The ACC has judiciously formed chemical categories and coordinated with other forthcoming High Production Volume (HPV) test plans. However, the problems that remain with the plan reflect the overall HPV program's flaws and failures. **Blindly conducting the SIDS battery of tests on mixtures of isoprene and 2-methyl-2-butene will contribute nothing to the understanding of these chemicals' toxicities, and indicates a complete disregard for existing data and the current level of knowledge about these and related chemicals.**

Dosing animals with an arbitrary selection of mixtures will provide no progress in understanding the toxicity of these mixtures. These tests, in fact, are a step backwards in scientific progress. The current research on these C5 compounds has far surpassed efforts in hazard identification and is focused on understanding and modeling the kinetics, metabolism, and toxicological mechanisms of these chemicals. The crude SIDS battery will offer nothing to the understanding of these chemicals, and if the EPA supports this research, it will set the state of the science back years.

Our main objections to this test plan are as follows:

**1. The test plan does not maximize the use of existing data.**

The ACC should employ a more thoughtful approach to understanding the systematic toxicity of the C5 alkane and alkene compounds in this category. Toxicity generally increases in these compounds with increasing molecular weight and increasing number of double bonds. Therefore, a comparison of the toxicity of these chemicals to alkanes and alkenes in other categories, such as the ACC's

Butadiene C4 category, would yield a greater level of understanding of the hazard posed by these substances, without conducting further tests.

Conducting animal tests with mixed streams does not enhance the understanding of the chemicals' potential hazards to human health. The proposal defies good science by ignoring evidence suggesting that the mixed streams would be less reactive than the pure stream of the most bioactive compound.

**2. The test protocol does not apply “thoughtful toxicology.”**

The existing data on the chemicals included in the test plan are sufficient to perform a basic hazard assessment of the industrial streams. Isoprene toxicity is well-understood at both an empirical and biochemical level and is considered a potential carcinogen in humans and other animals. Any additional testing of mixtures with a lower percentage of isoprene will not enhance the already comprehensive understanding of this chemical. 2-methyl-2-butene will behave very similarly to the well-studied butene, a simple asphyxiant. This chemical should also be less toxic than isoprene because it has one less double bond.

**3. Extreme species differences will obscure any SIDS test results.**

As with 1,3 butadiene, great inter- and intra-species differences in the adverse health effects of isoprene have hindered the understanding of the behavior of this potential carcinogen in humans.

**4. Extensive existing human exposure data are not considered.**

The primary sources of isoprene in the environment are natural emissions from vegetation. Exposure information on isoprene underscores a flaw in the underlying assumption of the HPV program: High production volume does not necessarily translate into high exposure. A study submitted to the EPA's Ozone Transport Assessment Group (OTAG) Air Quality Analysis Workgroup in 1997, revealed that environmental concentrations were typically orders of magnitude below recommended exposure limits.

**5. The ACC test plan does not maximize the use of nonanimal tests.**

The ACC is proposing to repeat *in vivo* genetic toxicity tests, even though the toxicity of isoprene has been established and it is an accepted animal carcinogen. Aquatic toxicity tests are proposed on mixed industrial streams, even though these substances are gases, are unlikely to undergo hydrolysis reactions, and have a low water solubility, resulting in rapid volatilization from water.

These concerns reflect specific violations of the EPA's Federal Register notice “Data Collection and Development on High Production Volume (HPV) Chemicals”<sup>1</sup> and the following key items of the EPA's October 14, 1999, letter to HPV participants,<sup>2</sup> which outlined certain principles to minimize animal tests in the program:

1. In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach.
2. Participants shall maximize the use of existing and scientifically adequate data.
3. Participants shall maximize the use of existing and scientifically appropriate

- categories of related chemicals and structure activity relationships.
5. Participants are encouraged to use *in vitro* genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.
  8. ...As with all chemicals, before generating new information, participants should further consider whether any additional information would be useful or relevant.”

For the HPV program to reduce the amount of repetitive, uninformative animal testing, the EPA must require that the ACC perform a more thoughtful review of existing data, expand the development of structure activity relationships, and specifically explain why any additional animal testing is necessary for these compounds. The current understanding of these chemicals has surpassed the stages of crude hazard identification. No further animal testing on these well-studied chemicals should be conducted under the HPV program.

I can be reached via telephone at 202-686-2210, ext. 302, or via e-mail at <[ncardello@pcrm.org](mailto:ncardello@pcrm.org)>. Correspondence should be sent to my attention at the following address: PCRM, 5100 Wisconsin Ave., Suite 400, Washington, DC 20016. I look forward to your response on this important issue.

Sincerely,

Nicole Cardello, MHS  
Research Coordinator

Attachment: Specific Comments

cc: The Honorable Robert C. Smith  
The Honorable F. James Sensenbrenner, Jr.  
The Honorable Ken Calvert  
The Honorable Jerry Costello  
Council on Environmental Quality  
Steve Russell, Esq. American Chemistry Council



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## General Comments on the C5 Test Plan Submitted by the American Chemistry Council

### 1. The test plan does not make adequate use of existing data.

The American Chemistry Council (ACC) has done an excellent job of grouping 11 different industrial streams with 16 separate CAS numbers into a single category, recognizing that isoprene and 2-methyl-2-butene are the primary bioactive agents in these mixtures. Toxicity generally increases in these compounds with increasing molecular weight and increasing number of double bonds. Therefore, a comparison of the toxicity of these chemicals to alkanes and alkenes in the ACC's Butadiene C4 category<sup>3</sup> would yield a greater level of understanding of the hazard posed by these substances, without conducting further tests.

The ACC's robust summary on isoprene clearly demonstrates that data exist for all SIDS endpoints for isoprene. Additional studies provide further understanding of isoprene, above and beyond information on the SIDS endpoints. The IARC has labeled isoprene a Group 2B carcinogen, which means, in its assessment, sufficient data exist to consider isoprene a possible human carcinogen and the number of experiments already conducted on animals is sufficient to determine that isoprene is an animal carcinogen.

Isoprene, the 2-methyl analogue of 1,3-butadiene, is estimated to be the most abundant hydrocarbon (anthropogenic or biogenic) across much of the eastern U.S.<sup>4</sup> Isoprene naturally occurs in the environment as emissions from vegetation. Isoprene is produced endogenously in humans and is the basic structural unit of countless natural products, including natural rubber, terpenes, vitamins A and K, and steroid sex hormones.

Isoprene toxicity is well understood at both an empirical and biochemical level. Further, the toxicity of other compounds in the industrial streams is well-characterized and are usually much less bioactive than isoprene. 2-methyl-2-butene should behave very similarly to the well-studied simple asphyxiant butene. Despite these facts, the ACC is proposing an extensive set of animal tests to evaluate potential health effects. This testing is wholly inappropriate and unnecessary.

The crude screening-level tests proposed in this test plan will provide no insight into the regulation of isoprene in the workplace, especially given the extensive toxicological work already being conducted on the metabolism and kinetics of isoprene. Table 1 presents a summary of some the available studies on the toxicological mechanisms of isoprene, including *in vitro* studies.<sup>5-22</sup> Instead of conducting the crude SIDS battery of tests on mixed isoprene streams, the issues of human metabolism, toxicological mechanisms, human biomarkers, and human PBPK models need further evaluation.<sup>5,7</sup> The metabolism of isoprene and the two isoprene monoepoxides by human cytochrome P-450 enzymes has been studied. Biomarkers of isoprene have already been studied *in vivo* and *in vitro*, pushing the science way beyond the crude endpoints measured in the SIDS battery.<sup>22</sup> The formation of hemoglobin adducts was measured in mice and rats, showing that measurement of these adducts are potential biomarkers of exposure. The detection of adducts and metabolites in humans should be the subject of further investigation.

The toxicokinetics of isoprene have been investigated and compared previously to butadiene.<sup>5,7,11,19</sup> This comparative information would be very helpful in structure activity modeling. The stereochemical course of the biotransformation of isoprene has been investigated *in vivo*<sup>10</sup> and *in vitro*.<sup>22</sup>

## **2. The test plan does not apply “thoughtful toxicology.”**

Based on the understanding of the structure and toxicity of the chemicals present in these mixed streams, the evidence suggests that the chemicals would not be more reactive, or synergistic than the primary bioactive agent. It is highly unlikely that the mixed stream with lower molecular weight alkanes and alkenes, with fewer functional groups would be more toxic than the most saturated component. Since the epoxide metabolites of isoprene are responsible for its toxicity and the other C5 alkene components of the stream are metabolized by the same metabolic pathway, it is likely that the mixed components will compete for the same active enzyme sites. Therefore, the mixed pyrolysis and hydro-treated C5 streams are likely to be less toxic than high purity isoprene. Data presented in the ACC’s C4 Butadiene test plan support this thoughtful analysis of chemical properties. Existing data show that mid-range butadiene streams are less toxic than one would calculate based on 1,3-butadiene content.<sup>3</sup>

However, if the EPA and ACC choose to ignore existing evidence and logic, it is still not necessary to perform a complete SIDS battery on a mixed stream. A cytotoxicity test or Ames assay will adequately show whether or not the mixed stream is more toxic than the most potent, bioactive agent and whether or not a potential for synergistic interactions exists. The check-the-box toxicity testing that the ACC is using in this case is inappropriate, unnecessary, and a waste of time, money, and animals’ lives.

The EPA Guidance documents for assessing the risk to environmental mixtures “Guidance for Conducting Health Risk Assessment of Chemical Mixtures” provide detailed descriptions of how to conduct a basic hazard identification assessment on a mixture with data on the components.<sup>23</sup> The available information in this case is more than adequate for completing a basic hazard identification evaluation. Testing mixtures on animals will not provide any greater understanding of the potential health effects in humans. The relative hazard of the different streams could be estimated based on their composition and existing toxicological and epidemiological data on crude mixtures and pure compounds. Data gaps or uncertainty in these calculations could be accounted for by including a toxicity equivalent factor or appropriate safety factor. In fact, an innovative toxicologist may even be able to accurately account for competitive binding of different C5 compounds, a toxicological mechanism that will likely reduce the toxicity of the mixed isoprene stream, compared to pure isoprene. By focusing efforts on interpreting the abundant existing data instead of conducting more animal testing, it is likely that a better understanding of the toxicity of these different C5 streams would be developed.

The study of mixtures is only useful if adequate physiological pharmacokinetic data and understanding exist so that data can be interpreted. The focus of any further research into these classes of chemicals should be on human PBPK models.

## **3. Extreme species differences will obscure any SIDS test results.**

Just as with butadiene, great variability in isoprene toxicity in different species casts doubt on the relevance of using these animal tests for predicting toxicity to humans. Several studies listed in Table 1 suggest substantial species differences in the toxicity and metabolism of isoprene and related compounds.<sup>6,8,9,11,15,16,19,20</sup> For example, according to the NIH database, isoprene was not mutagenic in *Salmonella typhimurian* and did not induce sister chromatid exchanges or chromosomal aberration in Chinese hamster ovary cells with or without exogenous metabolic activation. However, in mice, isoprene induced increases in the frequency of sister chromatid exchanges in bone marrow cells and the frequency

of micronucleated erythrocytes in peripheral blood. The site of tumors varied between the sexes of mice and between rats and mice in carcinogenicity testing of isoprene. In mice, isoprene exposure resulted in increased benign and malignant tumors of the lung and liver. Tumors have also been observed in heart and spleen and histiocytic sarcomas in male mice. In contrast, pituitary adenomas and Harderian gland adenomas were found in female mice. In both male and female rats, increased incidences in mammary gland tumors were found, whereas kidney tumors were found only in male rats.

The kinetics and metabolism of isoprene have also been explored. The rate of metabolism is more than three times greater in mice than in rats. *In vitro* studies and a physiological toxicokinetic model suggest that the rates of metabolism of isoprene in humans are lower.<sup>6</sup>

## **2. Exposure data are not considered.**

Isoprene is the single most important volatile compound contributing to formation of ground-level ozone. It constitutes about 40% of non-methane organic compound emissions to the atmosphere, and it is emitted almost exclusively by natural sources. Emission rates have been measured experimentally. Isoprene is produced endogenously in humans and is the basic structural unit of countless natural products, including natural rubber, terpenes, vitamins A and K, and the steroid sex hormones. Isoprene may be released to the environment as emissions during wood pulping, biomass combustion, and rubber abrasion, tobacco smoke, gasoline, wood smoke, turbine, and automobile exhaust.

The EPA even funded a project in 1995 that stated, "The primary purpose of this project is to advance fundamental knowledge of the kinetics and reaction mechanisms for the hydroxyl radical and ozone oxidation of isoprene, aromatic hydrocarbons and their daughter products...The results of this study will produce new observational and modeling data that will greatly expand knowledge of the detailed reaction processes and product production in the atmospheric oxidation of isoprene and aromatic hydrocarbons. These new data will permit the formulation of significantly more accurate photochemical reaction models for use in EPA policy decisions."<sup>24</sup> The results of this EPA-funded study should be made public and used.

Exposure information on isoprene underscores a flaw in the underlying assumption of the HPV program: high production volume does not necessarily translate into high exposure. A study submitted to the EPA's Ozone Transport Assessment Group Air Quality Analysis Workgroup in 1997, measured and modeled isoprene concentration across many sites. In general, results show a broad range of concentrations across sites. Mean observed isoprene ranged from 2.1 ppb-C in Maine to 28.6 ppb in Kentucky. Modeled isoprene concentrations ranged from 4.3 ppb-C in Pennsylvania to 48.2 ppb in Massachusetts.<sup>25</sup>

The American Industrial Hygiene Association recommends that an 8-hr TWA isoprene concentration not exceed 50 ppm. Isoprene is not a persistent environmental chemical; it is expected to have an atmospheric half-life on the order of hours, depending on the atmospheric concentration of hydroxyl radicals.<sup>26</sup>

Because of the widespread levels of isoprene in the atmosphere and the fact that its concentrations are largely affected by local climate and flora, isoprene is ideally suited to a hazard evaluation that uses existing human epidemiological data rather than solely relying on inaccurate animal testing.

## **3. The ACC does not maximize the use of nonanimal tests.**

Photolysis and hydrolysis are not expected to be environmentally significant fate processes in aquatic systems based on the UV spectra and lack of hydrolysable groups. Isoprene is expected to volatilize rapidly from environmental waters. Therefore, no aquatic toxicity tests on animals should be conducted. Only algal toxicity tests and structure activity relationships should be used to predict aquatic toxicity.

## **Summary**

The ACC has developed a costly (both in terms of dollars and animal lives) test plan for C5 compounds that will provide little information to improve our understanding of the toxicity of crude streams. Regardless of the outcome of these tests, the handling and emergency response of industrial streams of isoprene will be unchanged, as we already have an extensive understanding of its effects and its physical and chemical properties. The research on C5 and related compounds has far surpassed efforts at hazard identification and is currently directed toward improving understanding of metabolism, PBPK modeling, and biomarkers.

Given the interspecies variability and the state of the science on these chemicals, we urge the ACC to use the existing biochemical and toxicological information on compounds in these C5 streams rather than its proposed, rote check-the-box toxicity testing plan.

**Table 1. Existing Studies of the Toxicological Mechanisms of Isoprene and Related Compounds**

<b>Author</b>	<b>Reference</b>
Bird MG.	Future Directions—toxicology studies of 1,3-butadiene and isoprene. <i>Environ Health Perspect</i> 1990;86:99-102.
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Buckley LA, Coleman DP, Burgess JP, et al.	Identification of urinary metabolites of isoprene in rats and comparison with mouse urinary metabolites. <i>Drug Metab Dispos</i> 1999;27(7):848-54.
Chiappe C, De Rubertis A, Tinagli V, Amato G, Gervasi PG.	Stereochemical course of the biotransformation of isoprene monoepoxides and of the corresponding diols with liver microsomes from control and induced rats. <i>Chem Res Toxicol</i> 2000;13(9):831-8.
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