

**QUAT HPV CHALLENGE TASK GROUP RESPONSE TO  
EPA COMMENTS ON THE QUAT CATEGORY  
CHALLENGE SUBMISSION**

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The following responds to EPA's comments on the test plan and robust summaries for the "Quat Category," which the QUAT HPV Challenge Task Force submitted to EPA on April 27, 2004, as part of its commitment to the US HPV Challenge Program. For reference purposes, the Quat Category members are listed below:

Chemical Name	Abbreviation	CASRN
<b>Quaternary Ammonium Salts</b>		
Dimethylaminoethyl acrylate methyl chloride	ADAMMC	44992-01-0
Dimethylaminoethyl acrylate dimethyl sulfate	ADAMDMS	13106-44-0
Dimethylaminoethyl methacrylate methyl chloride	MADAMMC	5039-78-1
Dimethylaminoethyl methacrylate dimethyl sulfate	MADAMDMS	6891-44-7
<b>Esters of Acrylic and Methacrylic Acids</b>		
Dimethylaminoethyl acrylate	ADAM	2439-35-2
Dimethylaminoethyl methacrylate	MADAM	2867-47-2

The following text and the accompanying attachments (updated dossiers for ADAMMC, MADAMMC and MADAMDMS) respond to the various comments provided by EPA. For convenience, we have restated the comment from EPA in italics, which is then followed by an indented response.

**1. General**

*The submitter needs to provide a test plan that clearly shows the available data and how data gaps will be filled. The "Test Data" table on p. 5 is misleading, as it implies incorrectly that all endpoints have been satisfied with measured data on each chemical. Mammalian toxicity data available for MADAM were not discussed in the test plan nor compared with the ADAM data. The submitter needs to address all these deficiencies.*

All available MADAM data has been provided in the category test plan document. An updated test plan has been included in the revised test plan document (attached).

**2. Category Definition**

*The category definition is very clearly stated, although the nomenclature is misleading throughout because of improper spacing: for example, ADAMMC is correctly dimethylaminoethyl acrylate methyl chloride, not dimethylaminoethylacrylate methyl chloride.*

Nomenclature issues have been updated.

**3. Category Justification**

*The submitter bases the category on the structural similarity of the compounds and expected resulting similarities in their physicochemical and toxicological properties, as well as on limited toxicological data. The acute oral toxicities (an LD50 of 1600 mg/kg for ADAMMC and an LD50 of 1300 mg/kg for MADAMMC) are similar. Genetic toxicity data provided for three of the four sponsored compounds may support the category with negative responses, although the validity of the negative findings for ADAMMC and MADAMMC can only be verified after the submitter provides additional information on the use of positive controls. The measured ecotoxicity data provided, if confirmed by adequate revised robust summaries, support the submitter's conclusion that the sponsored substances have similar acute fish and invertebrate toxicities. LC50 and EC50 values are >100 mg/L for these two endpoints for all four sponsored substances. The data provided for the algae toxicity endpoint show a pattern of toxicities in which ADAM-based compounds have lower EC50 values than MADAM-based compounds (the submitter's explanation that this difference is associated with the toxicities of the hydrolysis products, acrylic acid and methacrylic acid, is not consistent with the estimated hydrolysis half-lives). Overall, the similar structures and measured data support the grouping of the category members.*

EPA raises the issue of positive controls in the Salmonella/Microsome assays performed on the quats. We have expanded the robust summary to include this information. All positive controls functioned as required by OECD standards.

We were clear in our test plan. We have adequate data for these materials. We have conducted valid tests and have excellent structural analogues which differ from the members of the quat series only by counter ions. The quats are site limited intermediates with very little general exposure. No further testing is needed at this time.

#### **4. Analog Justification**

*ADAM and MADAM, the non-quaternized parent chemicals of the sponsored substances, are proposed as data sources for the category members. However, no direct discussion that supports using these analogs was provided in the test plan; moreover, the submitter failed to use the abundant mammalian toxicity data on ADAM and MADAM to compare the two structure types. These deficiencies need to be addressed. Other information to help evaluate their suitability (e.g., pK values) also could have been provided.*

*The acute oral toxicity values of the category members and analogs are 455 mg/kg or higher. EPA believes that the use of the analogs, which represent both structural types in the category, is consistent with the close structural similarities and the available acute toxicity data. However, as data on the sponsored substances are not available on any repeated-dose mammalian toxicity endpoints to compare with analogs, and structural differences between acrylates and methacrylates may result in different biological reactivities, the mammalian toxicity data for ADAM should only be used to address ADAMMC and ADAMDMS; MADAM data need to be used only for MADAMMC and MADAMDMS.*

EPA correctly states that ADAM can be used as an analogue for ADAMMC and ADAMDMS. However, EPA asserts that only the mammalian toxicology data from MADAM, and not from ADAM, must be used for MADAMMC and MADAMDMS.

The QUAT HPV Committee believes that the data on ADAM is appropriate for use in assessing MADAMMC and MADAMDMS, particularly since there is limited data on MADAM. The literature is filled with examples where fully

substituting the  $\beta$  carbon eliminates toxicity. This substitution represents the difference between the MADAM series and the ADAM series. MADAM is fully substituted and ADAM is not.

We have added to the robust summaries a 3 week study on MADAMMC where the NOAEL was >1000 mg/kg (see Reference 8 in section 5.4 in attached, revised robust summary document). This is consistent with SAR from the literature on  $\beta$  substitution cited above. Use of ADAM as an analogue represents a worst-case scenario. Further testing of these site limited materials for which there is very low exposure, is not warranted based on the 21 day study on MADAMMC and the extensive testing of ADAM, a more toxic congener based on the NOAEL in the 3 week study of >1000 mg/kg. The NOAEL for ADAM over a similar period was 100 mg/kg.

## 5. Test Plan and Robust Summaries

### Physicochemical Properties (melting point, boiling point, vapor pressure, partition coefficient and water solubility)

*The estimated values provided are adequate for the boiling point and the octanol/water partition coefficient for the purposes of the HPV Challenge Program; these data are also needed for MADAMDMS. The estimated values for melting point, vapor pressure, and water solubility are inadequate. Estimated values are adequate only for melting points below 0 °C, vapor pressures below 10<sup>-5</sup> Pa, and water solubilities below 1 ug/L. The submitter needs to provide measured data following OECD guidelines for these endpoints.*

Estimated physical chemical data for MADAMDMS have become available with the release of ECOSAR 3.1 and are included in an updated robust summary. The QUAT HPV Challenge Task Force believes that there is inadequate justification to conduct additional physical property determinations given that these compounds are site limited, and manufactured/used as solutions in water, i.e., they are never present as pure substances. The relevant values for calculated physical parameters cited as criteria by EPA are delineated in the Table below:

End Point	ADAMMC	ADAMDMS	MADAMMC	MADAMDMS
MP (°C)	148.4	211.20	151.8	228.7
VP (Pa)	5.31E-7	3.18E-10	3.03E-7	1.03E-13
Water Solubility (mg/L)	1E06	Complete	Complete	1E06

Based upon EPA criteria that the estimated MP must be <0°C, VP <10<sup>-5</sup> Pa, and water solubility < 1µg/L, none of these materials qualifies for a requirement to develop measured data.

### Environmental Fate (photodegradation, stability in water, biodegradation, fugacity)

Additional information on MADAMDMS has been included in a revised robust summary based on ECOSAR 3.1.

*Photodegradation. The photodegradation data provided by the submitter for ADAMMC, ADAMDMS, and MADAMMC are adequate for the purposes of the HPV Challenge Program. The submitter needs to provide photodegradation data for MADAMDMS.*

Data are provided in the revised robust summary for MADAMDMS.

*Stability in water. The estimated data provided for ADAMMC, ADAMDMS, and MADAMMC are inadequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured hydrolysis data for the chlorides or the methylsulfates following OECD TG 111.*

Since these materials are site limited intermediates manufactured in water solutions, no stability data are needed.

*Biodegradation. The ready biodegradation data provided by the submitter for MADAMMC are adequate for the purposes of the HPV Challenge Program. The inherent biodegradation data provided by the submitter for ADAMMC are inadequate because inherent biodegradation tests allow for bacterial adaptation, which does not provide a conservative picture of the biodegradation of a chemical. The submitter needs to provide measured ready biodegradation data for ADAMMC following OECD TG 301.*

Based on Kow, these materials do not bioaccumulate and do not have significant aquatic toxicity. They are site-limited intermediates. No ready biodegradation assessment is necessary for ADAMMC.

*Transport between environmental compartments (fugacity). The fugacity data provided by the submitter are not adequate for the purposes of the HPV Challenge Program because they are estimated from default values. The submitter needs to calculate the fugacity values for all category members using the appropriate measured physicochemical values as inputs into the model. The use of estimated values introduces uncertainties that then become magnified in modeling applications.*

Based upon the table above, it is unnecessary to conduct laboratory determinations in order to generate the constants necessary to populate the fugacity model with measured values.

#### Ecological Effects (fish, invertebrates, and algae)

*EPA reserves judgement on the adequacy of the data for these effects because the robust summaries lack critical data elements. This information is particularly important because the toxicity of these substances may vary according to the test method used.*

*Summaries need to report critical data elements such as total organic carbon and pH. They also need to (1) clarify whether the substance was neutralized, (2) be corrected for 100% active ingredient, and (3) report the measured concentration of the test substance.*

This information is unavailable; however the Quat HPV Challenge Task Group believes the data provided in the test plan and robust summaries are sufficient.

#### Health Effects (acute toxicity, repeated-dose toxicity, genetic toxicity, and reproductive/developmental toxicity)

*Adequate data are available for the acute, repeated-dose, reproductive, and developmental toxicity endpoints for the purposes of the HPV Challenge Program, provided the concerns expressed under Analog Justification are addressed.*

*Acute Toxicity. The test plan (pages 8 and 9) cited acute oral toxicity test data for MADAMMC. Robust summaries of these data need to be provided.*

The MADAMMC robust summaries have been revised to include acute oral toxicity data under Section 5.1.1 and the test plan has been updated accordingly.

*The summary of the acute oral toxicity test by Collier (1985a) did not specify whether the clinical signs observed were seen in males, females, or both.*

An updated summary for this study is provided in the revised ADAMMC dossier (attached).

*Genetic Toxicity. EPA reserves judgement on the adequacy of the data submitted for the gene mutation (OECD TG 471 and 476) and chromosomal aberration (OECD TG 473) toxicity endpoints on the two sponsored chemicals, ADAMMC and MADAMMC, pending the receipt of revised summaries that provide critical missing details. The test plan (page 9) also cited negative in vitro gene mutation test results for MADAMDMS, but summaries of these studies need to be provided. The additional details for the sponsored chemicals need to be provided even though adequate analog data are available (see below), because adequate data on the sponsored substances are preferred over analog data. The test plan (page 9) reported that the analogs ADAM and MADAM were tested for genetic toxicity both in vitro and in vivo. Although both analogs were reportedly negative in the mouse micronucleus test in vivo, positive results were obtained for gene mutations in the Ames test (ADAM) and for chromosomal aberrations in cultured human lymphocytes (ADAM) and in Chinese hamster cells (MADAM). These data are adequate for the purposes of the HPV Challenge Program. If the additional details requested in the previous paragraph are not provided and these analog data are therefore needed to support this endpoint on the sponsored substances, a discussion of these test results and their comparison with the category members will need to be included in the test plan or final category analysis.*

*Some of the study summaries were missing critical details (i.e., the number of revertants seen at each test concentration, evidence of use of and appropriate response to positive controls, and statistical methods used).*

We have revised Sections 5.5 of the MADAMMC and ADAMMC robust summary documents to contain the requested information.

No in vitro gene mutation data are available for MADAMDMS, as was mistakenly reported in the original category test plan. The test plan has been revised to indicate that no data are available for this endpoint.

*Repeated-Dose, Reproductive, and Developmental Toxicity. The cited OECD TG's 408, 414, and 422 data on ADAM and the OECD TG 422 data for MADAM satisfy these endpoints. Data from the OECD TG 422 study on ADAM showed effects on fetal development (including malformations and variations) and viability, which does not support the submitter's conclusion of no teratogenic effects of ADAM stated on page 4 of the test plan. The test plan discussion needs to accurately reflect the data for this chemical.*

The EPA response discussed the results of the teratology study (OECD 414) conducted on ADAM. In our test plan, we concluded that this study was not indicative of a teratological response:

“In fetal observations: The following were found at 100 mg/kg/day. Twenty-seven/299 fetuses were malformed (14 fetuses from the same litter were dwarf, 13 other fetuses from another litter suffered aphyalangy). Two/144 fetuses were malformed (one fetus had a cleft palate, another fetuses presented hydrocephaly). Additionally, six dwarf fetuses suffered testicular ectopia. Reduced ossification or absence of ossification of many bones (head, vertebrae, sternebrae, limbs and paws) were also found at 30 mg/kg/day. The incidence for the absence of ossification of 6th sternebra was increase at 100 mg/kg/day. The NOAELs for development toxicity/teratogenicity test are considered to be 10 mg/kg/day for embryotoxicity and fetotoxicity, and to be 30 mg/kg/day for teratogenicity.”

Maternal toxicity was observed at 30 mg/kg and the NOAEL in the mothers was 10 mg/kg. Nevertheless, there was no significant increase in terata observed at 30 mg/kg, even though it was a maternally toxic dose. There were terata at 100 mg/kg as the EPA points out, but this was a very toxic dose where 3 out of 25 females (12%) had died. This is consistent with the reproduction study where no reproductive or developmental effects were seen at non-maternally toxic doses.