UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: February 4, 2008

SUBJECT: **Hexahydro-1,3,5-tris(2-Hydroxyethyl)-s-Triazine:** Toxicology Disciplinary Chapter for the Issuance of the Reregistration Eligibility Decision (RED) Document
Case No.: 3074
PC Code: 083301
DP Barcode: 346237

FROM: Jonathan Chen, Ph.D., Toxicologist
Jenny Tao, Toxicologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

TO: Lance Wormell, Chemical Review Manager
Diane Isbell, Team Leader
Mark Hartman, Chief
Regulatory Management Branch II
Antimicrobials Division

THROUGH: Nader Elkassabany, Team Leader
Team Two

And

Norm Cook, Chief
Risk Assessment and Science Support Branch
Antimicrobials Division

Attached is the Toxicology Disciplinary Chapter for hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine for the purpose of issuing a Reregistration Eligibility Decision (RED).

Hexahydro-1,3,5-tris(2-Hydroxyethyl)-s-Triazine

PC Code: 083301

Toxicology Disciplinary Chapter for
the Reregistration Eligibility Decision (RED) Document

2/4/2008

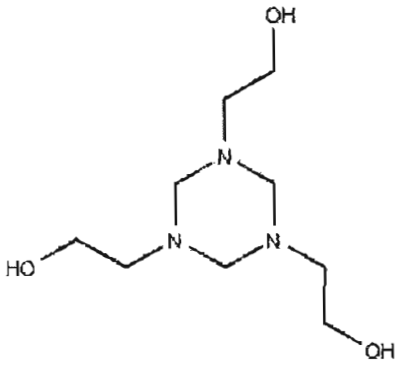
TABLE OF CONTENTS

0.0 BACKGROUND

Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine (HHT; Grotan, Triadine, Proxel, Onyxide, Myacide, Nipacide, and Surcide-P) is a bacteriostat and fungicide. It is used in adhesives, metalworking cutting fluids and chain lubricants, aqueous mineral slurries, paints, stains and coatings (in-can), surfactant/detergent, solutions and emulsions, chemical and clinical reagents, inks and dyes, fuel/oil storage, and construction compounds (caulks, spackling). It is also used in industrial processes and water systems as drilling mud, workover and completion fluids.

The structure of HHT is shown in Table 1 below.

Table 1. Chemical Structure of HHT

PC Code	CAS RN	Name	Structure
083301	4719-04-4	Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine	

1.0 HAZARD CHARACTERIZATION

Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine (HHT) has a moderate order of toxicity via the oral and dermal routes of exposure (Toxicity Category III) and is classified corrosive (Toxicity category I) by primary eye irritation studies. Dermal irritation studies classify HHT as a minimum irritant (Toxicity category IV), and it is not a dermal sensitizer.

In a 13-week oral toxicity study in rats, one male and seven females were found dead during the study period. Inflammatory changes and vascular congestions were presented in the thoracic viscera in these animals. In addition, all decedents from the high dose group showed a white discoloration and prominence of the limiting ridge of the stomach; such lesions were present in all high dose males and in the remaining high dose females which survived the duration of the study. Histopathological examination revealed treatment-related erosion of the glandular mucosa and lymphocytic infiltration in the stomach in both males and females.

Dermal administration of HHT to the intact skin of male and female rats for 21 days produced no mortality, no adverse effects on food or water consumption, or on hematological or serum biochemical parameters. Although body weight gain was reduced in the treated males when compared to the controls, the reduction was equal in all treated groups and no apparent dose-response pattern presented. Food consumption was reduced in all male groups over the course of the study, but more so in the treated animals. Dose-related histopathological changes at treated skin sites included epidermal ulceration, acanthosis and dermal inflammatory infiltration with occasional necrosis and hemorrhage in the treated animals.

Severe dermal irritation was observed in all high dose animals and they were sacrificed on day 36 of a 90-day dermal study in the rat. Dermal symptoms in these animals included erythema, eschar, desquamation, necrosis, exfoliation and ulceration. Females in the high dose group were reported to have the most severe dermal lesions. In these animals, no healing was observed whereas some healing was present in the lower dose groups after the weekends when the test material was not administered. Skin lesions including erythema with eschar, exfoliation, necrosis, desquamation and ulceration were also reported in the lower dose animals and were presented in a dose-response pattern. Histological findings of these dermal lesions revealed hyperplasia, hyperkeratosis, inflammation, sebaceous gland hyperplasia and eschar formation. A dose-related decrease (a linear response to the dose of the test material) in mean body weight was reported in males only and was statistically significant in the high dose group when compared to controls.

In developmental toxicity studies in the rat, maternal body weight gain ($p<0.01$) and food consumption ($p<0.001$) was significantly lower in the high dose females during the dosing period than the controls. Stomach lesions, characterized by ulceration and/or scarring of the mucosa were also observed in the high dose females. There were no any differences between the control and treated dams with respect to pregnancy rates, number of corpora lutea, implantation sites,

number of live fetuses, or early and late resorptions. No abortions or premature deliveries were reported. A statistically significant increase in the incidence of bilateral convoluted ureters and dilated renal pelvises were reported in the high-dose pups and a treatment related trend was observed.

HHT was found to be negative in Ames Salmonella assay and the mouse bone marrow chromosome aberration test, but it was positive for the unscheduled DNA synthesis assay.

There is no available data for reproductive toxicity, chronic toxicity and carcinogenicity for HHT.

2.0 TOXICOLOGY DATA REQUIREMENTS

The available toxicology data for HHT is listed below.

Table 2. Toxicology Data for HHT

Test		Technical		
		MRID	Required	Satisfied
870.1100	Acute Oral Toxicity	41675206 & 00155959	yes	yes
870.1200	Acute Dermal Toxicity	00155984	yes	yes
870.1300	Acute Inhalation	n/a	yes	no
870.2400	Primary Eye Irritation	00155985	yes	yes
870.2500	Primary Dermal Irritation	00155986 & 00155987	yes	yes
870.2600	Dermal Sensitization	00155987	yes	yes
870.3100	90-day oral (Rodent)	41483001	yes	yes
870.3150	90-day oral (Non-rodent)	n/a	yes	no
870.3200	21-day dermal (Rodent)	00155989	yes	no
870.3250	90-Day Dermal (Rat)	41483002 & 41858301	yes	yes
870.3465	90-Day Inhalation (Rat)	n/a	yes	no
870.3700	Developmental Toxicity (Rat)	41161801 & 41865701	yes	yes
870.3700	Developmental Toxicity (Rabbit)	n/a	yes	no
870.3800	Reproduction	n/a	yes	no
870.4100a	Chronic (Rodent)	n/a	yes	no
870.4100b	Chronic (Non-rodent)		yes	no
870.4200a	Carcinogenicity (Rat)		yes	no
870.4200b	Carcinogenicity (Mouse)		yes	no
870.5100	Mutagenicity – Bacterial Reverse Gene Mutation assay	41231702	yes	yes
870.5385	Mutagenicity – Bone marrow chromosome aberration test (Mouse)	41231701 & 41321501	yes	yes
870.5550	Mutagenicity – Unscheduled DNA synthesis in primary rat hepatocytes	41262301 & 43020001	yes	yes

n/a – not available

3.0 DATA GAPS

(1) Acute Inhalation study
(2) 90-day oral toxicity in nonrodents
(3) 90-day inhalation toxicity in rats
(4) Developmental toxicity in nonrodents
(5) 2-generation reproductive toxicity study
(6) Chronic studies and
(7) Cancer studies (Rat and Mouse)

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of Database for Acute Toxicity: The acute toxicity database for HHT is considered incomplete in the absence of acute inhalation exposure data. HHT has a moderate order of toxicity via the oral and dermal routes of exposure (Toxicity Category III) and is classified corrosive (Toxicity category I) by primary eye irritation studies. Dermal irritation studies classify HHT as a minimum irritant (Toxicity category IV) and the chemical is not a dermal sensitizer.

The acute toxicity data for HHT is summarized below in Table 3.

Table 3. Acute Toxicity Data for HHT

Guideline Number	Study Type/Test substance (% a.i.)	MRID Number	Results	Toxicity Category
870.1100 (§81-1)	Acute Oral – Rat Purity 79.4%	41675206	LD_{50} =1250 mg/kg (males) LD_{50} =763 mg/kg (females)	III
870.1100 (§81-1)	Acute Oral – Mouse (Supplemental) Purity 78.5%	00155959	LD_{50} = 1.30 mL/kg	III
870.1200 (§81-2)	Acute Dermal – Rabbit Purity 79.96%	00155984	LD_{50} > 2000 mg/kg	III
870.1300 (§81-3)	Acute Inhalation – Rat	n/a		
870.2400 (§81-4)	Primary Eye Irritation – Rabbit Purity 79.96%	00155985	Corrosive	I
870.2500 (§81-5)	Primary Dermal Irritation –Rabbit Purity 79.96%	00155986	Mild irritant	IV
870.2500 (§81-5)	Primary Dermal Irritation – Guinea pigs Purity 79.96%	00155987	Mild irritant	IV
870.2600 (§81-6)	Dermal Sensitization – Guinea pigs Purity 79.96%	00155987	Not a Sensitizer	N/A

4.2 Subchronic Toxicity

Adequacy of database for Subchronic Toxicity: The database for subchronic toxicity for HHT is considered incomplete in the absence of a 90-day oral toxicity study in non-rodents and a 90-day inhalation toxicity study in rats. The database for subchronic toxicity consists of four guideline studies in the rat.

870.3100 Subchronic (90-day oral) Toxicity – Rat

In a subchronic oral toxicity study (MRID 41483001), groups of Crl:CD(SD)BR rats (10/sex/group) received the test material Grotan (78.5% a.i.) via gavage at doses of 0, 10, 50, 100, or 250 mg/kg/day, 5 days/week for 13 weeks. Body weights and food consumption were recorded weekly. Hematology and clinical chemistry parameters were measured at study termination. Full necropsy was performed on all animals. Complete histopathology was performed on all control and high dose animals.

One male (from group 3) and seven females (one from each of groups 1, 3, and 4 and four from group 5) were found dead during the study period. With the exception of one female from the high dose group, the decedents had gross changes in the lung and/or thoracic cavity. The lungs in these animals did not collapse upon opening the thoracic cavity and adhesions or fluid was present in the area. Inflammatory changes and vascular congestions were present in the thoracic viscera. In addition to the thoracic lesions, all decedents from the high dose group showed a white discoloration and prominence of the limiting ridge of the stomach.

There were significant differences in the body weight gain and food consumption between treated and control rats. With regard to organ weights, absolute adrenal weights were increased for females in group 5; this increase was not significant. In addition, an increase in splenic weight was observed in all treated males. The sponsor attributes this difference to unusually low weights in control animals.

Grossly, a white discoloration and/or prominence of the limiting ridge of the stomach were present in 10/10 high dose males which were sacrificed at the end of the study. This was also present in the remaining high dose females which survived the duration of the study. In animals receiving 100 mg/kg/day, the change occurred in 3/10 males and 1/10 females.

A treatment-related distribution of lesions was in the stomach and to a lesser degree in the liver. In the stomach, erosion of the glandular mucosa was observed in 4/10 males and 2/10 females in the 250 mg/kg group. This finding was also present in one male from group 4 (100 mg/kg). Lymphocytic infiltration was reported in rats from group 4 (1/10 males and 2/10 females) and from group 5 (4/10 males and 6/10 females). Epithelial hyperplasia was reported in three animals from group 5 (1/10 males and 2/10 females) and in the controls (1/10 females), but there was no statistical significance for this finding.

In the liver, the incidence of hepatocyte vacuolation was increased in male rats from group 5 when compared to other groups. On the other hand, margination of hepatocyte cytoplasm was more pronounced in animals from the control groups and from group 2 (10 mg/kg) than in rats from groups 3, 4, and 5.

A high frequency of peribronchiolar lymphoid hyperplasia was reported for all groups. The incidence of this finding ranged from 70 to 100 percent on both sexes. While this finding is not believed to be treatment-related, the sponsor has not addressed its significance.

A statistically significant reduction in hematocrit was found in all treated males. Additionally, males in group 4 demonstrated a statistically-significant decrease in hemoglobin concentration. Although these findings were statistically significant, they were within the expected laboratory range.

Significant differences in blood chemistry values included reduced bilirubin in all treated groups, reduced total protein in both sexes of rats which received 250 mg/kg, and a statistically significant increase in sodium concentration for all treated males and for females in groups 3, 4, and 5. The bilirubin and total protein values were within the normal range and were not considered to be biologically significant.

Urinalysis was unremarkable and no treatment-related ocular lesions were present.

Based on the results of this study, **the systemic NOAEL is 50 mg/kg/day, and the systemic LOAEL is 100 mg/kg/day,** based on gastric lesions (erosion of the mucosa and lymphocytic infiltration) in males and females.

This study is classified as **Minimum.** While not expected to affect the outcome of this study, the sponsor should address the significance of the high incidence of peribronchiolar lymphoid hyperplasia as it related to the overall health of the test animals.

870.3200 Subchronic (21-day dermal) Toxicity – Rat

In a subchronic dermal study (MRID 00155989), Glokill 77 (78.5% a.i., batch TS-704) was administered dermally to groups of Sprague-Dawley rats (5/sex/dose) at concentrations of 0, 100, 500 or 1000 mg/kg/day for 21 days. The test material was used undiluted as supplied. One group served as controls and was treated with sterile distilled water in similar fashion as the test compound-treated animals. Identity and stability of the test material were not determined by the investigating laboratory. The test compound was held in contact with the skin by a porous gauze dressing covered with an Elastoplast elastic adhesive bandage. At the end of each 6-hour exposure period the bandage and dressing were removed and the exposure site washed and dried. The test site of each animal was observed daily for local dermal irritation. Animals were observed daily for toxic signs. Animals dying during the study were subjected to a detailed postmortem examination, and tissues were preserved for histopathological examination. Body

weight was recorded immediately prior to the start of the study, and twice weekly thereafter. Food consumption was recorded weekly. Water consumption was monitored daily. Hematology and serum biochemical determinations were conducted on a separate group of 10 animals (5 male, 5 female) from the same stock prior to start of the study, and on all test and control animals at termination of the study. Determinations were done for hemoglobin, erythrocytes, hematocrit, mean corpuscular volume, leukocytes (total and differential) and prothrombin time. Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was calculated. Blood chemistry values were determined for blood urea nitrogen, total protein, albumin, A/G ratio, creatinine, total bilirubin, alkaline phosphatase, SGPT, SGOT, gamma glutamyltranspeptidase, glucose, calcium, potassium, sodium chloride, and phosphate. At termination of the study, all surviving animals were necropsied and terminal weights taken on liver, kidney, adrenals, and gonads. Histopathology was done on liver, kidney, lymph nodes, skin (treated and untreated) and organs showing lesions or change in size. Skin was examined histologically in all treatment groups, since compound-related changes were observed in the high-dose group.

All animals survived the study. No toxic signs were reported except for local dermal irritation, which was dose-related and increased in severity with the duration of the study. All animals gained weight during the study; however, all groups of treated males gained less weight than corresponding controls. Male weight gain in all groups was approximately 70% of the control male weight gain. The weights observed for all three treatment groups was within the range normally observed in the investigators' laboratory, and therefore were not considered treatment related. Weight gain was comparable in control and treated females.

No difference in food consumption was reported between control and treated males, but during week 1, the mid and high-dose female food consumption was significantly decreased ($p < 0.05$) when compared to the controls. No intergroup difference in water consumption was reported. Mean corpuscular volume was reduced in the low and high-dose males, and hemoglobin was reduced in the low and high-dose females, but the reductions were not considered biologically significant since they were within the normal range of values for Sprague-Dawley rats in the investigators' laboratory.

The following statistically significant changes were noted in the treated animals when compared to the controls ($p < 0.05$): increased protein in mid and high dose males and high dose females, increased sodium in all treated males, increased phosphate in mid and high dose females, increased AST in mid and high dose males, decreased A/G ratio in mid and high dose males and high dose females, and decreased chloride in mid and high dose females. These changes were considered biologically insignificant because the values observed were within the range observed in the animals used in the hematology and biochemical screens. Except for multifocal scab formation at treatment sites at all dose levels, no macroscopic abnormalities were reported. Absolute adrenal weight was significantly decreased in low and high-dose males and was reported to be due to two control animals with high adrenal weights. No difference was reported in relative organ weights. Treatment-related histopathological changes were reported at treated

skin sites, but in no other tissues examined. Histopathological findings were: dose-related epidermal ulceration, acanthosis and dermal inflammatory infiltration with occasional necrosis and hemorrhage in the treated animals.

Based on the dose related epidermal ulceration and dermal inflammation reported at the treated skin sites, **a NOEL for local dermal toxicity was not obtained in this study. The NOEL for systemic toxicity is 1000 mg/kg**, the HDT (highest dose tested).

This study is classified **Minimum**

870.3250 Subchronic (90-day dermal) Toxicity – Rat

In a subchronic dermal study (MRID 41853801), Vanicide TH (96.6% a.i.) was administered dermally to groups of Crl:CD BR (Sprague Dawley) rats at concentrations of 0, 10, 30 or 100 mg/kg/day. Twenty four hours prior to the initial topical application, an area from the shoulders to the lumbar region was clipped on each animal. This clipped area was divided into quadrants. For the remainder of the study, the hair was clipped from this area on Sundays. The test material was applied to approximately 10% of the body surface area in one of the prepared quadrants. Daily applications were rotated from quadrant to quadrant. After the material was applied with a gauze patch, the patch was wrapped with COBAN. Material was left in contact with the skin for 6 hours and afterward, the residual material was removed with a paper towel and was washed with reverse osmosis water. This procedure was repeated for 5 days a week for the next 13 weeks. Animals were checked twice daily from Monday thru Friday for morbidity and mortality and once daily on holidays and weekends. Dermal irritation was observed and evaluated using the method described by Draize. Body weights were recorded on day 0 of the study and on Mondays prior to the administration of the test material. Food consumption was recorded weekly and ophthalmoscopic examinations were conducted prior to the initiation of the study and one week prior to the termination of the study. Hematology and serum chemistry samples were collected on day 31 and at study termination.

Three females died during the study (1 control, 1 mid and 1 high dose). The death in the control animal was believed to be caused by renal and urinary bladder problems. This was confirmed upon gross and histological examination of this animal. Mortalities reported in the mid and high dose groups were believed to be associated with dosing procedures. On day 36 of the study, all high dose animals were sacrificed because of the severity of the dermal irritation. Dermal symptoms in these animals included erythema, eschar, desquamation, necrosis, exfoliation and ulceration. Females in the high dose group were reported to have the most severe dermal lesions. In these animals, no healing was observed after the weekend periods when the test material was not administered. In animals in groups 2 and 3, some healing was present after the weekends. In group 2, slight erythema with eschar was observed. In group 3, severe erythema with eschar was observed. Exfoliation, necrosis, desquamation and ulceration were reported in the mid dose (group 3) animals. In the control group, irritation was minimal. These animals had only a few observations of slight erythema during the 13 weeks.

A dose related decrease in mean body weight was reported in males. A statistically significant decrease (7%) in body weight was reported for males in the high dose group when compared to controls. Although there was no statistical significance in mean body weights in the low and mid dose groups, there appeared to be a linear response to the dose of Vancide when these groups were compared to controls. No differences in mean body weights were reported for females in any of the treated groups when compared to controls.

In the mid and high dose males, mean weekly food consumption was statistically lower than controls on day 14. This was the only reported difference in food consumption reported for males. In females, a statistical significance was reported for animals in the mid and high dose groups on day 35; however, the significance was the result of an increase in food consumption reported for these animals.

Statistically significant differences were reported in the interim evaluation in both sexes of animals in the high dose group for hemoglobin and hematocrit. Red blood cell counts were also lower in the high dose group when compared to controls and platelet counts were higher. In high dose females, white blood cell counts were also higher than controls during the interim evaluation. At the end of the study, no significant differences were reported for any of the hematology parameters. In all treated males, albumen, calcium, total protein, sodium and chloride values were statistically lower than controls at the interim evaluation. Phosphorus values were statistically decreased in group 3 males. Potassium, AST and ALT values were lower than controls in group 4 animals. In females, significant differences reported for albumen, BUN calcium, glucose, total protein, sodium, chloride and potassium during the interim. Most of these values were higher than controls with the exception of albumen and total protein in high dose animals. Terminal serum chemistry values revealed lower albumen, sodium, calcium and total protein values in group 2 and 3 males. Group 2 males also had lower chloride and potassium levels when compared to controls. In females in group 3, phosphorus was increased and bilirubin and cholesterol were decreased when compared to controls. In group 2 females, decreases in total protein and cholesterol were reported.

No significant differences were reported for organ weights. Relative increases were reported for liver and lung weights in females without statistical significance when compared to controls. Histologically, there were no treatment related lesions reported with the exception of those reported for the skin. In the skin, there was an increased incidence in hyperplasia and hyperkeratosis of the epidermis of the skin in both sexes. Sebaceous gland hyperplasia and denial inflammatory cell infiltrations were also increased; other lesions included eschar formation, focal dermal fibrosis, focal epidermal necrosis and focal sebaceous gland necrosis.

Based on the results of this study the **dermal NOEL was < 10 mg/kg.** At the lowest dose tested there was dermal irritation and histological dermal lesions characterized by hyperplasia, hyperkeratosis, inflammation, sebaceous gland hyperplasia and eschar formation. Observations made with regard to serum chemistry and hematology values in both sexes appear

to be incidental findings that are not biologically significant. No significant differences were reported for the hematology values at the end of the study and the serum chemistry values that were statistically significant at both the interim and terminal evaluations, were within the range of normal values. The **systemic NOEL was 30 mg/kg and the systemic LOEL was 100 mg/kg** based on severe dermal irritation in both sexes and decreased body weight reported in high dose males. At the highest dose tested, both sexes of animals had to be sacrificed on day 36 due to the severity of the dermal lesions.

This study is classified **Minimum**

870.3250 Subchronic (90-day dermal) Toxicity – Rat

In a subchronic dermal study (MRID 41483002), groups of Crl:CD(SD)BR rats (10/sex/group) received the test material Grotan (78.5% a.i.) to the clipped dorsal area at doses of 0, 5, 50, or 250 mg/kg/day 6 hours/day, 5 days/week for 13 weeks. During the exposure period, the application sites were covered with semi-occlusive bandages and animals were fitted with Elizabethan collars to minimize ingestion of the test chemical. The test site was not washed between doses. Hematology and clinical chemistry parameters were measured at study termination. Full necropsy was performed on all animals. Complete histopathology was performed on all control and high dose animals. Normal and treated skin from all animals in all dose groups was examined histologically.

No deaths were reported during the study. The clinical signs observed during the study included periorbital, perianal, and cranial fur staining in all groups; yellow staining at the applications site in animals from groups 2 and 3; and skin reactions at the application site in animals treated with Triazine at doses of 50 and 250 mg/kg/day. Occasional incidences of erythema were observed in female rats which received 5 mg/kg/day.

In assessing the severity of the dermal lesions, erythema was scored as very slight to severe in both groups 3 and 4. An increase in the numbers of animals affected and an increase in the severity of the reported reactions occurred in group 4. Edema was present in animals in group 4 and was characterized as being very severe to moderate. In group 3, there were only isolated incidences of edema reported.

Statistically significant differences were found in blood chemistry values from individual animals. In group 2 the parameters included one male with increased alanine aminotransferase, one female with increased phosphorous and one female with increased chloride. In group 3, blood chemistry parameters were increased in two animals. One female in this group had increased sodium and one male had increased chlorine levels. In group 4, one female had elevated glucose levels and one male had elevated sodium levels.

Microscopically, there was an increase in the incidence of epidermal hyperplasia and ulceration of the treated area of the skin in animals treated with 50 or 250 mg/kg/day. Eighty to 100 percent

of both the treated and control animals were found to have peribronchiolar lymphoid hyperplasia in the lungs. The significance of the lesions was not discussed.

Based on the results of this study, **the dermal NOAEL was 5 mg/kg/day and the LOAEL was 50 mg/kg/day,** based on skin lesions observed at this level. **The systemic NOAEL was found to be greater than 250 mg/kg/day.**

This study is classified as **Minimum.**

4.3 Prenatal Developmental Toxicity

Adequacy of database for Prenatal Developmental Toxicity: The database for prenatal developmental toxicity is considered incomplete with no studies in rabbits being available. The database consists of two studies in the rat.

870.3700a Prenatal Developmental Toxicity (Gavage) Study – Rat

In a developmental toxicity study in rats (MRID 41161801), artificially inseminated female Sprague-Dawley rats (24/group) were administered Grotan (78.5%) by gavage at doses of 0, 250, 500, and 750 mg/kg/day in deionized water on gestation days 6 through 15.

One high-dose dam was sacrificed on GD 14. Prior to sacrifice on that day the animal exhibited labored breathing, rales, emaciation, piloerection, red staining around the mouth and nose and pale extremities. All other animals survived the study.

High dose females exhibited post dosing salivation. Rales, labored breathing, wheezing, and tachypnea were observed occasionally in the mid and high dose groups toward the end of the dosing period. No other clinical signs were reported.

Maternal body weight gain ($p<0.01$) and food consumption ($p<0.001$) was significantly lower in the high dose females during the dosing period than the controls.
Stomach lesions, characterized by ulceration and/or scarring of the mucosa were observed in 14 of 20 high dose females, including the dam sacrificed on day 14. No gross abnormalities were reported in the other dosage groups.

The data did not demonstrate any differences between the control and treated dams with respect to pregnancy rates, number of corpora lutea, implantation sites, number of live fetuses, or early and late resorptions. There were no abortions and no premature deliveries. In addition, those dosage levels did not produce any developmental toxicity as measured by fetal pup weight, external, or visceral, abnormalities. There were increased incidences of vestigial 14^{th} ribs and retarded ossification of the vertebral thoracic centra which appeared to be dose-related. However, since they were not statistically significant, and the incidence of these abnormalities is highly variable in rats, they are not considered treatment-related.

The maternal NOAEL is 500 mg/kg/day and the LOAEL is 750 mg/kg/day, based on decreased body weight gain, ulcerations and/or scarring of the stomach mucosa. **The Developmental toxicity NOAEL is greater than or equal to 750 mg/kg/day.**

This study is classified as **Minimum.**

870.3700a Prenatal Developmental Toxicity Study – Rat

In a developmental toxicity study in rats (MRID 41865701), Vanicide TH (96.6% a.i.) was administered via gavage to groups of female Crl:CD BR Sprague-Dawley rats (25/dose) at concentrations of 0, 10, 75 or 150 mg/kg/day from day 6 through 15 of gestation. Animals were checked twice daily for abnormalities and mortalities. Body weights and food consumption were recorded prior to selection and on days 0, 6, 9, 12, 15, 18 and 21 of gestation. Clinical signs were observed for daily and dams were sacrificed on day 21 of gestation by carbon dioxide asphyxiation. At sacrifice, uterine weights were recorded and their contents examined. The number of implants, live and dead fetuses, malformed fetuses, corpora lutea as well as late and early resorptions were observed for and recorded.

Live fetuses were euthanized by injection, weighed, their sex determined and observed for gross abnormalities. Half of the euthanized fetuses were utilized for skeletal observations while the rest were used to make visceral determinations.

Mortality was observed in the control (1/25), mid-dose (2/25) and high-dose (6/25) groups but these were thought to be associated with incorrect gavaging technique.

Body weight gain was significantly reduced in the high-dose group (39%) when compared with the control animals. Food consumption was higher for the low-dose and mid-dose groups through the dosing period and for the low-doe group during the post dosing period.

No treatment related lesions were observed in the maternal animals at sacrifice. Lesions observed in the cervical lymph nodes, liver, lung, kidney, skin and fur were reported as either single observances or at incidences that would not suggest an association with the compound.

Post implantation losses were higher in the treatment groups as compared with the controls but only the low-dose group was statistically significant.

No differences were reported for fetal body weights when control groups were compared with the treatment animals. External variations reported included protruding tongue; swollen hind paws with hematoma and stunted fetuses but there was no statistical significance in the treatment groups for the above mentioned observations. Malformations such as encephaly, micrognathia, omphalocele, and thread like tail were observed in the control, low-dose and mid-dose groups. No such malformations were seen in the high-dose group. An increase in the incidence of

hypoplastic sternebrae was reported for animals in the low- and mid-dose groups. There was a statistically significant increase in both the fetal and litter incidence of this anomaly. The fetal incidence of unossified sternebrae was statistically increased in the mid-dose group when compared to controls but this could be an incidental finding. Visceral variations were observed for all groups with dilated renal pelvises and convoluted and/or distended ureters being the most common variations observed. There was a statistically significant increase in this observation in the high-dose pups and a treatment related trend was observed for the occurrence of dilated renal pelvises and for bilateral convoluted ureters.

Based on the results of this study, the **maternal NOAEL was 75 mg/kg/day and LOAEL was 150 mg/kg/day**, based on decreased body weight gain. The **developmental NOAEL was <10 mg/kg/day**, based on the observed significant positive trend in the incidence of bilateral convoluted ureters and dilated renal pelvises.

This study is classified **Supplementary**. However, historical control data should have been provided to determine whether the renal observations in the pups were treatment-related.

4.4 Reproductive Toxicity

Adequacy of database for Reproductive: The database for reproductive toxicity of HHT is considered incomplete with no available data.

4.5 Chronic Toxicity

Adequacy of database for Chronic Toxicity: The database for chronic toxicity of HHT is considered incomplete with no available data.

4.6 Carcinogenicity

Adequacy of database for Carcinogenicity: The database for carcinogenicity of HHT is considered incomplete with no available data.

4.7 Mutagenicity

Adequacy of database for Mutagenicity Toxicity: The database for mutagenicity is considered complete with HHT being tested in the Ames Salmonella assay, the mouse bone marrow chromosome aberration test, and in the unscheduled DNA synthesis assay.

In a bacterial reverse gene mutation test (Ames) (MRID 41231702), following a range-finding toxicity testing with TA98 (only) at doses up to 5000 ug/plate, triplicate cultures of all five Ames strains (TA1535, TA1537, TA1538, TA98, TA100) were exposed to Triazine (78.5%) for 72 hours (extended from the customary 48 hours because triazine is bacteriostatic, thus slowing growth of revertent colonies) at each of five dose levels (0.32, 1.6, 8, 40, and 200 ug/ plate), both

in the absence and presence of a mammalian metabolic activation system consisting of the liver microsomal fraction (S9) prepared from male Fischer 344 rats induced by Aroclor 1254 and containing 44 mg protein per mL, plus generating cofactors.

In addition to solvent controls (DW), bacterial strains were exposed to their selectively appropriate active mutagens in the absence of activation (Sodium azide, 1 ug/plate for TA1535 and TA100; 9-amino-acridine, 50 ug/plate for TA1537; 2-nitrofluorene, 0.5 ug/plate for TA1538 and TA98), but all to 2-aminoanthracene (2-AA, 2 ug/plate) under activation, thus serving as positive controls. The entire experiment was repeated.

The number of revertent colonies per plate was counted electronically (Biotran III), treatment group means and standard deviations calculated, and resulting data statistically analyzed by ANOVA (F-statistic). If F was significant ($p < 0.05$) and dose-responsiveness apparent, a correlation coefficient was calculated over the response range, and its significance read from standard (published) tables. It's considered a substance positive if it produces a reproducibly statistically significant increase in the mean number of revertents that exceeds twice the concurrent solvent control, plus evidence for a dose- response. Plates showing evidence of severe toxicity (markedly reduced or absent background bacterial lawn) were excluded from analysis, since these may yield significantly decreased revertent colony counts (of no relevance to mutagenic activity), or increased colonies from surviving non-revertant cells incorporating the histidine available because of (toxic) lysis (i.e., a false-positive result).

In the preliminary toxicity test, triazine was toxic to both non-activated and activated TA98 cells at dose levels of 200 ug/plate and above, as revealed by reduced background lawn indicative of restricted growth. Hence, the five doses selected for the mutation assays were 200, 40, 8, 1.6, and 0.32 ug/ plate with or without S9 mix.

In both experiments, the test substance consistently reduced background lawns at the HOT, 200 ug/mL, in the absence of activation but with one exception did not induce significantly increased revertent colony counts. The exception was a significant F-value ($p < 0.05$) calculated for unactivated TA1537 cultures, derived from a wider-than-usual variation in mean numbers of revertents on test plates (3.3 to 7.7) compared to the concurrent mean control value (4.7). This was discounted by the investigator because there was neither a doubling of revertent rate nor evidence of a dose-relationship. Hence, the investigator concluded that triazine was **not mutagenic** in the Ames testing at levels up to levels of toxicity.

This study is classified as **Acceptable**. The study was well conducted with adequate procedures under controlled conditions such that the negative results for triazine in repeat experiments represent valid interpretations of the data.

In a mammalian bone marrow chromosome aberration test in mouse (MRID 41231701), Triazine (78.5%) was administered to groups of CD-1 mouse (5/sex/group) by gavage in a single dose at 200, 400, and 800 mg/kg, following a rang-finding study (at doses up to 2000 mg/kg) and were

sacrificed 24, 48, or 72 hours later. Control groups received 0.9% saline (solvent control), or cyclophosphamide (CPA, positive control); the latter sacrificed only at 24 hours. Femoral bone marrow harvested at scheduled sacrifice times was prepared for microscopic examination by conventional cytological techniques. At least 1000 polychromatic erythrocytes (PCE) per animal were scored for micronuclei (MN—PCE), and the ratio of PCE to normochromatic erythrocytes (NCE) also recorded for each animal. The micronuclei data were analyzed by the Mann—Whitney U—test, with probabilities for significant differences between test and control groups obtained from standard published tables.

In the range-finding test, deaths occurred at 1200 and 2000 mg/kg (respectively, 2 of 3 animals and 3 of 3 of each sex), but no mortalities at 800 mg/kg or below, providing an estimated LD_{50} of approximately 1000 mg/kg. Based upon the (OECD) convention of applying 80% of the LD_{50} as the MTD for this type of study, the HDT for the main study was selected as 800 mg/kg, with two lower doses of 400 and 200 mg/kg.

In the micronucleus assay itself, two high-dose (800 mg/kg) animals (one of each sex) died before their scheduled sacrifice at 48 hours. No cytotoxicty, as measured by reduction in PCE/NEC ratios, was found in any nabam-treated group. Random variation around control values for numbers of micronuclei was found at all sacrifice times and in both sexes of test animals. A singular statistically significant increase in micronuclei over concurrent control was calculated for the 400 mg/kg male group sacrificed at 72 hours (0.14 vs. 0.02). This increase was considered to be of no biological significance since it fell within solvent control ranges at other sampling times (notably 24 hours when optimally most of the micronuclei would be visualized).

Hence, the investigator concluded that triazine **did not induct micronuclei in bone marrow cells of mice** treated orally up to a nominal dose (800 mg/kg) considered a MTD by OECD (80% of the LD_{50}).

This study is classified as **Acceptable-Guideline,** as demonstrating no potential for the induction of micronuclei in CD-1 mice. This study was conducted in a manner consistent with adequate practices for this type of assay. Although the Agency's limit dose in the absence of any toxicity (1000 mg/kg) was not assayed (represented by the LD_{50} calculated in the range-finding study), we believe testing up to 800 mg/kg is sufficiently high to validate the negative result obtained.

Grotan was tested in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay (MRID 41262301). Based on the results of the preliminary cytotoxicity the test article, hexahydro-1, 3, 5-tris(2-hydroxyethyl)-s-triazine was tested at nine dose levels ranging from 0.0001 to 1.0 µL/mL in three replicate plates.

In the preliminary cytotoxicity assay, ten doses (0.0003 to 10 µL/mL) of the test material were examined. The study author stated that the pH of the stock concentration was adjusted to 7.4 with 1 N HCl prior to dilution. However, there was a basic shift in the pH of cultures treated with the two highest assayed levels (3.0 and 10 µL/mL) at initiation and termination of treatment.

The study author further stated that the LDH value could not be obtained for the highest test dose, presumably because of test material interference with the LDH determination. Cytotoxicity, as indicated by increased leakage of LDH into the culture medium, did not proceed in a conventional dose-related manner. At the highest assayed dose for which the LDH level could be determined (3.0 µL/mL), the percent relative cytotoxicity was 1%; however, as the dose was reduced, cytotoxicity increased to 52% at 1.0 µL/mL and 66% at 0.3 µL/mL. Although the percent relative cytotoxicity at 0.1 µL/mL was 8%, the microscopic evaluation of the hepatocyte cultures revealed a cytotoxic effect. Below this level, the test material was not cytotoxic.

Two UDS and parallel cytotoxicity assays were conducted with nine doses of the test material (0.0001 to 1.0 µL /mL). Because of unspecified technical difficulties, the first assay was terminated.

In the repeat assay, the two highest test doses (0.3 and 1.0 µL/mL) induced marked cytotoxicity (85%) and were, therefore, not scored for UDS activity. The five levels selected for the evaluation of UDS induction were 0.1, 0.03, 0.01, 0.003, and 0.001 µL/mL. The highest test material dose scored for UDS (0.10 µL/mL) induced a marked increase in the net nuclear grain count compared to the solvent control. Similarly, the percentage of cells with ≥5 net nuclear grains was markedly higher at this level compared to the solvent control value. However, the evidence for a genotoxic effect was confined to the 0.10 µL/mL dose group. The mean net nuclear grain counts and percentage of cells with ≥5 net nuclear grains for hepatocytes exposed to 0.001, 0.003, 0.01, or 0.03 µL/mL of the test material, although higher than the solvent control, did not indicate a positive or a dose-related response. The study author concluded that hexahydro-l,3,5-tris(2- hydroxyethyl)-s-triazine induced an equivocal response in this test system.

This study is classified as **Unacceptable**. In the absence of a dose-related effect, confirmation of a "significant" increase at a single dose is necessary to conclude a positive response in the UDS assay. However, the positive response was not dose related, was limited to the 0.10 µL/mL treatment group, and the results were not confirmed. In addition, the study author failed to provide information on test material purity, the batch number of the assayed sample, and analytical data to verify actual concentrations used in the assay. Therefore, it was concluded that the study is unacceptable, but the test material is classified as **presumptively genotoxic** in this test system.

In an unscheduled DNA synthesis (UDS) study (MRID 43020001), following preliminary cytotoxicity testing (by lactate dehydrogenase, LDH, release determinations), triplicate coverslip cultures (per does) of rat hepatocytes were exposed to hexahydro-l, 3, 5-tris(2-hydroxyethyl)-s-triazine for 18-20 hours to a series of nine concentrations of test article (ranging from 0.01 to 0.30 µg/mL), together with a constant dose of tritiated thymidine (HTdr, 10 µCi/mL). Other cultures were treated with 7, 12-dimethylebenz(a)anthracene (DMBA, 3 and 10 µg/mL), to serve as positive controls.

Fifty cells per slide were read; net nuclear counts (NNC) were calculated (crude nuclear count less mean of three cytoplasmic counts) and were averaged for each treatment. In addition, the percentage of cells in repair was recorded for each dose level. Means, standard deviations and percent survival were computed. The grain count results represent unscheduled DNA sysnthesis.

The preliminary cytotoxicity test demonstrated that the test article produced dose-related toxicities (based on LDH release) from >80% at 1.0 µg/mL down to 1% at 0.1 µg/mL. Hence, the HDT selected for the UDS assay itself was 0.3 µg/mL (at which related toxicity was 79%).

For the UDS assay, examination of the fixed and stained cells which had been treated at doses of 0.15, 0.2, 0.25, and 0.3 µg/mL revealed that these could not be evaluated for UDS "because of excessive cytotoxicity", resulting in relative cell survivals less than 35%. Although none of the remaining (lower) test article doses (0.01 to 0.1 µg/mL) caused a significant increase in mean NNC, according to the authors' criteria established for declaring a positive result (i.e., an increases of at least five counts over solvent control), there was a dose-related increase trend in both mean NNC, as well as percentage of cells showing DNA repair. On the other hand, DMBA-treated cultures showed a definitively significant mean NNC (>5 counts) in 99% of cells in repair.

Hence, the authors concluded that the test article was not considered positive according to their criterion for a genotoxic response.

This study is classified as **Unacceptable**, though "**equivocally (presumptively) positive**", because of slight, but dose-related, increases in both mean net nuclear silver grain counts (NGG), as well as percentage of cells in repair at the usable three highest dosage levels 0.05, 0.07 and 0.10 µg/mL, but which did not reach the criterion for significance proposed by the investigators, namely, increase of 5+ counts over control. Higher doses were stated to be "too toxic to be evaluated for UDS" (as suggested by LDI4 release at 30-60% lethality), necessitating in the reviewer's opinion a repeat assay to resolve this equivocation, together with (l) a finer sequence of dosages to recover viable cultures at higher concentrations, (2) testing hepatocytes from a female rat, and (3) providing the purity (% a.i.) of the test article.

In reviewing the data base for this substance, revealed a previous UDS study (MRID 41262301), conducted by the same laboratory. This study was also classified **Unacceptable**, requiring confirmation of a positive response (increased NNG >5, and increased % cells in repair) at the HDT of 0.10 µg /ml.

Thus, these two comparable assays, when considered together, constitute the recommended confirmation of singularly positive results, and may be re-classified ACCEPTABLLE, in ratifying that Grotan induces UDS (repair) above background at mildly toxic doses (<20% lethality).

4.8 Neurotoxicity

Adequacy of database for Neurotoxicity: The database for neurotoxicity of HHT is considered incomplete with no available data.

4.9 Metabolism and Pharmacokinetics

Adequacy of database for Metabolism and Pharmacokinetics: The database for metabolism and pharmacokinetics of HHT is considered incomplete. A test to study tissue and species variation in the *in vitro* metabolism of [^{14}C]-HHT was conducted with human blood and hepatocytes (MRID 47281205). This study is classified as unacceptable-nonguideline and does not satisfy the guideline requirement [OPPTS 870.7485, OECD TG 417].

5.0 Toxicity Endpoint Selection

5.1 Toxicological Doses and Endpoint Selection

5.1.1 Acute Reference Dose (aRfD)–all populations

Study Selected: Developmental Toxicity (Rat) § Number 870.3700a

MRID No.: 41161801

Executive Summary: Grotan [hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine], 78.5% of purity, was administered via gavage at doses of 0 (distilled water), 250, 500 and 750 mg/kg/day to 24 Sprague-Dawley (OFA-SD (IOP-Caw)) rats/group, days 6 through 15 gestation. Food consumption and body weight gain were reduced at 750 mg/kg/day. The incidences of post-dosing salivation and ulceration of the stomach were increased for high dose females. Maternal NOAEL and LOAEL are 500 mg/kg/day and 750 mg/kg/day, respectively, based on decreased body weight gain, ulcerations, and scarring of the stomach mucosa.

Dose and Endpoint for Risk Assessment: **Maternal NOAEL of 500 mg/kg/day** based on ulcerations and scarring of gastric mucosa.

Uncertainty Factor(s): **1000** (10x for interspecies extrapolation and 10x for intraspecies variations, and 10x for database uncertainty factor)

$$Acute\ RfD\ (GeralPopulation) = \frac{500\ mg/kg/day}{1000} = 0.5\ mg/kg/day$$

Comments about Study/Endpoint/Uncertainty Factor: Gastric ulceration and gastric mucosa scarring are considered as acute systemic effects through oral route. Because there

is only one developmental study and no appropriate second species developmental study and/or reproductive study available, a database uncertainty factor of 10 is applied.

5.1.2 Chronic Reference Dose (cRfD)

Study Selected: 90-day Oral (Rat) § Number 870.3100

MRID No.: 41483001

Executive Summary: In an oral toxicity study in the rat, Grotan [hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine], 78.5% of purity, was administered via gavage at does of 0 (distilled water), 10, 50, 100 or 250 mg/kg/day to 10 Crl: CD(SD) BR (VAF plus) rats/sex/group for 13 weeks. Noisy respiration occurred in 2/10 high-dose females with 4/10 deaths (not the same animals) from unexplained causes although there was evidence of triazine in the lungs. Stomach effects (erosion and lymphocytic infiltration) were seen at 100 and 250 mg/kg. There were other statistically significant findings but they were of doubtful toxicological significance. NOAEL = 50 mg/kg/day and LOAEL = 100 mg/kg/day, based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Dose and Endpoint for Risk Assessment: **NOAEL of 50 mg/kg/day** based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Uncertainty Factor(s): **100** (10x for interspecies extrapolation and 10x for intraspecies variations).

$$CHRONIC\ RfD = \frac{50mg/kg/day}{100} = 0.5\,mg/kg/day$$

5.1.3 Incidental Oral Exposure: Short–Term (1-30 days)

Study Selected: Developmental Toxicity (Rat) § Number 870.3700a

MRID No.: 41161801

Executive Summary: Grotan [hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine], 78.5% of purity, was administered via gavage at doses of 0 (distilled water), 250, 500 and 750 mg/kg/day to 24 Sprague-Dawley (OFA-SD (IOP-Caw)) rats/group, days 6 through 15 gestation. Food consumption and body weight gain were reduced at 750 mg/kg/day. The incidences of post-dosing salivation and ulceration of the stomach were increased for high dose females. Maternal NOAEL and LOAEL are 500 mg/kg/day and 750 mg/kg/day, respectively, based on decreased body weight gain, ulcerations, and scarring of the

stomach mucosa.

Dose and Endpoint for Risk Assessment: **Maternal NOAEL of 500 mg/kg/day** based on ulcerations and scarring of gastric mucosa.

Comments about Study/Endpoint/Uncertainty Factor: Decreased body weight gain, ulcerations, and scarring of gastric mucosa are considered appropriate for the incidental short-term oral end-points.

Because data gaps of the second species developmental study and/or reproductive study, a database uncertainty factor of 10 is applied. Therefore, the total margin of exposure should be 1000 (10x for interspecies extrapolation, 10x for intraspecies variations, and 10x for database uncertainty factor).

5.1.4 Incidental Oral Exposure: Intermediate-Term (1- 6 months)

Study Selected: 90-day Oral (Rat) § Number 870.3100

MRID No.: 41483001

Executive Summary: In an oral toxicity study in the rat, Grotan [hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine], 78.5% of purity, was administered via gavage at does of 0 (distilled water), 10, 50, 100 or 250 mg/kg/day to 10 Crl: CD(SD) BR (VAF plus) rats/sex/group for 13 weeks. Noisy respiration occurred in 2/10 high-dose females with 4/10 deaths (not the same animals) from unexplained causes although there was evidence of triazine in the lungs. Stomach effects (erosion and lymphocytic infiltration) were seen at 100 and 250 mg/kg. There were other statistically significant findings but they were of doubtful toxicological significance. NOAEL = 50 mg/kg/day and LOAEL = 100 mg/kg/day, based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Dose and Endpoint for Risk Assessment: **NOAEL of 50 mg/kg/day** based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Uncertainty Factor(s): **100** (10x for interspecies extrapolation and 10x for intraspecies variations).

5.1.5 Dermal Exposure: Short-Term (1-30 days)

A 21-day dermal toxicity study was available for HHT to address short-term dermal hazards. The results of this study (MRID 00155989/260195) showed no systemic toxicity

at doses up to and including 1000 mg/kg/day. Therefore, an endpoint for short-term dermal systemic risk assessment is not necessary.

5.1.6 Dermal Exposure: Intermediate- and Long-Term (30 days-6 months and ≥ 6 Months)

Study Selected: 90 -Day Dermal Study (Rat) § 870.3100

MRID No.: 41483002

Executive Summary: In a subchronic dermal study, groups of Crl:CD(SD)BR rats (10/sex/group) received the test material Grotan (78.5% a.i.) to the clipped dorsal area at doses of 0, 5, 50, or 250 mg/kg/day 6 hours/day, 5 days/week for 13 weeks. No deaths were reported during the study. The clinical signs observed during the study included periorbital, perianal, and cranial fur staining in all groups; yellow staining at the applications site in animals from groups 2 and 3; and skin reactions at the application site in animals treated with Triazine at doses of 50 and 250 mg/kg/day. Occasional incidences of erythema were observed in female rats which received 5 mg/kg/day.

In assessing the severity of the dermal lesions, erythema was scored as very slight to severe in both groups 3 and 4. An increase in the numbers of animals affected and an increase in the severity of the reported reactions occurred in group 4. Edema was present in animals in group 4 and was characterized as being very severe to moderate. In group 3, there were only isolated incidences of edema reported.

Microscopically, there was an increase in the incidence of epidermal hyperplasia and ulceration of the treated area of the skin in animals treated with 50 or 250 mg/kg/day. The dermal NOAEL is 5 mg/kg/day and the dermal LOAEL is 50 mg/kg/day, based on skin lesions observed at this level. The systemic NOAEL was found to be greater than 250 mg/kg/day.

Dose and Endpoint for Risk Assessment: **The systemic NOAEL of ≥ 250 mg/kg/day (HDT)**

Comments about Study/Endpoint/Margins of Exposure: There are no systemic effects noticed in this rat 90-day study tested up to 250 mg/kg/day. However, because the concern of potential endocrine disruption effects may associate with the triazine compounds, and the design of this study would not be able to evaluate the endocrine disruption concern, therefore, an extra safety factor of 10 should be applied. The Margin of exposure (MOE) of 1000 should be used in the risk assessment (10x for intra-species variation, 10x for inter-species variation, and 10x for database uncertainty factor).

5.1.7 Inhalation Exposure: All Durations

Study Selected: 90-day Oral (Rat) § Number 870.3100

MRID No.: 41483001

Executive Summary: In an oral toxicity study in the rat, Grotan [hexahydro-1,3,5-tris-(2-hydroxyethyl)-s-triazine], 78.5% of purity, was administered via gavage at does of 0 (distilled water), 10, 50, 100 or 250 mg/kg/day to 10 Crl: CD(SD) BR (VAF plus) rats/sex/group for 13 weeks. Noisy respiration occurred in 2/10 high-dose females with 4/10 deaths (not the same animals) from unexplained causes although there was evidence of triazine in the lungs. Stomach effects (erosion and lymphocytic infiltration) were seen at 100 and 250 mg/kg. There were other statistically significant findings but they were of doubtful toxicological significance. NOAEL = 50 mg/kg/day and LOAEL = 100 mg/kg/day, based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Dose and Endpoint for Risk Assessment: **NOAEL of 50 mg/kg/day** based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.

Comments about Study/Endpoint/Margins of Exposure: There is no appropriate inhalation study. Therefore, a route-to-route extrapolation from oral study to inhalation exposure route is applied. Because the potential endocrine disruption effect, data gaps from missing second species developmental study, reproductive study and longer term chronic studies and potential difference between oral and/or inhalation effects, a total MOE of 1000 is applied. (10x for intraspecies extrapolation; 10x for interspecies extrapolation; and 10x for data gaps uncertainty factor).

5.2 Dermal Absorption

Dermal Absorption Factor: A dermal absorption factor is not needed because a 90-day dermal study was used for selecting the dermal exposure risk assessment endpoints.

5.3 Classification of Carcinogenic Potential

There are no chronic and/or cancer studies for HHT. Because there are long term exposure scenarios (e.g. metal working fluid), endocrine disruption concern, some positive mutagenicity study findings, and a known formaldehyde producer, lack of chronic studies and cancer studies (two species) are considered as data gaps. These studies are required to support the current uses

of HHT.

The toxicological doses and endpoints selection are summarized below in Table 4.

Table 4. Summary of Toxicological Doses and Endpoint Selection for HHT			
Exposure Scenario	**Dose Used in Risk Assessment, UF**	**Special FQPA SF and Level of Concern (LOC) for Risk Assessment**	**Study and Toxicological Effects**
Dietary Risk Assessment			
Acute Dietary (All populations)	Oral NOAEL = 500 mg/kg/day UF = 1000 (10x – Inter; 10x – Intra and 10x – database UF)	NA	Rat Developmental Toxicity (MRID 41161801), based on ulcerations, and scarring of the stomach mucosa.
Chronic Dietary (All populations)	Oral NOAEL = 50 mg/kg/day UF = 100 (10x – Intra; 10x – Inter)	NA	Rat 90-day Oral Study (MRID41483001), based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males
Non-Dietary Risk Assessments			
Short-Term Incidental Oral (1-30 days)	Oral NOAEL = 500 mg/kg/day UF = 1000 (10x – Inter; 10x – Intra and 10x – database UF)	N/A	Rat Developmental Toxicity (MRID 41161801), based on decreased body weight gain, ulcerations, and scarring of the stomach mucosa.
Intermediate-Term Incidental Oral (30 days- 6 months)	Oral NOAEL = 50 mg/kg/day UF = 100 (10x – Intra; 10x – Inter)	N/A	Rat 90-day Oral Study (MRID41483001), based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males
Dermal Ab. Factor	Note required (dermal study is used for dermal end-points)		
Dermal Short-Term (1-30 days)	No risk assessment necessary. No effects observed in a 21-day dermal toxicity study up to 1000 mg/kg/day.		

Table 4. Summary of Toxicological Doses and Endpoint Selection for HHT			
Exposure Scenario	**Dose Used in Risk Assessment, UF**	**Special FQPA SF and Level of Concern (LOC) for Risk Assessment**	**Study and Toxicological Effects**
Dermal Intermediate- and long-term (30 days-6 months and >6 months)	Oral NOAEL = 250 mg/kg/day UF = 1000 (10x – Intra, 10x – Inter and 10x – database UF)	N/A	Rat 90-day dermal Study (MRID41483002), based on systemic NOAEL was found to be greater than 250 mg/kg/day.
Inhalation (all durations)	Oral NOAEL = 50 mg/kg/day UF = 1000 (10x – Inter 10x – Intra, and 10x – database UF)	NA	Rat 90-day Oral Study (MRID41483001), based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males
Cancer (oral, dermal, inhalation)	No cancer data are available.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

6.0 FQPA Considerations

There are no registered food-related uses for HHT; therefore, no FQPA concern is considered. However, some of the use (e.g., detergent) may trigger indirect food exposure concern.

7.0 REFERENCES

MRID

00155959 Consultox Laboratories (1973) Glokill 77: Acute Oral Toxicity. Unpublished study, 4 p.

00155984 Hughes, T. (1985) Acute Dermal Toxicity Screen in Rabbits of Bioban GK: Project No. 85-0866-21. Unpublished study prepared by Hill Top Research, Inc. 10 p.

00155985 Collier, T.; Axelrad, J. (1984) OECD Eye Irritation Test: Determination of the Degree of Ocular Irritation Caused by Glokill 77 in the Rabbit: Experiment No. 371/8408. Unpublished study prepared by SafePharm Laboratories Ltd. 11 p.

00155986 Guest, R.; Collier, T. (1985) Primary Dermal Irritation Test: Determination of the Degree of Primary Cutaneous Irritation Caused by Glokill 77 in the Rabbit: Experiment No. 317/8505. Unpublished study prepared by SafePharm Laboratories Ltd. 11 p.

00155987 Thomas, M. (1974) Glokill 77: Draize Skin Test, Guinea Pig Sensitization Test: CL 74: 74: 1024. Unpublished study prepared by Consultox Laboratories Ltd. 8 p.

00155989 Thomas, M.; Brooks, P. (1984) Glokill 77: 21 Day Dermal Toxicity Study in the Rat: Experiment No. 508/8411. Unpublished study prepared by SafePharm Laboratories Ltd. 89 p.

41161801 Ridgway, P. (1989) Triazine Rat Teratology Study: Reference No. LEF/8/89. Unpublished study prepared by Toxicol Laboratories Ltd. 117 p.

41231701 Asquith, J.; Phil, M. (1989) Triazine Joint Venture: Mouse Micronucleus Test: Triazine: Toxicol Study No. M/MMN/10659. Unpublished study prepared by Toxicol Laboratories Ltd. 40 p.

41231702 Griffin, T. (1991) Asquith, J.; Phil, M. (1989) Triazine Joint Venture: Bacterial Reverse Mutation Assay: Triazine: Toxicol Study No. M/AMES/10658. Unpublished study prepared by Toxicol Laboratories Ltd. 35 p.

41262301 Curren, R. (1988) Unscheduled DNA Synthesis in Rat Primary

Hepatocytes: Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triamine: Study No. T8102.380. Unpublished study prepared by Microbiological Associates, Inc. 27 p.

41483001 Hill, R.; Newman, A. (1990) Triazine: 13 Week Oral (Gavage) Toxicity Study in the Rat: Lab Project Number: LEF/3/90: LEF/3/89. Unpublished study prepared by Toxicol Laboratories, Ltd. 253 p.

41483002 Hill, R.; Newman, A. (1990) Triazine: 13 Week Dermal Toxicity Study in the Rat: Lab Project Number: LEF/4/89. Unpublished study prepared by Toxicol Laboratories, Ltd. 415 p.

41675206 Rush, R. (1989) Acute Oral Toxicity Study in Rats with Busan 1060: Lab Project Number: 3138.47. Unpublished study prepared by Springborn Laboratories, Inc. 59 p.

41858301 Trimmer, G. (1991). Vancide TH: 90 Day Subchronic Dermal Toxicity Study in Rats: Lab Project Number: 240810-MRD-89-408. Unpublished study prepared by Exxon Biomedical Sciences, Inc. 429 p.

41865701 Beyer, Bruce K.. (1991). Developmental Toxicity Study in Rats with Vancide TH. Exxon Biomedical Sciences, NJ. Study Number: 240834.

43020001 San, R.; Raabe, H. (1993) Unscheduled DNA Synthesis in Rat Primary Hepatocytes: Final Report: Lab Project Number: TC836.380: SPGT380. Unpublished study prepared by Microbiological Associates, Inc. 25 p.

8.0 APPENDICES

Toxicity Profile Summary

8.1 Toxicity Profile Summary Tables

8.1.1 Acute Toxicity Table – See Section 4.1

8.1.2 Subchronic, Chronic and Other Toxicity Table

Table 5. Subchronic, Chronic and Other Toxicity Profiles for HHT.

Guideline Number/ Study Type/ Test Substance (% a.i.)	MRID Number (Year)/ Citation/Classification/Doses	Results
870.3100 (§82-3) 90-day oral - Rat Purity: 78.5%	**MRID 41483001** (1990). Hill, R.; Newman, A. (1990). Triazine: 13 Week Oral (Gavage) Toxicity Study in the Rat: Lab Project Number: LEF/3/90: LEF/3/89. Unpublished study prepared by Toxicol Laboratories, Ltd. 253 p. **Minimum** Rats (10/sex/dose) 0, 10, 50, 100, or 250 mg/kg/day, 6 hrs/day, 5 days/week, 13 weeks	**Systemic Toxicity** NOAEL = 50 mg/kg/day LOAEL = 100 mg/kg/day, based on lymphocytic infiltration in females and erosion of gastric mucosa and prominence of limiting ridge of the stomach in males.
870.3200 (§82-3) 21-day dermal - Rat Purity: 79.96%	**MRID 00155989** (1984). Thomas, M.; Brooks, P. (1984). Glokill 77: 21 Day Dermal Toxicity Study in the Rat: Experiment No. 508/8411. Unpublished study prepared by SafePharm Laboratories Ltd. 89 p. **Minimum** Rats (5/sex/dose) 0, 100, 500, or 1000 mg/kg/day	**Systemic Toxicity** NOAEL >= 1000 mg/kg/day (highest dose tested); LOAEL not established.

Table 5. Subchronic, Chronic and Other Toxicity Profiles for HHT.		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/Classification/Doses**	**Results**
870.3250 (§82-3) 90-day dermal - Rat Purity: 78.5%	**MRID 41858301 (1991).** Trimmer, G. (1991). Vancide TH: 90 Day Subchronic Dermal Toxicity Study in Rats: Lab Project Number: 240810-MRD-89-408. Unpublished study prepared by Exxon Biomedical Sciences, Inc. 429 p. **Minimum** Vancide applied dermally at 0, 10, 30, or 100 mg/kg Rats (13sex/group)	**Dermal Toxicity** NOAEL < 10 mg/kg/day LOAEL = 10 mg/kg/day, based on the gross observation of dermal irritation which was confirmed histologically. **Systemic Toxicity** NOAEL >= 250 mg/kg/day (highest dose tested), LOAEL not established. Dermal irritation was characterized by hyperplasia, hyperkeratosis, inflammation, sebaceous gland hyperplasia and eschar formation.
870.3250 (§82-3) 90-day dermal - Rat Purity: 78.5%	**MRID 41483002** (1990). Hill, R.; Newman, A. (1990). Triazine: 13 Week Dermal Toxicity Study in the Rat: Lab Project Number: LEF/4/89. Unpublished study prepared by Toxicol Laboratories, Ltd. 415 p. **Minimum** Rats (10/sex/dose) 0, 10, 30, or 100 mg/kg/day, 6 hrs/day, 5 days/week, 13 weeks	**Dermal Toxicity** NOAEL < 10 mg/kg/day LOAEL = 10 mg/kg/day, based on the gross observation of dermal irritation which was confirmed histologically. **Systemic Toxicity** NOAEL >= 250 mg/kg/day (highest dose tested), LOAEL not established. Dermal irritation was characterized by hyperplasia, hyperkeratosis, inflammation, sebaceous gland hyperplasia and eschar formation.
870.3700 (§83-3) Developmental (gavage) - Rat Purity 78.5%	**MRID 41161801** (1989). Ridgway, P. (1989). Triazine Rat Teratology Study: Reference No. LEF/8/89. Unpublished study prepared by Toxicol Laboratories Ltd. 117 p. **Minimum** Female rats (24/dose) 0, 250, 500, or 750 mg/kg/day, GD 6-15, inclusive	**Maternal toxicity:** NOAEL = 500 mg/kg/day LOAEL = 750 mg/kg/day, based on decreased body weight gain, ulcerations, and scarring of the stomach mucosa. **Developmental toxicity:** NOAEL >= 750 mg/kg/day, highest dose tested. LOAEL not established.

Table 5. Subchronic, Chronic and Other Toxicity Profiles for HHT.		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/Classification/Doses**	**Results**
870.3700 (§83-3) Developmental (gavage) - Rat Purity 96.6%	**MRID 41865701** Beyer, Bruce K. (1991). Developmental Toxicity Study in Rats with Vancide TH. Exxon Biomedical Sciences, NJ. Study Number: 240834. **Supplementary** Vancide TH administered orally at 0, 10, 75 and 150 mg/kg/day, GD 6-15 25 rats/group	**Maternal toxicity:** NOAEL = 75 mg/kg/day LOAEL 150 mg/kg/day (HDT), based on decreased body weight gain **Developmental toxicity:** NOAEL < 10 mg/kg/day LOAEL not established.
870.5100 Bacterial reverse mutation test Purity 78.5%	**MRID 41231702** Asquith, J.; Phil, M. (1989) Triazine Joint Venture: Bacterial Reverse Mutation Assay: Triazine: Toxicol Study No. M/AMES/10658. Unpublished study prepared by Toxicol Laboratories Ltd. 35 p. **Acceptable** Guideline TA1535, TA1537, TA1538, TA98, or TA100 up to 200 ug/plate with and without S9 metabolic activation	**Negative** Grotan was found to be negative for inducing increases in reverse gene mutation in the standard Ames Salmonella strains exposed to test compound up to toxic levels (200 ug/ plate), with and without metabolic activation.
870.5385 Mammalian bone marrow chromosome aberration test (Mouse) Purity: 78.5%	**MRID 41231701** Asquith, J.; Phil, M. (1989). Mouse Micronucleus Test [On] Triazine: Toxicol Study No. M/MMN/10659. Unpublished study prepared by Toxicol Laboratories Ltd. **Aceptable** Guideline Grotan administered by gavage in a single oral dose at 200, 400 and 800 mg/lg 5 mice/sex/dose	**Negative** Grotan has not been found to be a mutagen when tested by the micronucleus test in the bone marrow of CD-1 mice dosed at concentration up to 855.3 mg/kg (80% of the LD_{50}).

Table 5. Subchronic, Chronic and Other Toxicity Profiles for HHT.		
Guideline Number/ Study Type/ Test Substance (% a.i.)	**MRID Number (Year)/ Citation/Classification/Doses**	**Results**
870.5550 Unscheduled DNA synthesis (Rat) Purity 78.5%	**MRID 41262301 and 43020001** **MRID 41262301**. Curren, R. (1988) Unscheduled DNA Synthesis in Rat Primary Hepatocytes: Hexahydro-1,3,5-tris(2-hydroxyethyl)-s-triazine: Study No. T8102.380. Unpublished study prepared by Microbiological Associates, Inc. 27 p. **MRID 43020001**. San, R.; Raabe, H. (1993) Unscheduled DNA Synthesis in Rat Primary Hepatocytes: Final Report: Lab Project Number: TC836.380: SPGT380. Unpublished study prepared by Microbiological Associates, Inc. 25 p. **Acceptable** Guideline 0, 0.001, 0.03, 0.01, 0.03, or 0.1 ug/mL (MRID 43020001) 0, 0.03, 0.10, 0.30, 1.00, or 3.00 uL/mL (MRID 41262301) Rat hepatocyte cells	**Positive** Increased net nuclear grain count; increased percent of cells with > 5 nng at 0.10 ug/mL. These two comparable assays, when considered together, constitute the recommended confirmation of singularly positive results, and may be re-classified ACCEPTABLLE, in ratifying that Grotan induces UDS (repair) above background at mildly toxic doses (<20% lethality).