AMINO ALCOHOL

201-16553A

HPV Challenge Program

TEST PLAN
For
Tris(hydroxymethyl)aminomethane (77-86-1)

Submitted to the U.S. Environmental Protection Agency
Under the High Production Volume (HPV) Chemicals Challenge Program

By

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I. INTRODUCTION

The Dow Chemical Company has committed to sponsor TRIS AMINO in the EPA High Production Volume Chemical Program.

II. IDENTIFICATION OF THE SUBSTANCE

For more than 50 years, Tris(hydroxymethyl)aminomethane (TRIS AMINO) CAS# 77-86-1, technically described as 2-Amino-2 (Hydroxymethyl)-1,3-Propanediol, has been used in applications ranging from pharmaceuticals, biological buffers, and transport of live aquatic species, to emulsifying agents in cosmetics. TRIS AMINO is widely known and used as a therapeutic biological buffer, predominantly in patients suffering a decrease in blood pH due to diabetes or a wide array of respiratory diseases and conditions. It is also used clinically as a drug for the treatment of metabolic acidosis where it is injected intravenously or via other routes. It is listed in both the U.S. Pharmacopeia and the National Formulary of drugs for therapeutic drug applications. TRIS AMINO is administered in human patients primarily via the intravenous route regardless of the cause of the acidosis. It has been rigorously evaluated in numerous clinical experiments with animals and humans, and has consistently shown a low incidence of long-term toxicological effects at effective doses with relatively few and minor side-effects. TRIS AMINO is also used in the anti-inflammatory Keterolac® to improve drug solubility. In this form, it has been approved for use in treating acute post-operative pain via an intramuscular injection. Keterolac® has been widely used due to its outstanding pain control properties, and is a nondependency-forming alternative to opiates with a low occurrence of side-effects. TRIS AMINO is also listed in the CTFA International Cosmetic Ingredient Dictionary (page 628, entry name of Tromethamine), reflecting its use as an ingredient in various cosmetic formulations.

Because of its low aquatic toxicity, TRIS AMINO is also an effective buffer used to regulate the pH of the water during the transport of live aquatic species. TRIS AMINO is also widely used as a buffer, solubilizer and neutralizing agent in cosmetic creams, solutions, and lotions, mineral oil and paraffin wax emulsions, polishes and cleaning compounds. Finally, TRIS AMINO is also often used as a buffer in various biological test systems and life science applications such as cell culture media.

III. JUSTIFICATION FOR THE USE OF SURROGATE SUBSTANCES TO SUPPORT THE SUBMISSION FOR TRIS AMINO

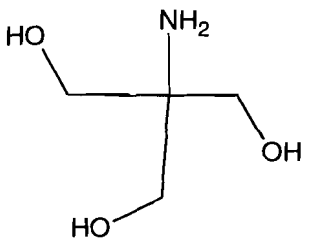
Table 1: Identification of the surrogate substances

CAS No.	Chemical Acronym	Chemical Name
000077-86-1	TRIS AMINO, THAM, TA, TRIS, TROMETHAMINE	Tris (hydroxymethyl) aminomethane (2-Amino-2-hydroxymethyl-1,3-propanediol)
000115-69-5	AMPD	2-Amino-2-methyl-1,3-propanediol
000124-68-5	AMP, AMP-95	2-Amino-2-methyl-1-propanol

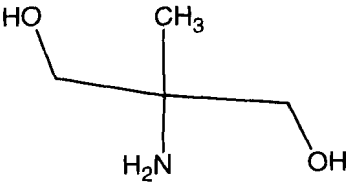
A common mechanism of action, based on structural and chemical similarity, is one of the bases that the EPA has provided under the HPV program to use structurally-similar substances as data surrogates (U.S. EPA, 1999). We propose to utilize surrogate chemicals to fill gaps in our HPV data base for TRIS AMINO.

TRIS AMINO (2-Amino-2-hydroxymethylpropanediol) and the proposed surrogate compounds 2-Amino-2-methyl-1,3-propanediol (AMPD) and 2-Amino-2-methyl-1-propanol (AMP) possess an identical molecular "backbone" structure and common functional groups, forming a continuum from triol to diol to mono-alcohol, respectively. These structures are shown in Figure 1. TRIS AMINO, AMPD and AMP all contain a primary amine connected to a tertiary carbon, with one or more terminal primary alcohol groups

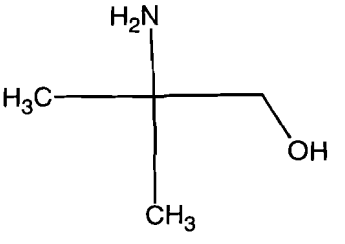
Figure 1. Representative Structures of the Surrogate Substance(s)



TRIS AMINO



AMPD



AMP

Table 2: Chemical Properties of TRIS AMINO and of the Surrogate Substances

Test Chemical	CAS No.	Molecular Wt. g/mol	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (hPa)	Partition Coefficient Log K_{ow}	Water Solubility (g/L)
TRIS AMINO	77-86-1	121.14	171-172	219-220 @ 10 mm Hg	3.04×10^{-6}	-2.31	800
AMPD	115-69-5	105.14	110	151-152 @ 10 mm Hg	8.71×10^{-3}	<-3.8	2500
AMP	124-68-5	89.14	30.5	165.5 @ 760 mm Hg	$4.5.3 \times 10^{-1}$	-0.63	Miscible all proportions

Consistent with the general structural and chemical similarities of TRIS AMINO, AMPD and AMP, studies have demonstrated a similar pattern of physical chemistry, environmental fate, and ecological and mammalian toxicity profiles. The compounds are solid crystalline masses in the pure or neat state, and possess common general physical/chemical properties. Because of their structure, all the substances are highly soluble in water, have very low vapor pressures, possess relatively low partition coefficients (log K_{ow}'s), and similar dissociation constants, making them likely to remain dissolved in the water compartment upon the event of an environmental release, where biodegradation is ultimately expected. MacKay Level III fugacity modeling predicts that TRIS AMINO and the surrogate substances will tend to partition predominately to water Gonsior (2006). There is also a low potential to bioaccumulate in aquatic organisms based on low log K_{ow} values. The high water solubility and negligible vapor pressure of all three substances support the low estimated Henry's Law Constants (4.54×10^{-8} and 6.48×10^{-10} Pa m^3/mol for TRIS AMINO and AMP, respectively, and 8.67 $\times 10^{-13}$atm-m^3/mole for AMPD).

The data on fish, aquatic invertebrate, and algal toxicity, in addition to the use of TRIS AMINO as a buffer for live fish transport, indicate a pattern of low toxicity for this chemical and the surrogate substances. These materials are of low toxicity to aquatic organisms with EC50s greater than 100 mg/L.

Mammalian toxicity studies have displayed similar results. The oral LD_{50} value for TRIS AMINO is 5500 mg/kg in the mouse, and its surrogates range from 2150 to greater than 5000 mg/kg in the rat and mouse. TRIS AMINO was non-irritating to eyes when a 40% aqueous solution was applied to the eyes of rabbits (pH 10.4 for 0.1M aqueous solution). In contrast, 95% AMP in water was severely irritating to the eyes, presumably due to the severely alkaline pH of the test solution used (pH 11.3 for 0.1M aqueous solution); however, more neutral cosmetic formulations containing lower concentrations of AMP are only minimally irritating. There is no sensitization data available for TRIS AMINO; however, based on the following data, TRIS AMINO is not expected to be a sensitizer. Laboratory animal test samples of AMP did not cause allergic skin reactions when tested in guinea pigs following topical or intradermal administration. In patch tests with humans, AMP and cosmetic formulations containing either AMP or AMPD were negative for dermal sensitization.

Repeated-dose mammalian toxicity studies conducted on TRIS AMINO and the two surrogate chemicals indicate that the compounds are generally well-tolerated at concentrations as high as 500 mg/kg/day via IV infusion for TRIS AMINO and ingestion of up to 3200 ppm in the rodent diet (250-750 mg/kg/day for rats and mice, estimated). A number of human clinical trials of the IV infusion of TRIS AMINO have also been successfully conducted. In all studies, the only target tissue, when observed at all, has been the liver with AMP. Human clinical studies with Keterolac® (a major component of which is TRIS AMINO) have suggested that patients with decreased liver function not be given the drug over extended treatment periods based upon changes in several clinical chemistry parameters. Ingestion of relatively high dosages of AMP has caused liver histopathological changes in rats and dogs. The most significant toxicological activity has been a fetotoxic effect of AMP when ingested at relatively high levels by pregnant rats. Subsequent dermal exposure to comparable dosages failed to elicit a developmental effect in rats. Overall, there have been no consistently-noted observations or treatment-related findings among the numerous repeated-dose mammalian toxicity studies that have been conducted over the last 50 years on these compounds that would indicate long-term significant toxicity of either compound at typical human exposure levels. Reflective of these findings is the fact that both TRIS AMINO and AMP display similar patterns of excretion from the body, being

primarily eliminated unchanged via the urine over a relatively short period of time. Further, no evidence of either direct reactivity or metabolism to reactive species toward genetic material has been observed. Genetic toxicity studies conducted on the TRIS AMINO and the surrogate substances in the presence or absence of mammalian metabolic enzymes have all been negative.

Finally, TRIS AMINO and the surrogate chemicals have displayed little if any toxicity to humans during their long history of use as human drugs and/or in personal care products and cosmetics. TRIS AMINO has found use as an IV drug for the management of acidosis in humans for many years and the toxicity of AMPD and AMP have been reviewed by the Cosmetic Ingredient Review Expert Panel which concluded that these materials are safe as used in cosmetic formulations up to 1% (CIR, 1990).

Based upon the properties, uses and toxicities of TRIS AMINO, AMPD and AMP, the use of AMPD and AMP as surrogate substances to fulfill any data gaps for TRIS AMINO is warranted.

IV. DEVELOPMENT OF ROBUST SUMMARIES AND STUDY SCORING CRITERIA

The Dow Chemical Company has chosen to use the IUCLID (International Uniform ChemicaL Information Database) format for preparation of robust summaries for the HPV program. Because many of the fields in the IUCLID database program are outside the scope of the HPV program, these fields are typically left blank in the IUCLID robust summary. Scoring of studies from company files or from the literature for reliability to fulfill the testing requirement for each endpoint used a system similar to that published by Klimisch *et al.* (1997). Studies were given a score of "1" if the data could be considered valid without restriction based on the completeness of the protocol and adequate details in reporting. Studies were given a score of "2" if the data and study design could be considered scientifically valid to address the endpoint but with restrictions due to lack of various technical or reporting details or deviations from current OECD guidelines. Studies were given a score of "3" if their conduct was not acceptable and "4" if there wasn't enough information present to assign a reliability

rating. However, a study receiving a score of "4" could provide supplementary information that could be used to address the endpoint in a weight of evidence evaluation in the absence of other data.

V. TEST ENDPOINT RESULTS FOR TRIS AMINO and SURROGATE SUBSTANCES

A summary of the data on HPV/SIDS endpoints for TRIS AMINO and structurally similar amino alcohols can be found in Table 3: Summary of the Endpoints. Evaluation of this data leads to the conclusions that (1) a substantial quantity of data currently exist to adequately represent the toxicological and ecological profile, (2) there is concurrence and similarity among the existing data for the various HPV/SIDS endpoints, (3) available data from TRIS and the structurally similar substances can be used to adequately represent the various HPV/SIDS endpoints that may not have been subjected to the same level of testing, and (4) utilization of these data support the conclusion that no further testing is needed for most of the HPV/SIDS categories. The data for each of the HPV/SIDS endpoints are discussed in the following section.

A. Melting Point

IUCLID 2.1: TRIS AMINO is crystalline masses in the neat or pure state with melting points of 171-172°C (Merck, 1989). The data suggest that decomposition is unlikely, and because the melting point is well-documented in peer-reviewed literature and public databases, **further testing for this endpoint would not be productive**.

B. Boiling Point

IUCLID 2.2: The boiling point for TRIS AMINO is well documented (Merck, 1989). **No further testing is planned for this endpoint.**

C. Vapor Pressure

IUCLID 2.4: The vapor pressure for TRIS AMINO was estimated by EPIWIN MPBPWIN program, and although estimations are not considered reliable for this category, both the experimental or estimated values for the structurally similar amino alcohols are extremely low at 3.0×10^{-6} (TRIS AMINO) hPa at 20-25°C. The vapor pressure, for the structurally related substances have been well-documented in either

measured reports, published literature or chemical handbooks. This result is consistent with the physical/chemical nature of the amino alcohols and suggests a very low degree of volatility; there is **no further testing planned for this endpoint**.

D. Partition Coefficient

IUCLID 2.5: The value for TRIS AMINO is –2.31(Lhotak, 1996). The partition coefficient is consistent with high water solubility and which by definition would be indicative of low log K_{ow} values. Based on the structure, and the low log K_{ow} values, **no further testing of this endpoint is necessary.**

E. Water Solubility

IUCLID 2.6.1: Measured data indicate that TRIS AMINO is highly soluble to miscible in water. Measured aqueous solubilities of at least 800 g/l at 25 ^{o}C for TRIS AMINO further document its very high water solubility (L. Troester, personal communication, 2006). Furthermore, TRIS AMINO is sold as an aqueous solution, and is formulated as part of the drug Keterolac® to enhance its solubility. Sufficient data exist for this endpoint to characterize water solubility for the TRIS AMINO, such that **no further testing is needed**.

F. Photodegradation

IUCLID 3.1.1: TRIS AMINO does not absorb light >290 nm, and therefore direct photolysis is not possible. Indirect photolysis (hydroxyl radicals) is possible, however. Modeling has shown that the half-life of TRIS AMINO in the atmosphere is approximately 3.8 hours (West, 2004a). Since volatilization is not an important environmental fate process for the surrogate substances, testing for photolysis in the atmosphere is not relevant, and therefore **no further testing for this endpoint is necessary**.

G. Stability in Water (Hydrolysis)

IUCLID 3.1.2: TRIS AMINO does not possess molecular structures that contain functional groups subject to hydrolysis under neutral ambient conditions (Reaction Mechanisms in Environmental Organic Chemistry, 1994). It is commercially available

as an aqueous mixture, further documenting high stability in water, and is reported stable for several years <200°C. This testing endpoint is well characterized, and **no additional testing is required.**

H. Environmental Transport

IUCLID 3.3.1: Environmental transport data were obtained for the TRIS AMINO by estimation using the EPIWIN Level III Fugacity program (Canadian Environmental Modeling Center). Measured water solubility data and the previously-reported values for vapor pressure were used as the model input. The program also used input values for octanol/water partition coefficients, air-water coefficients, melting point and molecular weight that were either calculated by other programs in the EPIWIN suite or used as reported in peer-reviewed literature or databases.

The fugacity mass amount (partitioning) values obtained indicate that distribution into the air would be negligible (<0.1%), as would be expected for primary amino alcohols compounds with low volatility and high solubility in water. Likewise, the distribution to sediment was estimated to be <0.1%. The calculated mass amounts in the soil environmental compartment was <0.1%; the predicted mass amounts distributed to water was 100.0%, a high level that is consistent with the known high water solubility of members of this category (Gonsior, 2006).

Although the values obtained using this model should not be regarded as quantitative, the model results are consistent with the known physical and chemical properties of TRIS AMINO, i.e., the known high water solubility and negligible volatility. **Further testing for this endpoint is not needed.**

I. Biodegradation

IUCLID 3.5: Biodegradation is the conversion of a chemical by microorganisms in the environment into its simpler components and ultimately to carbon dioxide and its other constituent molecules. Chemicals are classified as readily biodegradable by the Organization for Economic Development (OECD) guidelines if there is a 70% degradation of dissolved organic carbon within a 10-day period during a typical 28-day laboratory protocol.

There is a biodegradation study, OECD 301D, for TRIS AMINO which indicates that it is not readily biodegradable (IWL, 1990). There is evidence based on studies with structurally-related compounds to suggest that TRIS AMINO would biodegrade under favorable conditions. There is an OECD 302C study on AMPD which showed 96.7% biodegradation after 22 days. It should therefore be considered inherently biodegradable, which indicates that it would not persist indefinitely in the environment and that it would exhibit similar biodegradation potential based on structure-biodegradability relationships measured for similar compounds (West, 2004b).

These data indicate that there is sufficient information on the biodegradation potential of TRIS AMINO and of the surrogate substances, and justifies that **no additional testing is needed.**

J. Acute Fish Toxicity

IUCLID 4.1: The LC_{50} value of TRIS AMINO for 29 species of fish was reported to be greater than 4400 mg/L when observed for a period of 30 days; it is used as a buffering agent for shipping live fish at dose levels ranging 440-1100 mg/L under aerated or static conditions (McFarland and Norris, 1958). AMP has been evaluated in 96-hour LC_{50} testing with *Lepomis macrochirus*, where the LC_{50} under semi-static conditions was reported to be approximately 190 mg/L (Parekh, 1980a). **No additional testing is required.**

K. Aquatic Invertebrates

IUCLID 4.2: Based on toxicity trends in fish, and comparable physical-chemical properties, TRIS AMINO is expected to be as non-toxic to aquatic invertebrates as AMP. Aquatic invertebrate toxicity data are available for AMP only, and the authors report 48- & 96-hour LC_{50} values for *Crangon crangon* to be 179 mg/L (Young and Tapp, 1983). In *Daphnia magna*, AMP was reported with a 48-hour EC_{50} of 193 mg/L (Parekh, 1980b). **No additional testing is required.**

L. Aquatic Plants

IUCLID 4.3: TRIS AMINO was tested with *Selenastrum Capricornutum*, and a NOEC of >100 mg/L in a 96-hour growth rate test (Adams, *et al.*, 1985). **No additional testing is considered necessary.**

M. Acute Oral Toxicity

IUCLID 5.1.1: Acute oral toxicity studies were conducted with TRIS AMINO using a gastric tube in the Swiss mouse and Wistar rat. Solutions of 20% and 5% were given via the gastric tube to rats and mice respectively at doses of 1000, 2000, and 3000 mg/kg as a solution. The LD_{50} for rats and mice are estimated to be >3000 mg/kg in solution. There was no toxicity noted in either species at the top dose levels, although abundant urine output was noted for some animals (Giroux and Beaulaton, 1962). **Additional testing is not considered necessary**.

N. Acute Inhalation Toxicity

IUCLID 5.1.2: There is no available data on TRIS AMINO or the surrogate substances for this endpoint. There is, however, a low potential for inhalation exposure based on known use patterns, physical state of the pure materials, and vapor pressure, and **therefore testing is considered unnecessary**.

O. Acute Dermal Toxicity

IUCLID 5.1.3: TRIS AMINO has been evaluated in the rat and mouse via intradermal injections, and the LD_{50} for both species exceeded 1000 mg/kg body weight, the highest dose tested. At that dose level, lesions were noted, but no other signs of toxicity were reported (Giroux and Beaulaton, 1962). AMP was evaluated in rabbits via a 24-hour skin patch test, and the LD_{50} was reported greater than 2000 mg/kg (Parekh, 1980c). There were no signs of systemic toxicity, however the treatment sites were necrotic within 2-3 days, and remained so at study termination. Treated groups exhibited a loss in body weight over the 14-day post-treatment period.

No additional testing for this endpoint is considered necessary, as reliable data is available for TRIS AMINO and the surrogate compound AMP.

P. Skin Irritation

IUCLID 5.2.1: Skin irritation tests following the Draize Method have been performed on TRIS AMINO, although documentation is not sufficient for full assessment (reliability 4). It was found to be moderately irritating on abraded rabbit skin, but resolved within 48 hours (Baldwin, 1961). There was no noticeable irritation on unabraded skin sites. In a more reliable study, AMP was found to be irritating to rabbits, with burrowing lesions noted when applied to abraded skin sites (Machle *et al.*, 1940); there was mild irritation noted when applied to unabraded skin. The severity of irritation is directly related to the base strength of the amino alcohol. At 25°C the pKa of TRIS AMINO is 8.03, the pKa of AMPD is 8.76, and the pKa of AMP is 9.72. Since limited data exists for the compound, but exists for the surrogate substances, **additional testing is not considered necessary**.

Q. Eye Irritation

IUCLID 5.2.2: Undiluted TRIS AMINO was found to be essentially non-irritating to rabbits, although documentation is not sufficient for full assessment (reliability 4). Another test conducted on a 40% solution of TRIS AMINO in water has shown this material to be non-irritating to rabbits (Power, 1975). N**o additional testing is necessary.**

R. Skin Sensitization

IUCLID 5.3: While the toxicological/safety profile of TRIS AMINO has been extensively established for many endpoints, especially those associated with its use in the treatment of metabolic acidosis, no laboratory studies of skin sensitization have been reported for this material, and the assessment for dermal sensitization has been based on the favorable use experience associated with various uses, such as applications where it is an ingredient in formulations associated with anticipated dermal contact. In addition, structurally related surrogates AMPD and AMP have been negative in laboratory animal and/or human patch tests for sensitization. AMP did not cause allergic skin reactions when tested in guinea pigs following topical or intradermal administration. In patch tests with humans, cosmetic formulations containing either 0.22% AMP or 0.5% AMPD or 0.075% AMPD were negative for dermal sensitization (Kociba, 2003; CIR, 1990). **Based upon extensive**

clinical use and surrogate related chemical activities no further testing is recommended.

S. Repeated-Dose Toxicity

IUCLID 5.4: TRIS AMINO was administered to various species of animals and humans in multiple studies and trials primarily via the intravenous route, since it is delivered in that manner to human patients suffering acidosis and requiring treatment. The test durations ranged from 10 to 99 days in rats, mice, and rabbits. In all studies, regardless of duration or species, the observations consistently noted were lesions and / or gangrene at the infusion sites, and, in many cases, increases in white blood cell counts associated with gangrene. Sporadic kidney and liver lesions were noted in the longer studies. Because the studies were generally conducted at one dose level (plus control) comparable to human therapeutic doses, no-observed-adverse effect level (NOAEL's) / lowest-observed-adverse effect level (LOAEL's) are not useful to report. It is reasonable to assume, however, that since the studies were conducted under very likely human exposure scenarios, the relative toxicity of TRIS AMINO to humans is low. The assumption of low toxicity is also supported by multiple clinical trials with both healthy and ailing human patients treated with TRIS AMINO at similar dose levels. In these clinical trials, the most common observations included a transient decrease in respiratory rate, an increase in urine output, hyperkalemia, and hypoglycemia. In cases where the dose level far exceeded the recommended therapeutic dose, vomiting, sweating, hunger, dizziness, and diarrhea occurred in a small subset of the test group. There are no documented long-term effects of TRIS AMINO treatment, and no serious side-effects on record that are directly attributed to treatment with the compound.

The surrogate chemical AMP has been more thoroughly tested in mammalian species via more traditional industrial safety and handling toxicity test than TRIS AMINO. The repeated dose toxicity of AMP has been well-studied and documented. These dietary studies ranged from eight weeks to one year in multiple species. The primary treatment-related effect noted has been histopathological changes consisting of

increased liver weights and vacuolation of the liver in rats and dogs (Parekh, 1981; 1981b; Griffin, 1990). **No further testing of TRIS AMINO is recommended.**

T. Genetic Toxicity: Gene Mutations and Chromosome Aberrations

IUCLID 5.5 and 5.6: TRIS AMINO was negative in a gene mutation assay in *Saccromyces cerevisiae*, and negative in the bacterial gene mutation assay with *E.coli* (Livtak and McEvoy, 1990). **No additional testing is considered necessary**.

U. Carcinogenicity

IUCLID 5.7: TRIS AMINO did not induce tumors when tested in male Syrian golden hamsters receiving 0.2 ml of a mixture of Tris buffer and 0.9% NaCl intratracheally into the lungs weekly for life (Ketkar, M. *et al.*, 1979). The long history of safe use of TRIS AMINO as a biological buffer in the treatment of acidosis, its use in Keterolac®, an anti-inflammatory drug, and the compound's use in cosmetics over many years suggest that TRIS is not carcinogenic. Repeated dose toxicity studies of TRIS and the structurally related primary amino alcohol, AMP, do not show any evidence of any preneoplastic lesions, and mutagenicity studies are all negative; a one-year study of AMP in dogs (Griffin, 1990), indicated no evidence of carcinogenicity. **These data suggest that the materials are not carcinogenic. No additional testing is considered necessary.**

V. Reproductive Toxicity

IUCLID 5.8.1: There have been no reproduction studies conducted with TRIS AMINO. Data are available to indirectly evaluate the potential for reproductive effects from exposure via chronic studies that include histological examination of gonadal tissues for evidence of adverse effects. No adverse clinical, histological, or hematological effects were noted in more than a dozen repeated-dose toxicity studies conducted with AMP and AMPD that would indicate toxicity to the reproductive organs. Likewise, there have been no reports of any reproductive effects in the multiple studies conducted on human patients with TRIS AMINO. However, in a recent rat reproductive/developmental screening study, the HCl salt of AMP has been observed to be fetotoxic in rats. An OECD 421 study was conducted using AMP-HCl in which male and female CD rats were fed diets supplying 0 (control), 100, 300, or 1000 mg/kg/day of AMP-HCl (Carney *et al.*, 2005). Males were exposed for at

least two weeks prior to breeding and continuing throughout breeding for 37 days. The females were exposed for two weeks prior to breeding, continuing through breeding (up to two weeks), gestation (three weeks), and lactation (four days). Evidence of complete litter resorption (100% post-implantation loss) was seen at 1000 mg/kg/day, and significant resorptions were seen at 300 mg/kg/day. Effects associated with, or secondary to the post-implantation loss increase at 300 mg/kg/day included decreased litter size, increased pup body weight, and decreased gestation body weight and body weight gain. The no-observed effect level (NOEL) for systemic toxicity in males was 100 mg/kg/day, but could not be established for females due to liver effects in the low dose group; the NOEL for reproductive toxicity was 100 mg/kg/day. There were no treatment related effects on reproductive performance in the 100 mg/kg/day group. The NOEL for general toxicity in males was 300 mg/kg/day, while the general toxicity NOEL for females could not be determined, based upon the presence of very slight microscopic liver effects. The NOEL for reproductive effects was considered to be 100 mg/kg/day.

In light of the history of TRIS AMINO use in pharmaceuticals and no direct evidence of reproductive toxicity associated with TRIS AMINO, we believe that reproduction/developmental toxicity data may exist. We plan to explore this further by filing a Freedom of Information Act (FOIA) request with the US Food and Drug Administration (FDA) to determine if such testing has been conducted. In the event that we do not obtain this data, then we commit to conducting an OECD 421 reproductive and developmental screening study similar to that conducted on AMP to fully evaluate any potential fetotoxicity of TRIS AMINO in rats.

W. Developmental Toxicity

IUCLID 5.82: Data are not available for TRIS AMINO. However, an OECD 414 GLP guideline study on AMP is available. In this study, female rats were exposed 6 hours daily dermally to 0, 30, 100, or 300 mg AMP/kg/day from gestation days (GD) 6-20 (Carney and Thorsrud, 2006). Dermal administration of 300 mg/kg/day of AMP produced significant effects at the test site, as evidenced by scabbing and moderate to severe scaling. The dermal finding of slight scaling at 30 and 100 mg/kg/day was not considered adverse, as the observation was transient in nature and relatively low in incidence. There was no evidence

of test article related systemic maternal or developmental toxicity at any dose level tested. Under the conditions of this study, the NOAEL for maternal toxicity based on dermal effects was 100 mg/kg/day. The NOEL for developmental toxicity was 300 mg/kg/day, the highest dose level tested. While this study is believed to represent a definitive evaluation of potential developmental toxicity for TRIS AMINO in addition to AMP, it is believed that given the extensive use of TRIS AMINO as a therapeutic agent data exist within US FDA archives to address this endpoint (FOIA reference noted in Reproduction Section and below). Should no data be identified, a proposed OECD 421 reproductive and developmental screening study will provide an additional evaluation of any potential for developmental toxicity TRIS AMINO.

VI. CONCLUSIONS

Evaluation of the existing data leads to the conclusions that [1] a substantial quantity of data currently exist to adequately represent the toxicological and ecological screening profile of the category, [2] there is a concurrence and similarity among the existing data for the various HPV/SIDS endpoints [3] available data from previously studied structurally similar primary amino alcohols can be used to adequately represent the majority of the various HPV/SIDS endpoints for the compound that was not subjected to the same level of testing (with the exception of acute inhalation and developmental toxicity), and [4] utilization of these data support the conclusion that no further testing is needed to satisfy endpoints for HPV/SIDS with the exception of Reproductive toxicity. (Table 4: Test Plan Matrix). However, TRIS AMINO has a long history of safe use in pharmaceutical products intended for use internally in humans. We believe that reproduction/developmental toxicity data may exist, so the FOIA inquiry with the FDA will determine if such testing has been conducted. If no data is found we will then commit to conducting an OECD 421 reproductive and developmental screening study. Further, a proposed OECD 421 reproductive/developmental toxicity screening test will provide data on the potential developmental toxicity of TRIS AMINO.

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Table 3: Summary of the Data

		SURROGATE SUBSTANCES	
	TRIS AMINO (77-86-1)	***AMPD (115-69-5)***	**AMP (124-68-5)**
PHYSICAL CHEMISTRY			
Melting point, °C	*171-172*	*110*	*30.5*
Boiling point, °C	*219-220°C @ 133.3hPa 290.9°C @ 1013hPa*	*151-152*	*165°C @ 1013hPa*
Vapor Pressure @ 20°C	*$3.0x10^{-6}$hPa*	*$8.71x10^{-3}$ hPa*	*$4.5.3x10^{-1}$ hPa*
Water Solubility @ 25°C	*800 g/L*	*2500 g/L*	*Miscible all proportions*
Log K_{ow}	*-2.31*	*<-3.8*	*-0.63*
Estimated LogD (log K_{ow} @ pH 7)	*-2.22*	--	*-2.77*
Density	*0.932 g/cm^3 @ 20°C*	---	*0.928 g/cm^3 @ 20°C*
ENVIRONMENTAL FATE			
Biodegradation	*Not inherently*	*inherently*	*Not inherently; does not biodegrade*
Hydrolysis	*Not expected*	*Not expected*	*Not expected*
Photodegradation	*Direct photolysis not expected.*	*Direct photolysis not expected.*	*Direct photolysis not expected.*
Transport between Environmental Compartments: (**Fugacity Level III Model**) Default assumption: 1000 kg/hr released simultaneously into air, water, and soil.	*<0.1% to air 68.6% to water 31.4% to soil <0.1% to sediment*	---	*0.07% to air 73.9% to water 26.0% to soil 0.03% to sediment*
ECOTOXICITY			
Acute Toxicity to Fish (LC_{50})	*Non-toxic to 29 species in concentrations up to 4400 mg/L. Used as a buffering agent at 440-1100 mg/L for shipping live fish*	*96-h static LC50 in zebrafish is >10,000 mg/L*	*Marine LC_{50}(96h)= 184 mg/L in Pleuronectes platessa* *Freshwater LC_{50}(48h) = 331mg/L in Leuciscus idus*

Table 3 (Cont'd): Summary of the Data

		SURROGATE SUBSTANCES	
	TRIS AMINO (77-86-1	***AMPD (115-69-5)***	**AMP (124-68-5)**
ECOTOXICITY (Cont'd)			
Acute Toxicity to Aquatic Invertebrates (48hr LC_{50})	---	---	*LC_{50} = 179 mg/L in Crangon crangon* *EC_{50} = 193 mg/L in Daphnia magna*
Toxicity to Aquatic Plants	*72-hour growth rate NOEC > 200 mg/L; 96-hour growth rate NOEC > 100 mg/L in Selenastrum capricornutum*	---	*72-hour EC_{50} = 520 mg/L in Scenedesmus sp.*
TOXICOLOGICAL DATA			
Acute Toxicity (oral)	*LD_{50} > 3000 mg/kg in rats (20% solution) & mice (5% solution)*	*LD50 was 3500 - 4400 mg/kg in mice* *LDLo=140 mg/kg in mice* *LDLo= 1500 mg/kg in rabbits*	*LD_{50} = 2900 ± 140 mL/kg in rats* *LD_{50} = 2150 mL/kg in mice* *LDlo = 1000-2000 mL/kg in rabbits*
Acute Toxicity (dermal) mg/kg	*LD_{50} > 1000 mg/kg in rats & mice*	---	*LD_{50} > 2000 mg/kg in rabbits*
Acute Toxicity (inhalation)	---	---	---
Acute Toxicity (other routes)	*LD_{50} = 3280-4040 mg/kg in rats via tail vein* *LD_{50} = 6000 mg/kg in rats via tail vein* *LD_{50} = 6100 mg/kg in mice via tail vein*	---	*LC_{50} = 325 mg/kg in mice i.p. dose*
Acute Skin Irritation	*In rabbits, a mild irritant on abraded skin, resolved within 48 hours*	---	*In rabbits, an irritant in abraded (burrowing lesions) or intact (dermatitis) skin.*

Table 3 (Cont'd): Summary of the Data

		SURROGATE SUBSTANCES	
	TRIS AMINO (77-86-1	***AMPD (115-69-5)***	**AMP (124-68-5)**
TOXICOLOGICAL DATA (Cont'd)			
Acute Eye Irritation	*In rabbits, saturated TRIS AMINO solution (40%) is essentially non-irritating.*	---	*In rabbits, vision was destroyed if eyes were flushed or unflushed. Neat AMP is highly irritating likely due to high pH.*
Sensitization	---	*0.1% negative on Guinea Pig*	*Intracutaneous test and a later patch test with guinea pigs found AMP to be a non-sensitizer.*
Repeated Dose Toxicity	*NOAEL = 500 mg/kg of 0.3M THAM in Sprague-Dawley rats for 20 days of infusions.* *NOAEL > 0.5mg/kg of 0.3M THAM for 4 weeks via IV in rabbits*	*20-Day dermal rabbit – No effects*	*LOAEL < 25ppm via diet for 12 weeks in Sprague-Dawley rats (increased liver weights)* *LOAEL~1000ppm via diet for 8 weeks in Sprague-Dawley rats (alopecia & focal skin erosions)* *NOAEL > 3200ppm via diet for 8 weeks in CD-1 mice* *NOAEL = 25ppm via diet for 13 weeks in dogs (clin chemistry & organ weight changes suggesting liver as target organ)* *NOAEL > 110ppm via diet for 1 year in dogs*

Table 3 (Cont'd): Summary of the Data

		SURROGATE SUBSTANCES	
	TRIS AMINO (77-86-1	***AMPD (115-69-5)***	**AMP (124-68-5)**
TOXICOLOGICAL DATA (Cont'd)			
Genetic Toxicity-Mutation	*Bacterial cell gene mutation assay (bacterial strains)- negative*	*Not mutagenic in 4 strains of Salmonella, with or without metabolic activation*	*AMES Test – negative Mammalian cell gene mutation assay – negative Bacterial Reverse Mut. Assay – negative*
Genetic Toxicity-Chromosomal Aberrations	---	---	*Mouse Micronucleus Assay- negative*
Toxicity to Reproduction	---	---	*A 1 year dietary dog study revealed no gross or histopathologic effect on testes, uteri, or ovaries (NOAEL > 110ppm)* *NOEL for general toxicity in male rats was 300 mg/kg/day; the general toxicity NOEL for female rats could not be determined, based upon the presence of very slight microscopic liver effects. NOEL for reproductive effects is 100 mg/kg/day. (OECD 421 study)*
Developmental Toxicity	---	---	*NOAEL for maternal toxicity based on dermal effects was 100 mg/kg/day. The NOEL for developmental toxicity was 300 mg/kg/day, the highest dose level tested. (Developmental study in rats OECD 414)*

Table 4: Test Plan Matrix

	TRIS AMINO (77-86-1)	***AMPD (115-69-5)***	**AMP (124-68-5)**
PHYSICAL CHEMISTRY			
Melting point, °C	*171-172 (measured)* A No Data	*110 (measured)* No Data	*30.5 (measured)* No Data
Boiling point, °C	*219-220 (measured)* A No Data	*151-152 (measured)* No Data	*165 (measured)* No Data
Vapor Pressure @ 20°C	*$3.0x10^{-6}$hPa (calculated)* Calc No Data	*$8.714x10^{-3}$ hPa (calculated)* Calc No Data	*$4.5.3x10^{-1}$ hPa (calculated)* Calc No Data
Water Solubility @ 25°C	*800 g/L (measured)* A No Data	*2500 g/L (measured)* Exp No Data	*Miscible all proportions (measured)* No Data
Log K_{ow}	*-2.31(measured)* A No Data	*<-3.8 (measured)* No Data	*-0.63* Calc No Data
ENVIRONMENTAL FATE			
Biodegradation	*Not Inherently(measured)* A No Data	*Inherently(measured)* No Data	*Not inherently; does not biodegrade Calc* No Data
Hydrolysis	*Not expected* NA No Data	*Not expected* NA No Data	*Not expected* NA No Data
Photodegradation	*Direct photolysis not expected.* NA No Data	*Direct photolysis not expected.* NA No Data	*Direct photolysis not expected.* NA No Data
Transport between Environmental Compartments: (**Fugacity Level III Model**) Default assumption: 1000 kg/hr released simultaneously into air, water, and soil.	*<0.001% to air 68.6% to water 31.4% to soil <0.1% to sediment* Calc No Data	---	*0.07% to air 73.9% to water 26.0% to soil 0.03% to sediment* Calc No Data
ECOTOXICITY			
Acute Toxicity to Fish (LC_{50})	*Non-toxic* A No Data	*LC_{50} (96-h) static in zebrafish is >10,000 mg/L*	*LC_{50}(48h) in Bluegill sunfish* Y
Acute Toxicity to Aquatic Invertebrates (48hr LC_{50})	*R*	*R*	*EC_{50} in Daphnia magna* Y

Table 4 (Cont'd): Test Plan Matrix

	TRIS AMINO (77-86-1)	*AMPD (115-69-5)*	AMP (124-68-5)
ECOTOXICITY (Cont'd)			
Toxicity to Aquatic Plants	*72-hour growth rate NOEC in Selenastrum capricornutum* $A_{\text{No Data}}$	---	*72-hour EC_{50} in Scenedesmus sp.* No Data
TOXICOLOGICAL DATA			
Acute Toxicity (oral)	*LD_{50} in rats* $A_{\text{No Data}}$	*LD_{50} in mice and rats*	*LD_{50} in mice and rats* N
Acute Toxicity (dermal) mg/kg	*LD_{50} in rats & mice* A_N	---	*LD_{50} in rabbits* N
Acute Toxicity (inhalation)	*NA*	*NA*	*NA*
Acute Skin Irritation	*Irritant* A_N	---	*Irritant* N
Acute Eye Irritation	*Non-irritating* $A_{\text{No Data}}$	---	*Highly irritating likely due to high pH.* N
Sensitization	*Non-sensitizer* *R*	*Non-sensitizer*	*Non-sensitizer* N
Repeated Dose Toxicity	*20-day IV in rats* *4-week IV in rabbits* *R*	---	*Diet (12 weeks) in rats* No Data *Diet (8 weeks) in rats* No Data *Diet (8 weeks)in mice* No Data *Diet (13 weeks) in dogs* No Data *Diet (1 year) in dogs* Y N, Y
Carcinogenicity	*Test* *Negative for tumors* *A*		*Negative*
Genetic Toxicity-Mutation	*Negative* A_N	*Negative*	*All negative* Y
Genetic Toxicity-Chromosomal Aberrations	*R*	---	*Negative* Y
Toxicity to Reproduction	*Test (Dependent on FOIA result)*	---	*One year dietary dog study* Y *OECD 421 in rats* Y

Table 4 (Cont'd): Test Plan Matrix

	TRIS AMINO (77-86-1)	***AMPD (115-69-5)***	**AMP (124-68-5)**
TOXICOLOGICAL DATA			
Developmental Toxicity	*Test (Dependent on FOIA result)*	---	*OECD 414 in rats* Y

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using Structurally related information
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
Exp	Endpoint requirement fulfilled via experimentation
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
Y	Yes (GLP)
N	No (GLP)
No Data	No data on GLP status