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ROHM AND HAAS COMPANY

TDL-008

March 18, 2004

Mr. Mike Leavitt, Administrator
U.S. Environmental Protection Agency
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right to Know Program

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RE: Revisions/Updates to the Test Plan and IUCLID/Robust Summaries for Primene™ 81-R Amines (C12 to C14 t-alkyl amines; CAS No. 68955-53-3)

Dear Mr. Leavitt:

This letter is submitted by the Rohm and Haas Company in response to comments received from the U.S. Environmental Protection Agency (EPA) dated December 29, 2003 following EPA's review of the Test Plan and IUCLID/Robust Summaries for Primene™ 81-R Amines (C12 to C14 t-alkyl amines; CAS No. 68955-53-3).

Rohm and Haas Company would like to thank the EPA for their careful review of the Test Plan and Robust Summaries. We have provided responses for each of EPA comments. In addition, we have provided a detailed rationale supporting our decision for not conducting a Primene™ 81-R hydrolysis study that was suggested by EPA.

In addition to comments to your points we have included our responses to other agencies.

The modified Test Plan and Robust Summaries (the Export file and the .rtf and .pdf formats), as well as this cover memo are being transmitted electronically via e-mail.

Best regards,

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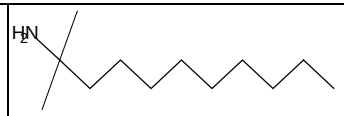
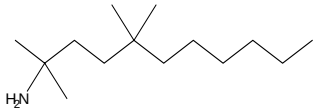
Response to Comments on Chemical RTK HPV Challenge Submission: Primene™ 81-R Amines (CAS No. 68955-53-3)

Table 1. Structure and Corresponding SMILES Notation for the C12-C14 Primene™ Tertiary Alkyl Primary Amines

Primene™ 81-R Amine		
	12Cs, -NH_2	14Cs, -NH_2
SMILES Notation	CC(N)(C)CCCCCCCCC	CCCCCCC(C)(CCC(C)(N)C)C

HYDROWIN was unable to estimate the hydrolysis rate for C12 and C14 Primene™ 81-R Tertiary Alkyl Primary Amines, suggesting their stability.

In summary, the general literature, Rohm and Haas Company experience with the Primene™ Amines and QSAR model estimates suggest that these tertiary alkyl primary amines are hydrolytically stable. As such, the conduct of a hydrolysis study to corroborate the known characteristics of the Primene™ 81-R Tertiary Alkyl Primary Amines would not provide any new information in the context of EPA's RTK Challenge Program.

References

Lyman W.J., Reehl, W.F. and D.H. Rosenblatt. (1982). Handbook of Chemical Property Estimation Methods NY: McGraw-Hill p. 7-4.

Meylan, W.M. and P.H. Howard. 1999a. User's Guide for EPIWIN, EPI suite: EPI-Estimation programs interface for Microsoft Windows. Syracuse Research Corporation, North Syracuse, NY. 33 pp.

Mill, T., Haag, W., Penwell, P., Pettit, T. and H. Johnson. (1987). Environmental Fate and Exposure Studies Development of a PC-SAR for Hydrolysis: Esters, Alkyl Halides and Epoxides". EPA Contract No. 68-02-4254. Menlo Park, CA: SRI International.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Fugacity. The submitter needs to provide the input values used for the calculation of its fugacity model.	See Primene™ 81-R structures and SMILES notation in previous response regarding stability in water and Section 3.3.1, transport between environmental compartments, in the robust summaries. SMILES notation used as an input for EPIWIN and subsequent Mackay Fugacity modeling.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Acute Toxicity. A robust summary for an acute oral toxicity study (Ref. 24) omitted the method for calculating the LD_{50}. In addition there is a discrepancy in LD_{50} values given in the Values field (>500 mg/kg) and those given in the Conclusions field (1177 mg/kg for males and 612 mg/kg for females). Please note that the robust summary for the acute oral toxicity study is actually reference 25.	
Original Text	**Revised Text**
Conclusion: Since there was a statistically significant sex-related difference in mortality observed, the LD50 was calculated separately for males and females. The acute oral LD50 in male rats was 1177 mg/kg with 95% confidence limits of 974 and 1422 mg/kg. The acute oral LD50 in female rats was 612 mg/kg with 95% confidence limits of 442 and 848 mg/kg.	Conclusion: Since there was a statistically significant sex-related difference in mortality observed, the LD50 was calculated separately for males and females. The acute oral LD50 in male rats was 1177 mg/kg with 95% confidence limits of 974 and 1422 mg/kg. The acute oral LD50 in female rats was 612 mg/kg with 95% confidence limits of 442 and 848 mg/kg. The LD_{50} 95% confidence limits were calculated from the logarithm of the doses and the incidence of mortality using the Thompson moving average procedure (W.R. Thompson, "Use of Moving Averages and Interpolation to Estimate Median-Effective Dose", Bacteriol. Rev. 11, pp. 115-145, 1947). Slope estimates were obtained using the methods outlined by Weil (C.S. Weil, "Economical LD50 and Slope Determinations", Drug and Chemical Toxicology 6(6), pp. 595-603, 1983). All calculations were performed using the Statistical Analysis System on an IBM mainframe computer (SAS Institute Inc. SAS User's Guide: Basics, Version 5 Edition, Cary, NC:SAS Institute Inc., 1985).
In addition there is a discrepancy in LD_{50} values given in the Values field (>500 mg/kg) and those given in the Conclusions field (1177 mg/kg for males and 612 mg/kg for females).	LD50 value removed from Values field. See conclusions for study results.
Acute Toxicity. A summary for an acute oral toxicity study (reference 19) incorrectly flagged the study as a critical study, although it assigned Klimisch code 4 (critical studies can only receive a code of 1 or 2).	Deleted "Critical End Use Study" from reference 19. Not cited by EPA, but "Critical End Use Study" deleted from reference 27 (assigned Klimisch code 4) and reference 22 (assigned Klimisch code 3).

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Repeated-Dose Toxicity. The submitter needs to provide the magnitude of the observed reductions in body weight gain in the robust summary for a 4-week inhalation study in rats. **Original Test** - Body weight gain: Statistically significant decreases in body weight occurred in 537 mg/m3 males during week 1, and in females during weeks 1 and 2. Males exposed to 129 mg/m3 showed statistically significant decreases in body weight and body weight change during weeks 2, 3, 4. The females exposed at 129 mg/m3 showed statistically significant decreases in body weight and body weight change during weeks 3 and 4. At 19 mg/m3 statistically significant decreases in body weight and body weight change were seen in the females during week 2. Females in the 2 mg/m3 exposure group showed statistically significant decreases in body weight and body weight change during weeks 2, 3, and 4.	**Revised Text** - Body weight gain: Statistically significant decreases in body weight (15.9 %) and body weight change (25.8 %) occurred in 537 mg/m^3 males during week 1, and in females during weeks 1 (6.4 %; 14.4 %, body weight change) and 2 (10.7 %; 21.6 %, body weight change). Males exposed to 129 mg/m^3 showed statistically significant decreases in body weight (6.8 %, 7.2 %, 6.1 %) and body weight change (11.1 %, 10.8 %, 8.7 %) during weeks 2, 3, and 4, respectively. The females exposed at 129 mg/m^3 showed statistically significant decreases in body weight (5.8 %, 5.2 %) and body weight change (9.6 %, 8.0 %) during weeks 3 and 4, respectively. At 19 mg/m^3 statistically significant decreases in body weight (3.9 %) and body weight change (8.7 %) were seen in the females during week 2. Females in the 2 mg/m^3 exposure group showed statistically significant decreases in body weight (5.6 %, 8.3 %, 9.9 %) and body weight change (11.7 %, 16.3 %, 18.6 %), during weeks 2, 3, and 4, respectively.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
***Genetic Toxicity*.** A robust summary for a positive mutagenicity assay (Ref. 8) omitted information on the cytotoxic concentrations and the concentration at which positive results were observed in TA1535 without metabolic activation.	
Original Test	**Revised Text**
GENOTOXIC EFFECTS: - With metabolic activation: not mutagenic - Without metabolic activation: mutagenic in strain TA1535	GENOTOXIC EFFECTS: - With metabolic activation: not mutagenic - Without metabolic activation: mutagenic in strain TA1535 at 250, 375, and/or 500 ug/plate
CYTOTOXIC CONCENTRATION: - With metabolic activation: mg/plate - Without metabolic activation: mg/plate	CYTOTOXIC CONCENTRATION: - With metabolic activation: cytotoxicity in all strains at 2000 ug/plate; cytotoxicity in TA1535 at 1000 and 5000 ug/plate; cytotoxicity in TA100 at 500 ug/plate - Without metabolic activation: cytotoxicity in all strains at 2000 ug/plate; cytotoxicity in TA1535 at 750 and 5000 ug/plate; cytotoxicity in TA100 at 500 ug/plate
- Positive and negative control groups and treatment: In the presence of metabolic activation, 2-anthramine was used as the positive control, for all strains. In the absence of metabolic activation, the positive controls used were: 2-nitrofluorene (TA98), sodium azide (TA100 and TA1535) and 9-aminoacridine (TA1537).	- Positive and negative control groups and treatment: In the presence of metabolic activation, the positive controls used were: 2-anthramine (TA1537, TA1535, TA100) and 2-acetamidofluorene (TA98). In the absence of metabolic activation, the positive controls used were 2-anthramine (TA1537, TA1535, TA100) and 2-acetamidofluorene (TA98).

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Genetic Toxicity. The robust summary for a negative micronucleus assay in rats lacks information on the number of erythrocytes examined per dose. **Original Test** - Criteria for evaluating results: the test article is considered positive in this assay if it elicits a dose-response or a statistically significant increase in the number of micronucleated cells over that of the concurrent vehicle control at one or more dose levels. In the event that the test article elicits a significant increase in the number of MN-PCE due to an unusually low number of MN-PCE in the concurrent vehicle control, the data from that dose may be compared to historical vehicle control data.	**Revised Text** - Criteria for evaluating results: the test article is considered positive in this assay if it elicits a dose-response or a statistically significant increase in the number of micronucleated cells over that of the concurrent vehicle control at one or more dose levels. In the event that the test article elicits a significant increase in the number of MN-PCE due to an unusually low number of MN-PCE in the concurrent vehicle control, the data from that dose may be compared to historical vehicle control data. For each animal, a total of at least 1000 erythrocytes were recorded to calculate the PCE/NCE ratio. For each animal, the remaining number of polychromatic erythrocytes were recorded to total at least 2000 and were scored for the presence or absence of micronuclei. The frequency of micronucleated polychromatic erythrocytes and the PCE/NCE ratio were calculated on these data.

EPA COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
***Reproductive Toxicity*.** The missing information from the one-generation reproductive toxicity study summary includes exact dosing regimen and the magnitude of body weight reduction in parents and pups.	
Original Text	**Revised Text**
Test condition: Parental rats: 26/sex/dose, 6-7 weeks old at initiation ADMINISTRATION / EXPOSURE - Type of exposure: dietary - Duration of test/exposure: 1 generation - Treatment:: Primene™81-R Amine was administered in the diet to groups of rats at 0, 250, 750, or 1500 ppm.	**Test condition:** Parental rats: 26/sex/dose, 6-7 weeks old at initiation ADMINISTRATION / EXPOSURE - Type of exposure: dietary - Duration of test/exposure: 1 generation - Treatment: – Parental animals were dosed daily seven days per week in the diet beginning approximately six weeks of age. Parental animals were mated after at least ten weeks of exposure to treated diet. Treatment continued throughout gestation, lactation, and until terminal necropsy. F1 animals may also be exposed *in utero* and during lactation via the dam's milk.
- Control group and treatment: yes, Sham control - Vehicle: none - Doses: 0, 250, 750, or 1500 ppm - Concentrations: 0, 19.1, 55.6, 107.3 mg/kg/day (males); 0, 21.0, 62.8, 124.1 mg/kg/day (females) - dietary concentrations	- Control group and treatment: yes, Sham control - Vehicle: none - Doses: 0, 250, 750, or 1500 ppm - Concentrations: 0, 19.1, 55.6, 107.3 mg/kg/day (males); 0, 21.0, 62.8, 124.1 mg/kg/day (females) - dietary concentrations
- Body weight: No body weight effects in parental animals at 250 ppm. Cumulative body weight gain decreased after first week of treatment in males and throughout most of premating period in females at 750 ppm. Decreased mean body weight and cumulative body weight gain during premating period in both sexes at 1500 ppm. No body weight effects on pups at 250 ppm. Decreased mean body weight in pups from postnatal day 4 on at 750 ppm and at all intervals at 1500 ppm.	- Body weight: No body weight effects in parental animals at 250 ppm. Cumulative body weight gain decreased after first week of treatment in males (9%) and throughout most of premating period in females (7-18%) at 750 ppm. Decreased mean body weight (6-12%) and cumulative body weight gain (16-82%) was evident during the premating period in both sexes at 1500 ppm. No body weight effects on pups at 250 ppm. Mean body weight was decreased in pups at 1500 ppm (7-42%) throughout lactation and in pups at 750 ppm (9-12%) from postnatal day 4 through the remainder of lactation period.

ENVIRONMENTAL DEFENSE COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
The robust summary on page 8 states that Primene™ 81-R amine is very toxic to aquatic organisms. However, the test plan characterizes aquatic toxicity as moderate. Since the LC 50's are approximately 1 mg/L, Primene is at least moderately toxic. In any event, the available data on aquatic toxicity exceeds HPV requirements by including data on hatchability and fish behavior. **Original Text** **1.6.2 Classification** **Classified:** as in Directive 67/548/EEC **Class of danger:** other: toxic; corrosive; hazardous to the environment **R-Phrases:** (22) Harmful if swallowed (23/24) Toxic by inhalation and in contact with skin (34) Causes burns (43) May cause sensitization by skin contact (48/23) Toxic: danger of serious damage to health, by prolonged exposure through inhalation **(50/53) Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment** **Source:** Rohm and Haas Company, Spring House, PA, USA	Based on results of acute toxicity testing in aquatic organisms, algae appear to be the most sensitive species (72hr EC50: = 0.2 mg/L – nominal). Additionally, measured aquatic toxicity values for fish and daphnia are in the moderate toxicity category (EC50 > 1.0 mg/L and ≤ 100.0 mg/L). Therefore, the acute aquatic toxicity of Primene™ 81-R may be better stated as ranging from moderate – very toxic to aquatic organisms but the algal value triggers the R-phrase and that cannot be changed. Fish 96hr LC50: = 1.3 mg/L - nominal Daphnia 48hr EC50: = 4.1 mg/L - nominal Algae 72hr EC50: = 0.2 mg/L – nominal

ENVIRONMENTAL DEFENSE COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
The variability of the amine mixture is not well described in the test plan and robust summaries. Information in Sections 1.1-1.4 of the robust summary indicates that over 95% of the mixture is comprised of C10-C14 aliphatic amines. However, no information is provided on the degree of branching or the quantities of possible contaminants such as olefins and formamide. This information needs to be supplied in the robust summaries. Also, if the mixture is variable, the extent and nature of the variability need to be described for studies on separate endpoints.	The Rohm and Haas Company asserts that the variability of the composition of the amine mixture composition of Primene™ 81-R Amine is well described given the nature of the mixture under § 1.1.2: Spectra, of the IUCLID Summary. The NMR analysis states that: The [13]C NMR spectrum shows that Primene™ 81-R Amine consists of a mixture of branched (primary, secondary and tertiary) alkyl amine species. The resonances observed between 55 and ~ 47 ppm are due to primary, secondary and tertiary carbons bearing nitrogen as well as some highly branched alkyl species. Excess overlap of the amine and alkane resonances in the NMR spectra prohibit calculation of the quantity of specific species. The [1]H NMR spectrum confirms that Primene™ 81-R Amine consists of a mixture of branched (primary, secondary and tertiary) alkyl amine species. Since all of the resonances observed are between 1.8 and 0.8 ppm range, this suggests that the amine nitrogen is attached to tertiary alkyl carbons only. The general chemical shifts of protons on a carbon atom attached to nitrogen are 2.5 for methyl, 2.7 for methylene, and 3.1 for methine. The absence of peaks in this region confirms the lack of such amines. Using a GC method of analysis the Primene™ 81-R Amine samples were shown to contain approximately 200 components. Most of the components were C11-C14 isomers of tertiary alkyl primary amines (major C12; 66%). A sample of the Primene 81-R Amine was analyzed via GC/MS. Based on this analysis, it is concluded that the amount of C12 amine in Primene™ 81-R Amine is at least 70% and C12 – 14 t-alkyl amines are at more than 80%. This data was calculated based on the total area of the protonated molecular ion for each amine, and then divided by the sum of the areas for all of the amines. It is also assumed that response factors for all amines are the same and no other components are present in the product. This assumption may not be correct, as other chemical analysis of Primene 81-R have shown the presence of minor amounts of olefins (~1-2%) and N-t-alkyl formamides (~0.5%) in the product. This method cannot detect these components. It is most likely that the low molecular weight amines (C9-11) detected here are either incorrect or at a much lower actual concentration than reported.

ENVIRONMENTAL DEFENSE COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
On page 20 of the robust summary, it is stated that Primene™ 81-R does not partition well into the atmosphere, while on page 21 [*sic 20*] it is stated that the substance easily partitions into the atmosphere. Which statement is correct?	We have clarified the text to state that based on estimates from QSAR models given their accuracy, partitioning into the atmosphere is moderate to low. We agree this section seemed contradictory and tried to clarify. The propensity of Primene™ 81-R Amine to partition into the atmosphere was based on results of the Quantitative Structure Activity Relationship (QSAR) model used to estimate volatility, Henry's Law constant and fugacity as well as the assumed accuracy of the QSAR model. At issue is the interpretation of moderate and low potentials for volatility and partitioning into the atmosphere. The paragraphs below excerpted from the Primene™ 81-R Amine robust summaries/SIAR illustrate the outcomes of the EPIWIN QSAR model (Meylan and Howard, 1999a). *Measured data indicate that that the Primene™ 81-R Amine exhibits moderate volatility, the measured vapor pressure equaling 0.114 and 0.167 mm Hg at 19 and 24°C, respectively. The QSAR estimated Henry's Law constants equal 1.71E-04 and 3.01E-04 atm-m^3/mole for the C_{12} and C_{14} isomers, respectively, indicating that Primene™ 81-R Amine will exhibit a moderate tendency to partition out of the water phase into the atmosphere.* *For the C_{12} and C_{14} isomers, according to the Mackay level III fugacity model (Mackay, Paterson and Shiu, 1992) partitioning into the air would account for a small amount of applied material equaling 1.51 and 0.54%, respectively.* Highly volatile compounds such as methylchloroform exhibit a vapor pressure of 123 torr (mm Hg). Moderately volatile materials such as 2-chlorobiphenyl exhibit a vapor pressure of 0.0084 torr and low volatility materials of which DDT is representative have vapor pressures approximating 1.98E-07 torr. The predicted volatility of the C12 and C14 alkyl amines comprising Primene™ 81-R Amine expressed as vapor pressure falls in the moderate range. From the fugacity estimate the partitioning of the Primene™ 81-R Amine components would be low. The assumed acceptable accuracy of QSAR models is 1 - 2 orders of magnitude. Thus, volatility estimates of Primene™ 81-R Amine at 19°C, for example, could range from 11.4 to 0.00114 torr, i.e., moderate to approaching low. Fugacity estimates could vary similarly from 15.1 – 0.0151% atmospheric partitioning for the C12 isomers. From these data one can draw the conclusion that the volatility and fugacity of Primene™ 81-R Amine could

	range from 'moderate to low' perhaps the qualitative descriptions of both the volatility and fugacity terms could be better characterized as: moderate to low given the assumed accuracy of the model estimates.

ENVIRONMENTAL DEFENSE COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
The acute toxicity and other mammalian toxicity studies demonstrate that Primene™ 81-R is more toxic when administered via the dermal route than the oral route. Is this because of metabolic, toxicokinetic and/or cell sensitivity mechanisms? We commend the sponsor on conducting the mammalian toxicity studies using multiple routes of administration.	Although metabolism and/or toxicokinetic studies have not been conducted with Primene™ 81-R, the corrosivity of the test material may inherently make it more toxic following dermal application.

ENVIRONMENTAL DEFENSE COMMENT(S)	ROHM AND HAAS COMPANY RESPONSE(S)
Neurotoxicity (ataxia, hyperactivity, tremors, etc.) is the most sensitive response in repeat dose studies regardless of the route of administration. Are there available studies that identify specific neurological lesions that might account for these effects?	We have not conducted any special studies to identify neurotoxicity, however, in repeated dosing toxicity studies, no changes were observed microscopically in tissues from the central (brain and spinal cord) and peripheral (sciatic nerve) nervous systems under H and E staining, nor in skeletal muscle corresponding to the behavioral effects noted.