

201-16105

## DADMAC HPV COMMITTEE

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November 22, 2005

Dr. Oscar Hernandez  
Director, Risk Assessment Division  
Office of Pollution Prevention and Toxics  
US Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, DC 20460  
Via E-mail: [hernandez.oscar@epa.gov](mailto:hernandez.oscar@epa.gov)

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**Re: Response to July 21 EPA Comments on the April 2004 Submission of Test Plan and Robust Summaries for Diallyldimethylammonium chloride (DADMAC), CAS RN 7398-69-8**

Dear Dr. Hernandez:

The DADMAC HPV Committee has reviewed the Agency's July 21, 2005 comments on its test plan and robust summaries for diallyldimethylammonium chloride (DADMAC; CAS RN 7398-69-8), which the Committee submitted to EPA on April 27, 2004. In response, the Committee is respectfully submitting the attached information.

Please do not hesitate to contact me at 202-419-1500 or [rfensterheim@regnet.com](mailto:rfensterheim@regnet.com) if you have any questions.

Sincerely,

Robert J. Fensterheim  
Executive Director

cc: [oppt.ncic@epa.gov](mailto:oppt.ncic@epa.gov)  
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## ATTACHMENT I - DADMAC HPV COMMITTEE - NOVEMBER 22, 2005

**Pollution Prevention (P2) Considerations for Dimethyldiallylammonium chloride****PBT Profiler Estimate = P B T**

Chemicals that are persistent, bioaccumulative, and toxic have the potential to concentrate to levels that may cause significant adverse impact on human health and the environment. Orange or red highlights for the above "PBT Profiler Estimate" indicates the EPA criteria have been exceeded for persistence (P), bioaccumulation (B), or toxicity (T). The PBT Profiler estimates are designed for screening-level assessments to help identify Pollution Prevention (P2) opportunities for chemical substances when no experimental data are available. Experimental data should always be used in preference to the results of the PBT Profiler.

**PBT Profiler Physical/Chemical Property Estimates**

Property	Value	Type	Units
Molecular Weight	161.68		
Melting Point	130	Estimated	degrees C
Vapor Pressure	0.0000035	Estimated	mm Hg at 25 degrees C
Log K <sub>ow</sub>	-2	Estimated	at 25 degrees
Water Solubility	10000	Estimated	mg/L at 25 degrees C
Henry's Law Constant	0.0000000000072	Estimated	atm/m <sup>3</sup> mole at 25 degrees
Hydroxyl Radical Reaction Rate Constant	0.0000000000072	Estimated	cm <sup>3</sup> /molecule-sec at 25 degrees C
Ozone Reaction Rate Constant	Not Estimated		
Ultimate Biodegradation Survey	2.842 ( Weeks )	Estimated	

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DADMAC**Persistence Summary**

**Partitioning:** The PBT Profiler uses three environmental compartments (water, soil, and sediment) to determine the persistence of a chemical in the environment. If released to the environment, Dimethyldiallylammonium chloride is expected to be found predominantly in soil. It is also expected to be found in water, but not in sediment.

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The PBT Profiler does not explicitly consider a chemical's fate in the atmosphere in its persistence estimate. It also does not consider a chemical's potential to enter groundwater. Important P2 considerations in these media may be discussed on a chemical by chemical basis in the sections that follow.

### Transformation and

**Persistence:** The PBT Profiler has estimated that Dimethyldiallylammonium chloride is expected to be found predominantly in soil and its persistence estimate is based on its transformation in this medium. Its half-life in soil, 30 days, does not exceed the EPA criteria. Therefore, Dimethyldiallylammonium chloride is estimated not to be persistent in the environment.

The PBT Profiler calculates a chemical's atmospheric half-life from the estimated gas-phase reaction rate with hydroxyl radicals and ozone. The vapor pressure of Dimethyldiallylammonium chloride, 0.0000035 mm Hg, suggests that it will exist as a particulate in the atmosphere. Since particulates react slower with hydroxyl radicals and ozone (relative to a gas-phase reaction), the atmospheric lifetime of Dimethyldiallylammonium chloride is expected to be longer than that predicted by the PBT Profiler. As a result, the distribution of Dimethyldiallylammonium chloride in the various environment compartments may be different than that predicted by the PBT Profiler. This should be considered when identifying P2 opportunities.

### Pollution Prevention

**Considerations:** The PBT Profiler estimates persistence in sediment by its potential for biodegradation in anaerobic (oxygen free) environment. Dimethyldiallylammonium chloride is not expected to be present in sediment, however, groundwater is also an anaerobic compartment. Chemicals may leach through soil and enter groundwater depending on their physical and chemical properties. The PBT Profiler has estimated that the physical and chemical properties of Dimethyldiallylammonium chloride indicate that it may have the potential to leach through soil and enter groundwater. Pollution Prevention (P2) opportunities for this compound should also consider its potential transport to and persistence in groundwater. The PBT Profiler does not explicitly consider groundwater in its persistence estimate.

### Overall

**Persistence:** The overall persistence is a calculated term that allows the persistence of different chemicals to be compared using a single value. Even though the units of the overall persistence are the same as those used for a chemical's half-life (hrs), these two terms are not inter-convertible. The overall persistence takes into account both a chemical's media-specific half-life as

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well as its rate of transport into (and out of) that compartment. Because the overall persistence takes into account transport, its value will likely be different than any of the media-specific half-lives.

The overall persistence can only be calculated in a mass-balance multimedia model. These models calculate the overall persistence by determining the weighted average of the residence time in each compartment.

The overall persistence for Dimethyldiallylammonium chloride is 29 days using the default emission scenario of the level III multimedia model. The overall persistence using different release scenarios is provided in the following section.

### Release

#### Scenarios:

The PBT Profiler estimates persistence based on a standard release scenario emitting equal amounts to soil, water, and air. A more in-depth P2 assessment may utilize a release scenario that is more representative of an individual chemical's life cycle. This section of the PBT Profiler provides seven different release scenarios to help identify P2 opportunities for Dimethyldiallylammonium chloride. The seven release scenarios are based on a more realistic total release of 300 kg/hr to the environment and not the 1,000 kg/hr shown on the PBT Profiler results page. Since the fugacity model is linear, the percent in each compartment does not change based on the total release to the environment, but only on the relative amount released to air, water, and soil.

The following table provides the percent estimated in each environmental compartment using different release scenarios.

Dimethyldiallylammonium chloride: The media (water, soil, and sediment) the chemical is expected to be found in predominantly (the predominant compartment) is underlined. The color of each estimate indicates if the EPA criteria have been exceeded in that specific medium. Therefore, by determining the color of the underline value in each row, the persistence ranking for each different scenario can be compared directly to the default persistence value, P, estimated by the PBT Profiler.

The overall persistence, P<sub>o</sub> (days), calculated for each release scenario is also provided.

Release to each medium (Kg/hr)			Percent in each medium				P <sub>o</sub>
Air	Water	Soil	Air	Water	Soil	Sed	
100	100	100	0	39	<u>61</u>	0	29
150	0	150	0	24	<u>76</u>	0	33

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300	0	0	0	26	<u>74</u>	0	32
150	150	0	0	<u>51</u>	49	0	27
0	150	150	0	47	<u>53</u>	0	27
0	300	0	0	<u>100</u>	0	0	22
0	0	300	0	22	<u>78</u>	0	33

### **Bioaccumulation Summary**

#### Bioconcentration:

Bioaccumulation is the process by which the chemical concentration in an aquatic organism achieves a level that exceeds that in the water, as a result of chemical uptake through all possible routes of exposure.

Biomagnification, refers to the concentration of a chemical to a level that exceeds that resulting from its diet. Bioaccumulation includes both biomagnification and bioconcentration.

In general, chemicals that have the potential to bioconcentrate also have the potential to bioaccumulate. Since a bioconcentration factor (BCF) in fish can be readily measured in the laboratory and bioaccumulation is much more complicated to determine, the BCF is frequently used to predict the importance of bioaccumulation. The estimated bioconcentration factor (BCF) for Dimethyldiallylammonium chloride, 3.2, does not exceed the EPA bioconcentration criteria.

#### Bioaccumulation

Estimate: The PBT Profiler estimates that Dimethyldiallylammonium chloride is not expected to bioaccumulate in the food chain because it does not exceed the BCF criteria.

### **Toxicity Summary**

#### Fish

#### Chronic

#### Toxicity:

PBT chemicals are those that persist in the environment, bioconcentrate in aquatic organisms, and may bioaccumulate in humans, birds, and wild mammals. Exposure to PBT chemicals will result in chronic exposures which, in turn, leads to chronic toxicity. The PBT Profiler uses an estimated fish chronic toxicity value (ChV) to allow organic chemicals lacking experimental data to be screened for P2 opportunities. A more in-depth P2 assessment requires that the potential toxicity of Dimethyldiallylammonium chloride to other aquatic organisms (and at other duration of exposure) be determined.

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The PBT Profiler estimates that Dimethyldiallylammonium chloride is not chronically toxic to fish. It is important to note that these results do not suggest that Dimethyldiallylammonium chloride will not be toxic to all aquatic organisms. Some aquatic organisms, such as daphnids, may be more sensitive to both acute and chronic exposures to Dimethyldiallylammonium chloride. To help assess the toxicity of Dimethyldiallylammonium chloride to other aquatic organisms, the PBT Profiler provides the complete ECOSARTM estimates for this compound, as discussed below.

### Other Toxicity

**Information:** Unlike persistence and bioaccumulation, there is a wide range of different aquatic toxicity endpoints that may be of concern when assessing a chemical for P2 opportunities. The PBT Profiler determines the fish chronic aquatic toxicity for its toxicity ranking. Endpoints specific to humans, avian and terrestrial species, benthic organisms, and other aquatic animals are not included in the PBT Profiler toxicity ranking that appears on the initial results page. Other endpoints associated with the acute, sub chronic, and chronic toxicity of Dimethyldiallylammonium chloride should be considered for the above organisms in light of its persistence, potential for bioaccumulation, release to the environment, and life cycle when performing an in-depth P2 Assessment.

To help address some of these toxicity issues, the following information may be useful when identifying P2 opportunities for Dimethyldiallylammonium chloride:

- **Complete ECOSARTM Results.** Depending on the structure of Dimethyldiallylammonium chloride, EPA's ECOSARTM estimation program may provide a variety of aquatic toxicity endpoints for a number of different organisms. The PBT Profiler's link to the ECOSARTM results for Dimethyldiallylammonium chloride will open another window and display the estimated results. When investigating P2 opportunities for Dimethyldiallylammonium chloride, its potential toxicity to other aquatic organisms should be considered.
- **EPA Chemical Categories:** In the review of over 20,000 chemicals under the New Chemical Program, the Office of Pollution Prevention and Toxics (OPPT) of EPA has identified a number of chemical classes that may have human health concerns. The PBT Profiler has been programmed to identify chemicals that may belong to one of these chemical categories based on the presence of specific functional groups and features.

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The PBT Profiler has determined that the chemical structure of Dimethyldiallylammonium chloride indicates that it is not a member of one of the 19 EPA chemical categories that may have human health concerns. This indicates that no relationship between the structure of Dimethyldiallylammonium chloride and known human health affects has been developed to date. It should be noted that Dimethyldiallylammonium chloride may be toxic to more sensitive organisms. More information on the Chemical Categories is available on EPA's web site.

**Conditions for *Salmonella Typhimurium* Microsomal Mediated Reverse Mutation Assay**

The request for clarification of the *Salmonella* mutagenicity test and the mouse lymphoma mammalian cell genotoxicity assay was surprising as these tests are standardized. Nonetheless, we have expanded the robust summary for these studies to provide the requested information.

<b>Type</b>	:	Ames test
<b>Reference</b>	:	De Jouffrey (1996a).
<b>System of testing</b>	:	<i>Salmonella</i> microsome plate test.
<b>Test concentration</b>	:	312.5, 626, 1,250, 2,500 and 5,000 $\mu\text{g}/\text{plate}$ in the presence/absence of S9.
<b>Cycotoxic conc.</b>	:	No increase in mutations in presence or absence of S9.
<b>Metabolic activation</b>	:	With and without.
<b>Result</b>	:	Negative.
<b>Method</b>	:	OECD Guidelines for the Testing of Chemicals, Guideline No. 471, May 1983 (revised September, 1995): "Genetic Toxicology: <i>Salmonella Typhimurium</i> Reverse Mutation Assay".
<b>Year</b>	:	1996.
<b>GLP</b>	:	Yes.
<b>Method</b>	:	The day before treatment, tester strains of <i>Salmonella typhimurium</i> , TA1535, TA1537, TA98, TA100 and TA102 were infected into nutrient broth and placed under agitation in an incubator at 37°C for 14 hours.

S9 fraction was purchased from Motox and obtained from the liver of rats treated with Aroclor 1254 (500 mg/kg). S9 mix contained per ml:

5 $\mu\text{moles}$ glucose-6-phosphate	100 $\mu\text{moles}$ sodium phosphate pH 7.4
4 $\mu\text{moles}$ NADP	100 $\mu\text{l}$ S9 fraction
33 $\mu\text{moles}$ KCl	Sterile distilled water to make up to 1 ml
8 $\mu\text{moles}$ $\text{MgCl}_2$	

The experiments were performed according to:

- direct plate incorporation method (preliminary toxicity test, both experiments without S9 mix, first experiment with S9 mix): test substance solution (0.05 to 0.1 ml), S9 mix (0.5 ml) when required and bacterial suspension (0.1 ml) were mixed with 2 ml of overlay agar (containing traces of the relevant amino acid and biotin and maintained at 45°C). After rapid homogenization, the mixture was overlaid onto a Petri plate containing minimum medium.
- pre-incubation method (second experiment with S9 mix): test substance solution (0.05 to 0.1 ml), S9 mix (0.5 ml) and bacterial suspension (0.1 ml) were incubated for 60 minutes at 37°C before adding the overlay agar and pouring onto the surface of a minimum

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agar plate. After 48 to 72 hours of incubation at 37°C, revertants were scored with an automatic counter (Artek counter, model 880, O.S.I., 75015 Paris, France).

In each experiment, the following controls were included using triplicate plates:

- vehicle controls: each bacterial tester strain treated with the vehicle,
- positive controls: each bacterial tester strain treated with appropriate reference mutagens.

The sterility of the S9 mix was checked before the beginning and at the end of each experiment and was found to be satisfactory.

### Result

- Revertant frequencies for all doses of the test compound in all strains with and without S9 approximated or were less than those observed in the concurrent negative control cultures. All positive and negative control values were within acceptable limits.

Tester Strain	Test Substance	Dose µg/plate	S9	Revertants	S9	Revertants
TA 1535	DADMAC	0	-	21	+	21
		312.5	-	22	+	23
		625	-	21	+	16
		1250	-	22	+	23
		2500	-	18	+	19
		5000	-	22	+	18
	NaN <sub>3</sub>	1	-	437	+	
	2AM	2	-		+	342
TA 1537	DADMAC	0	-	10	+	15
		312.5	-	10	+	13
		625	-	12	+	12
		1250	-	11	+	9
		2500	-	12	+	14
		5000	-	14	+	12
	9AA	50	-	427	+	
	2AM	2	-		+	350
TA 98	DADMAC	0	-	26	+	22
		312.5	-	21	+	23
		625	-	27	+	23
		1250	-	26	+	24
		2500	-	22	+	29
		5000	-	22	+	31
	2NF	0.5	-	122	+	
	2NF	0.5	-		+	1874
TA 100	DADMAC	0	-	141	+	118
		312.5	-	128	+	113
		625	-	141	+	124
		1250	-	131	+	110
		2500	-	132	+	120
		5000	-	139	+	107
	NaN <sub>3</sub>	1	-	629	+	
	2AM	2	-		+	1373
TA 102	DADMAC	0	-	308	+	346
		312.5	-	319	+	316

<b>Test substance</b>	: DADMAC (50% solution in water).
<b>Conclusion</b>	: Under the experimental conditions, the test substance (DADMAC) did not show mutagenic activity in this bacterial reverse mutation test of <i>Salmonella typhimurium</i> .
<b>Reliability</b>	: (1) valid without restrictions. Guideline study.
<b>Type</b>	: Ames test
<b>Reference</b>	: San, R. (1991)
<b>System of testing</b>	: Salmonella microsome plate test.
<b>Test concentration</b>	: 167, 500, 1670, 5,000, 7,500, and 10,000 µg/plate in the presence/absence of S9.
<b>Cycotoxic conc.</b>	: No cytotoxicity was observed in this study.
<b>Metabolic activation</b>	: With and without
<b>Result</b>	: Negative.
<b>Method</b>	: As described in Ames (1975) and Maron (1983).
<b>Year</b>	: 1991
<b>GLP</b>	: Yes.
<b>Method</b>	: The test compound was evaluated in triplicate cultures in strains TA1535, TA1537, TA1538, TA98 and TA100 in the presence and absence of S9 at the above doses. (Ames <i>et al</i> , 1975).  Overnight cultures were prepared by removing a colony of tester strain from the appropriate master plate and transferring it to a vessel containing ~50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a resting shaker/incubator at room temperature. The shaker/incubator was programmed to begin shaking at approximately 125 rpm at 37±2 °C approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of approximately 1-2 x 10 <sup>9</sup> cells per milliliter.  Tester strain titers were determined by viable count assays on nutrient agar plates.
<b>Result</b>	: The ration of revertants in treated plates versus controls never exceeded 1.4. No significant increase in mutations either in presence or absence of S9.

Positive Controls and Their Responses

Strain	Act.	Positive Controls see below for CAS No. and grade	Conc Per Plate	Positive Control Responses				
				Plate No.	Rever-tants per plate	Bkgrnd. Bacterial Eval.	Avg. Rever-tants	Std Dev
TA98	+	2-aminoanthracene	0.5 µg	1	889	normal	982	92
				2	1072	normal		
				3	984	normal		
	-	2-nitrofluorene	1.0 µg	1	232	normal	218	12
				2	210	normal		
				3	213	normal		
TA100	+	2-aminoanthracene	0.5 µg	1	2100	normal	1957	125
				2	1870	normal		
				3	1900	normal		
	-	sodium azide	1.0 µg	1	452	normal	473	32
				2	510	normal		
				3	456	normal		
TA1535	+	2-aminoanthracene	0.5 µg	1	87	normal	84	6
				2	77	normal		
				3	87	normal		
	-	sodium azide	1.0 µg	1	373	normal	389	19
				2	410	normal		
				3	385	normal		
TA1537	+	2-aminoanthracene	0.5 µg	1	94	normal	125	32
				2	124	normal		
				3	157	normal		
	-	ICR-191	2.0 µg	1	155	normal	141	12
				2	135	normal		
				3	133	normal		
TA1538	+	2-aminoanthracene	0.5 µg	1	1490	normal	1474	169
				2	1634	normal		
				3	1297	normal		
	-	2-nitrofluorene	1.0 µg	1	381	normal	366	13
				2	357	normal		
				3	361	normal		

Positive Control CAS No. and Grade

Positive Controls	CAS No.	Grade
2-aminoanthracene	613-13-8	practical grade
2-nitrofluorene	607-57-8	98% pure
sodium azide	26628-22-8	practical grade
ICR-191	1707-45-0	95% pure

- Test substance** : DADMAC (solution in water).
- Conclusion** : Under the experimental conditions, the test substance (DADMAC) did not show mutagenic activity in this bacterial reverse mutation test of *Salmonella typhimurium*.
- Reliability** : (1) valid without restrictions.  
Guideline study.

**Mouse Lymphoma**

- Type** : Mammalian cell gene mutation assay.
- Reference** : De Jouffrey (1996b).
- System of testing** : Mouse lymphoma (TK<sup>+/+</sup>) L5178Y cells
- Test concentration** : 625, 1,250, 2,500 and 5000 µg/plate.

- Metabolic activation** : With and without.
- Result** : Negative.
- Method** : OECD Guidelines for the Testing of Chemicals, Number 476, April 4, 1984: "Genetic Toxicology: *In Vitro* Mammalian Cell Gene Mutation Test"
- Year** : 1996.
- GLP** : Yes.
- Method** : After a preliminary toxicity test, DADMAC was tested in 2 independent experiments with and without a metabolic activation system, the S-9 mix, prepared from a rat liver microsomal fraction (S9; final concentration of S9 fraction 2%) for 3 hours at 37°C. S9 mix (5ml) contained 1 ml glucose-6-phosphate (180 mg/ml), 1 ml NADPH (25 mg/ml), 1 ml KCl (150 mM), and 2 ml S9. L1210 cells, originally obtained from through ATCC, were supplied by Dr. Oudelkhim-Diot. Cytotoxicity was then determined using cloning efficiency ( $CE_0$ ) before expression of the mutant phenotype. Cell viability (using clonal efficiency  $CE_2$ ) and number of mutant clones (differentiating small and large colonies) were checked after the expression of the mutant phenotype. The test substance was dissolved in distilled water (500 mg/ml) and tested undiluted. The dose-levels for the positive controls were as follows:
- without S9 Mix; 25µg/ml of methylmethane sulfonate
- with S9 mix; 3µg/ml of cyclophosphamide
- Cells were seeded in 50 ml RPMI 1640 medium containing 10% horse serum, L-glutamate (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), and sodium pyruvate (200 Mg/ml). Cells were incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> /95% air. After 24 hour incubation, cells were removed and counted.
- Approximately 0.5X10<sup>6</sup> cells/ml in 20, ml culture medium with 5% horse serum. Were exposed to the test or control substances in the presence or absence of S9 mix (3 hours @ 37°C).
- Treatment medium was as follows:
- Viability: 1.6 cells/well (one 96 well-well plate per culture=2 plates per dose level) to determine Cytotoxicity using cloning efficiency ( $CE_0$ ). After 12±1 days of incubation at 37°C, clones were counted.
- Mutant plates: 2000 cells/well to select the trifluorothymidine resistant mutant cells ( $CE_{mutant}$ ). After 12 ± 1 days, at 37°C in the presence of 4 µg of trifluorothymidine/ml culture medium, small (<25% of the diameter of the well) and large (>25% of the diameter of the well) clones were counted.
- Result** : The highest concentration applied produced a decrease of cell culture growth and the cell growth observed at the lowest concentration was approximately in the range of the negative control. No precipitation of test article was observed. No substantial and reproducible increase in mutant colony numbers was observed at any valuated concentration neither in the

presence or absence of metabolic activation. Furthermore, there was no indication of a dose-dependant increase in the number of spontaneous mutant colonies in the solvent control. The results of this study are shown below:

Concentration (mg/ml)	S-9	Viability % Survival	Mutant Frequency per 10 <sup>6</sup> Survivors
0.00	-	100	53
	+	100	75
0.625	-	105	66
	+	103	62
1.25	-	74	68
	+	103	80
2.50	-	83	63
	+	103	80
5.00	-	89	57
	+	120	54
MMS	-	50	506
CPA	+	61	853

The material did not significantly increase the mutant frequency in this test.

- Test substance** : DADMAC (50% solution in water)
- Conclusion** : Under the experimental conditions, the test substance (DADMAC) did not show mutagenic activity in mouse lymphoma cells in the presence or absence of S9 metabolic activation.
- Reliability** : (1) valid without restrictions.  
Guideline study.

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DADMAC HPV COMMITTEE RESPONSE TO  
EPA COMMENTS ON THE DIALLYLDIMETHYLAMMONIUM CHLORIDE  
CHALLENGE SUBMISSION

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November 22, 2005

On July 21, 2005, EPA provided comments on the test plan and robust summaries for diallyldimethylammonium chloride (DADMAC, CAS No. 7398-69-8), which the DADMAC HPV Committee submitted to EPA on April 27, 2004, as part of its commitment to the US HPV Challenge Program. The DADMAC HPV Committee was pleased that EPA was generally supportive of the submission and has developed this response to address those issues and deficiencies identified by the Agency.

The following responds 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.

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

*The submitted data for boiling point (estimated value above the 300 °C criterion) and partition coefficient are adequate for the purposes of the HPV Challenge Program.*

*Melting point, vapor pressure and water solubility. The submitter needs to provide measured values for these endpoints because the use of estimated values introduces uncertainties that then become magnified in modeling applications. Estimated values are acceptable only for melting points below 0 °C, vapor pressures below  $10^5$  Pa, and water solubilities below 1 µg/L.*

EPA has requested measured rather than estimated values for melting point, vapor pressure and water solubility given concern that the estimated values introduce uncertainties that can become magnified in modeling applications. After considering the Agency's comments, the DADMAC HPV Committee has arrived at the following conclusions.

**Melting point:** There is no need to conduct a measurement of melting point given that the substance is produced and used in a liquid state.

**Vapor pressure:** DADMAC is a salt and as such would be expected to exhibit a very low vapor pressure due to its ionic nature. This interpretation of physical chemistry is confirmed by EPA modeling. According to EPIWIN, its vapor pressure is estimated to be  $3.53 \times 10^{-6}$  mm Hg at 25°C or  $47 \times 10^{-5}$  Pa.

**Water solubility:** As noted in the robust summaries, DADMAC is completely soluble in water. The DADMAC HPV Committee sees little need to accurately measure the water solubility as it is for all practical purposes infinite.

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

*The submitted data for photodegradation, stability in water, and fugacity are adequate for the purposes of the HPV Challenge Program.*

*Biodegradation. The anaerobic data provided by the submitter are not adequate for the purposes of the HPV Challenge Program. The submitter needs to provide measured aerobic ready biodegradation data following OECD TG 301.*

Based on the EPA PBT Profiler Estimate (see Attachment I), DADMAC is predicted to have a half-life in soil of 30 days. This does not exceed the EPA criteria; therefore it is not expected to be persistent in the environment. Considering this, plus the estimated ultimate biodegradation survey of 2.842 weeks, the Committee feels the material is biodegradable and further testing is unwarranted.

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

*The test plan states that DADMAC production is carried out in a closed system and that exposure to DADMAC is not significant but did not claim closed-system intermediate reduced-testing status. Information on CSI submissions is on the EPA Web site at <http://www.epa.gov/chemrtk/closed9.htm>.*

*The submitted data for acute, repeated-dose, and genetic toxicity endpoints are adequate for the purposes of the HPV Challenge Program. The submitter needs to address deficiencies in the robust summaries.*

*Reproductive/Developmental Toxicity. The submitted data are for a homopolymer of DADMAC (99% polyDADMAC containing approximately 1% DADMAC) and showed no effects. Because the animals were exposed to approximately 100 times less DADMAC—only a few mg/kg/day—the results do not assess its toxicity. The submitter needs to provide reproductive/developmental toxicity data on DADMAC following OECD TG 421.*

**Closed system intermediate:** The DADMAC HPV Committee has reviewed the applicability of EPA's "Guidance for Testing Closed System Intermediates for the HPV Challenge Program." Based on that review, the Committee believes that DADMAC clearly qualifies for reduced testing as a closed system intermediate. There is little doubt that DADMAC is an intermediate chemical as it has no specific end uses in its monomeric form; rather, the compound is polymerized to produce polyDADMAC. According to EPA Guidance, there are two types of intermediates that qualify for reduced testing because of limited potential for exposure:

- a) isolated intermediates which are stored in controlled on-site facilities; and,

- b) isolated intermediates with controlled transport, i.e. to a limited number of locations within the same company or second parties which use the chemical in a controlled way as an intermediate with a well-known technology.

These circumstances directly describe the situation with DADMAC. DADMAC is manufactured in three facilities in the U.S. by three companies: Ciba Specialty Chemicals, Nalco and SNF Inc. These companies are recognized as employing responsible product stewardship and industrial hygiene programs. The production of DADMAC by these companies is conducted in rigorous controlled, closed systems. It is worth noting that some of the controls used are directed at reducing exposure to allyl chloride, a volatile compound that has an OSHA Permissible Exposure Limit of 1 ppm (3 mg/m<sup>3</sup>), which is used in the manufacture of DADMAC. Once the DADMAC is produced, typically it is either:

- immediately polymerized in the same reactor to polyDADMAC;
- isolated (and maintained in a controlled environment) at the same facility in order to remove sodium chloride (a by-product of manufacturing) and then polymerized; or,
- shipped to a different facility owned by the manufacturing company for processing/polymerization.

A relatively small percentage of the DADMAC produced is transported to a small number of facilities (less than 10) that are owned by other large chemical companies with comparable high quality product stewardship/industrial hygiene programs. These companies convert the DADMAC to polyDADMAC. The polymerization of DADMAC to polyDADMAC is similarly conducted in a controlled environment and as such there are essentially no emissions of DADMAC to air, soil or water. The quality of the polyDADMAC is impeded in the presence of the monomer and therefore manufacturers strive for residual monomer levels of < 0.5%.

Employees working in DADMAC production and polymerization facilities wear personal protective equipment including gloves, safety glasses and uniforms that are long-sleeved and laundered. Nonetheless, the potential for worker exposure is considered very low given that DADMAC is a large molecule with a molecular weight of 161.8 and has essentially no volatility since its vapor pressure is  $3.53 \times 10^{-6}$  mm Hg at 25°C. The only potential exposure points are material handling for transportation, sampling or cleaning. It is further worth noting that DADMAC is lipophobic and has a high affinity for water. The octanol/water partition coefficient of DADMAC is so low (-2.49) that there will be no uptake by fauna or flora in the aquatic compartment.

The DADMAC HPV Committee recognizes that, according to EPA's guidance, to be eligible for the reduced testing for closed system intermediates "it is necessary to establish that all sites in the United States manufacture and handle the chemical in a manner consistent with the definition of closed-system intermediate." In order to develop the assurance necessary that DADMAC qualifies for reduced testing, the three member companies of the DADMAC HPV Committee confirmed that controlled environments are maintained by the facilities within their own company as well as the few customer facilities that receive this compound that are under another company's control. If necessary, the specific names and locations of these facilities can be provided to EPA on a confidential basis.

**Reproductive/Developmental Toxicity:** The DADMAC HPV Committee has considered EPA's concerns regarding the adequacy of the available data to address the teratogenic/reproductive effects endpoint. As already noted, DADMAC appears to qualify for the reduced testing requirements for "closed system intermediates under the HPV program." For this reason alone, no additional reproductive effects testing is warranted. Nonetheless, the Committee believes that even if the substance was not a closed system intermediate, the available data should be considered adequate given that:

- there were no histological, clinical, and hematological effects at the LOAEL in the subchronic rat and dog studies and chronic rat study;
- there was no teratogenic or reproductive effects as a result of treating rats with polymers containing DADMAC monomer;
- DADMAC is poorly transported across membranes; and,
- DADMAC is negative in *in vivo* and *in vitro* cytogenicity tests and tests for gene mutations.

These studies have clearly shown that DADMAC at doses of 1.25 mg/kg and lower has no effect on the morphology of reproductive organs (from repeat dose DADMAC studies) or from a 2-generation rat study of polyDADMAC containing 1% DADMAC. A summary of the data to substantiate this position follows at the end of this section.

The DADMAC HPV Committee has also considered the potential for general population exposure to DADMAC. The only possible exposure route appears to be through drinking water given that the major use of polyDADMAC coagulants (> 95%) is in the clarification of drinking water.

Assuming a "worst-case" scenario, at the maximum permitted use level of 10 mg/L and a residual monomer content of 2000 ppm, the amount of DADMAC found in drinking water can be calculated as follows:

Maximum use level of polyDADMAC	: 10 mg/L ( $10^{-6}$ )
Max. DADMAC concentration in polyDADMAC	: 2000 mg/kg ( $2 \times 10^{-3}$ )
Max. DADMAC in drinking water	: $[(2 \times 10^{-3}) \times (10 \times 10^{-6})]$ kg/L $2 \times 10^{-8}$ kg/L 20 $\mu$ g/L
Consumption of drinking water per day	: 2 liters
Worst-case daily exposure to DADMAC	: [(2 liters) X (20 $\mu$ g/L)] 40 $\mu$ g/day

Since polyDADMAC, containing at least 1% DADMAC monomer as residual, resulted in a NOEL of 125 mg/kg bw/day in a 2-generation study rat feeding study for reproductive effects, an implied NOEL for DADMAC monomer of 1.25 mg/kg bw/day can be calculated. For a 60 kg adult, this equates to a daily dose of 75 mg/day (75,000  $\mu$ g/day). Taking into account this worst-case estimate of exposure of 40  $\mu$ g/day, one can clearly see that this value is approximately 2000 times less than the given NOEL.

### **Assessment of reproductive performance**

**Subchronic Dog Study (Monomer):** Groups of 4 male and 4 female beagle dogs were fed diets which provided doses of 0, 50, 200 or 800 mg/kg DADMAC (Tegeris, A. (1976). *DADM: Ninety Day Feeding to Dogs*. Pharmacopathics Research Laboratories, Laurel, MD). The only effect observed in this study was a decrease in body weight gain at the high dose of 800 mg/kg. The NOAEL was 200 mg/kg, which is approximately 100-fold higher than the NOAEL in the rat teratology study. There were no adverse histological effects in males in the testes or accessory sex glands. In females the reproductive tract was normal including ovaries, tubules, etc. This study clearly supports the absence of any adverse reproductive outcomes from DADMAC.

**Subchronic Rat Study (Monomer):** Groups of 15 male and 15 female rats were provided diets which supplied doses of 0, 5, 50 or 500 mg/kg of DADMAC (Sterner, W. (1976). *13 Weeks Oral Toxicity Feeding Study with Monomer in Rats*. International Bioresearch Laboratories, Hanover, Germany). Groups of 5 rats were sacrificed for histopathology at 4, 8, and 13 weeks. No adverse histological observations were reported on any aspect of the reproductive system in either treated males or females. Interestingly, at the end of the study, liver microsomal mixed function oxidase activity was determined *in vivo* via hexobarbitone sleeping time revealing no change in metabolic profile. This is significant as modifying hormone metabolism is a potential site of toxicological action on the reproductive system. Hormone metabolism was not modified based not only on this observation but also on the absence of morphological changes in the reproductive system. The NOAEL in this study was 50 mg/kg and was based on decreased body weight gain.

**Two-Generation Rat Feeding Study (Polymer):** A two-generation reproduction study was carried out on poly-DADMAC in rats. Poly-DADMAC is a water soluble polymer so that the monomer present in this polymer is totally bioavailable. This polymer contained at least 1% DADMAC monomer. The monomer doses were 0.00375, 0.125 and 1.25 mg/kg. The high polymer dose was toxic to the rats, inducing mottled kidneys in the F1 males. There were no adverse reproductive outcomes in this study. This includes number of live births, stillbirths, total litter size, nor any effect on maternal instinct and raising of the pups.

**Mutagenicity (Monomer):** Adverse reproductive outcomes can result from mutagenic responses. Even though there were no adverse reproductive or carcinogenic outcomes, a battery of mutagenicity studies was performed. As expected there were no adverse chromosomal effects from DADMAC treatment and no gene mutations.

**Conclusion:** Rodent studies have clearly shown that DADMAC at doses of 1.25 mg/kg and lower has no effect on the morphology of reproductive organs as observed in subchronic and chronic studies or their function as measured in a 2-generation rat study or cell genotype as measured by genotoxicity studies. There is no reason to elevate the test dose just to fill in a data point. The NOAEL of 1.25 mg/kg provides a substantial margin of safety for DADMAC.

**Teratology:** As stated above, there were no morphological changes in the reproductive system of female rats and dogs administered DADMAC. There were also no adverse reproductive outcomes which would indicate terata in the offsprings. Nevertheless, a teratology study has been conducted on a DADMAC homopolymer (Palmer, K. (1991). *Poly (dimethyl diallyl ammonium chloride) (PDADMAC) - Oral (Gavage) Rat Teratology Study*. Toxicol Laboratories, Ltd., Ledbury, UK.). The polymer contained at least 1% residual DADMAC monomer. There were no adverse effects.

**Rat Teratology Study (Polymer):** Rats were treated by gavage with polyDADMAC containing at least 1% DADMAC for gestational days 6-15. Doses were of 50, 150, 450 and 600 mg/kg (corresponding to doses of 0.5, 1.5, 4.5 and 6.0 mg/kg DADMAC). No adverse effects were observed on maternal clinical signs or body weight. The NOAEL for DADMAC in rats was 6 mg/kg/day. This is 4 times higher than the NOEL accepted for the reproduction study.

**Conclusion:** DADMAC was tested for teratological effects as part of a polymer that was the subject of a rat teratology study. Since exposure to DADMAC would typically be experienced as a residual in a polymer, this is an appropriate study for investigation. At a DADMAC dose of 6 mg/kg, no adverse effects were observed.

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

*EPA agrees with the submitter's proposal to test for these endpoints according to OECD TG's 203, 202, and 201, respectively.*

The DADMAC HPV Committee is currently exploring conducting studies regarding ecological effects as noted in the original test plan submission. The industry is further exploring whether these studies might be available through producers in other parts of the world. At the same time, the Committee has begun to question the appropriateness of conducting additional aquatic toxicity tests in light of the existing data on this material (already provided as part of the initial robust summary submission), as well as the information attached from the EPA PBT profiler (Attachment I); which estimated DADMAC was "not chronically toxic to fish." A decision will be made in the near future and the Committee will notify EPA at that time.

### **Repeated-Dose Toxicity**

*A 13-week repeated-dose toxicity study in dogs omitted details of clinical observations, whether or not ophthalmological examination was performed, frequency of weight measurements, effect on food consumption, hematology and clinical chemistry parameters evaluated, organs weighed at necropsy and organs examined histopathologically.*

A revised robust summary is being prepared and will be submitted in the coming weeks.

### **Genetic Toxicity**

*The in vitro bacterial reverse mutation assays in Salmonella typhimurium were missing details such as culture conditions and the identity of the positive controls and their responses.*

*The in vitro mammalian cell gene mutation test in mouse lymphoma cells was missing study details such as culture conditions and the number of replicates/concentration.*

As suggested, robust summaries for genetic toxicity have been revised to include information on culture conditions, the identity of the positive controls and their responses and the number of replicates/concentration; see Attachment II.