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US EPA HPV Chemical Challenge Program

ROBUST SUMMARIES FOR TRIGLYCIDYL ISOCYANURATE

CHEMICAL NAME: S-TRIAZINE-2,4,6(1H,3H,5H)-TRIONE, 1,3,5-TRIS(2,3-EPOXYPROPYL)-
(CAS No. 2451629)

CONSORTIUM NAME: ***HUNTSMAN-NISSAN-TGIC***
CONSORTIUM NUMBER:

Toxicology Summary and

Test Plan

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June 25, 2006

(This document contains a total of 40 pages)

Test Plan Summary:

Triglycidylisocyanurate (TGIC) is a trifunctional epoxide resin used primarily as a hardener for polyester-based powder coatings. TGIC is also known by the chemical name: 1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-tris(oxiranyl-methyl)- , identified by Chemical Abstract Service (CAS) No. 2451-62-9.

The following table provides an overview of the studies reviewed for the Robust Summary Document. These studies were generally selected to conform to the data requirements described in the EPA's "Draft Guidance on Developing Robust Summaries" (October 22, 1999). The reference section at the end of this document lists all of the reports included in the Robust Summary and also describes several additional studies for TGIC. The additional studies were conducted by the sponsors, as well as other interested parties, in order to evaluate various health related endpoints associated with the manufacture and occupational use of TGIC. Some of these studies may not have been discussed in detail in this document in an effort to avoid redundancy and to help maintain focus on the requirements described in the Robust Summary.

All SIDS Level I endpoints have been adequately addressed. Definitive developmental toxicity/teratogenicity studies, conducted in accordance with OECD guidelines, do not appear to be available. However, the lack of findings in the 13-week reproductive toxicity screening study provides sufficient data to address any concerns on reproductive and developmental toxicity endpoints. In addition, the negative results in the Dominant Lethal Assay and Mammalian Spot Test and the lack of any significant histopathological findings in the reproductive organs of the test animals in the two year chronic exposure study provide additional scientific evidence for the lack of reproductive and development toxicity from exposures to TGIC. The weight of this scientific data fully supports the conclusion that TGIC is not likely to be a reproductive/developmental toxicant.

The lack of carcinogenic responses in the cancer screening endpoint in a 13 week subchronic study, the 30 week dermal initiation-promotion study and the

two year cancer bioassay, fully support the position that TGIC is unlikely to be a carcinogen.

Thus, the necessary toxicological endpoints for TGIC have been addressed, and no further testing is necessary or proposed. The HPV testing commitment for TGIC is complete.

SUMMARY FOR TGIC TEST PLAN

STUDY	Data Available?	Data Adequate?	Testing Required?
	Y/N	Y/N	Y/N
Physical-Chemical Data			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
Environmental Fate & Pathway			
Photodegradation	N	N	N
Stability in Water	Y	Y	N
Transport Between Environ. Compartments (Fugacity)	Y	Y	N
Biodegradation	Y	Y	N
Ecotoxicity			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	N
Toxicity to Algae	Y	Y	N
Toxicity			
Acute Oral Toxicity	Y	Y	N
Acute Inhalation Toxicity	Y	Y	N
Acute Toxicity to the Eye	Y	Y	N
Acute Dermal Toxicity	Y	Y	N
Genotoxicity *in vivo* (Chrom. Aberrations)	Y	Y	N
Genotoxicity *in vitro* (Gene Mutation)	Y	Y	N
Genotoxicity i*n vitro*	Y	Y	N
Repeated Dose Toxicity	Y	Y	N
Reproductive Toxicity	Y	Y	N
Developmental Toxicity/Teratogenicity	N	Y	N

INTRODUCTION

Triglycidylisocyanurate (TGIC) is a trifunctional epoxide resin used primarily as a hardener for polyester-based powder coatings. TGIC is also known by the chemical name: 1,3,5-Triazine-2,4,6(1H,3H,5H)-trione, 1,3,5-tris(oxiranylmethyl)-, identified by Chemical Abstract Service (CAS) No. 2451-62-9.

The purpose of this document is to describe the available toxicology data for TGIC (which may or may not be contained in the regulatory files of the OECD member countries), provide a summary of the potential toxicological effects of TGIC, discuss the Use and Exposure Profile for TGIC as it is used in commerce, identify what significant toxicology data gaps exist, if any, and note in the Test Plan the investigations which the sponsors are proposing to conduct.

Historically, TGIC has been safely used in commerce throughout the world for more than twenty years. During this time, more than eighty toxicology evaluations of TGIC have been conducted. A summary of the toxicological effects is presented below and a more comprehensive discussion appears at the end of this document.

Physical-Chemical Parameter Summary

Unless otherwise indicated, the toxicology testing described in this Test Plan Summary and in the compilation of Robust Summaries has been conducted using technical grade TGIC. Technical grade TGIC contains two isomers of TGIC, with typical concentrations of the α-isomer ranging from 76 to 80%, and concentrations of the β-isomer ranging from 20 to 24%. The relative amount of the isomers found in technical grade TGIC is apparently not influenced by the method of manufacture. Technical grade TGIC may contain up to 100 ppm of

excess epichlorohydrin reactant (oxirane, [chloromethyl], CAS No.106-89-8), as an impurity related to manufacture of the product.

The sponsor companies have provided melting point (95ºC), vapor pressure (7.2 x 10^{-4} pascal), partition coefficient (-0.8) and water solubility (9 g/L) data.

Environmental Fate and Pathway Summary

TGIC will hydrolyze in water to form 1,3,5-tris (2,3-dihydroxypropyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione. Typical half-life in water (at room temperature) is approximately 160 hours. At higher temperatures (60ºC) the half-life is approximately 4.5 hours. Fugacity data (transport between environmental compartments) was modeled using the EPIWIN computer program. Due to the limited distribution of unreacted TGIC in commerce, it's low vapor pressure and it's ability to hydrolyze in water, there is only very limited applicability of the modeled data.

TGIC is not readily biodegradable when tested under OECD 301 and modified Sturm test protocols.

Ecotoxicity Summary

In the environment, TGIC has no significant toxicity to aquatic organisms. The 96-hour LC_{50} for Zebrafish is greater than 77 mg/L, 77 mg/L being the saturation concentration. The 24-hour EC_{50} for Daphnia magna was greater than 90.6 mg/L and the EC_0 was calculated to be 58 mg/L.

Mammalian Toxicity Summary

Six different assays have shown TGIC to be of moderate acute oral toxicity with LD_{50} values ranging from 88 to 1450 mg/kg, with a mean value of 391 mg/kg.

Four assays have demonstrated the low dermal toxicity of TGIC with results ranging from greater than 2000 mg/kg to greater than 3000 mg/kg. Two acute inhalation studies have shown TGIC to be of moderate toxicity, with LC_{50} values greater than 309 mg/m^3 air. The intravenous LD_{50} is approximately 150 mg/kg.

In rabbit eye irritation studies, the instillation of TGIC has shown marked to severe irritation of the cornea, iris and conjunctiva, independent of whether the eye was rinsed or not. The results of several dermal irritation studies using rabbits have shown that TGIC has minimal skin irritation potential.

The results of guinea pig studies have demonstrated that TGIC is a dermal sensitizer. The observed potency of the sensitization response has been shown to be protocol dependent.

Repeated exposure to TGIC, either by the oral or intravenous route, caused lymphoid depletion of spleen and thymus, as well as lymphoid tissues such as lymph nodes. Dose-levels of 5-50 mg/kg were well tolerated by rats over an extended period of time. During subchronic exposure, the results of acute studies were confirmed.

Chronic exposure to TGIC in a 13 week dermal initiation-promotion assay and in a two year chronic bioassay (dietary) did not result in increased tumor frequencies in the study animals. TGIC can be considered as non-carcinogenic in animal studies.

A preliminary reproductive toxicity study in rats (effects produced only at the high dose of 7.32 mg/kg/day) showed that apart from slightly lower sperm counts (without effect on the sperm viability) all reproductive parameters were comparable to that of the controls and no changes in fertility or litter parameters were observed. Thirty ppm or 2.08 mg/kg/day was considered to be the overall NOEL for this study.

At least eight different *in vitro* bacterial and mammalian mutagenicity tests were conducted to evaluate TGIC. In two Ames Assays, TGIC induced increased numbers of revertant colonies/plate. These results are observed for molecules containing reactive epoxide groups and are not unexpected. However, in two cell transformation assays using Balb 3T3 mouse fibroblasts, no increases in cell transformations were recorded. A Mouse Lymphoma Forward Mutation Assay showed a slight increase in mutation frequency compared to control cells. Additionally, a chromosome aberration study conducted using isolated human lymphocytes *in vitro* did not show any mutagenic or clastogenic activity.

The above assays suggest that humans and rodents may respond differently to molecules containing epoxide groups, such as TGIC. In comparative DNA Repair Assays using human fibroblasts and rat hepatocytes, the human fibroblasts were exposed to TGIC at concentrations up to 20 times greater than the rat hepatocytes. The human fibroblasts did not show any unscheduled DNA synthesis, while unscheduled DNA synthesis was identified in the rat tissue. This comparison clearly demonstrates that rat cells are more sensitive to TGIC than human cells. This result may be directly correlated with much higher concentrations of epoxide hydrolases in human tissue as opposed to rat tissue. These data support the negative result in the two year cancer bioassay in rats.

Fourteen separate *in vivo* mammalian genotoxicity studies have been conducted to evaluate TGIC. Two Sister Chromatid Exchange (SCE) studies in Chinese Hamster cells were conducted. The results indicate that TGIC can induce SCE at doses of 140 mg/kg and higher, but will not induce SCE at doses below 70 mg/kg. A Nucleus Anomaly Test offered similar results. In this test, doses below 140 mg/kg did not show nuclear anomalies, whereas, doses above 280 mg/kg did.

Four chromosome aberration tests were performed in male mice. Oral administration of TGIC resulted in a dose dependent rate of aberrations, with a NOEL of 5 to 10 mg/kg. In an inhalation study, the cytotoxicity observed at 10 and 50 mg/m^3 rendered analysis of chromosomal aberrations impossible. A dose of 2.5 mg/m^3 showed no increase in chromosome aberrations or cytotoxicity. An additional chromosome aberration study in mice was designed to measure the effect of TGIC on primary and secondary spermatocytes. The study indicated that TGIC was not clastogenic in primary or secondary spermatocytes at doses up to 96 mg/kg.

Three dominant lethal studies, two oral and one inhalation, have been conducted using TGIC at doses of 137 to 550 mg/kg, or 2.5 to 50 mg/m^3. The primary conclusion from these four studies was that “despite the toxic effects on mature sperm, spermatids and spermatogonia, there was no increase in embryonic death, e.g. no sign of induction of dominant lethal mutations.” This conclusion supports the hypothesis that effects on the spermatogonia by TGIC during spermatogonial development may lead to non-viable sperm, but not heritable genetic damage.

A mammalian spot test in mice did not induce somatic cell recessive gene mutations. A study designed to measure the pH-dependent alkylation potential of TGIC indicated that TGIC can be hydrolyzed or inactivated with regard to it’s mutagenic potential when passing through the stomach.

A study to evaluate the DNA-binding potential of TGIC was also performed. Results of this study indicate that at a dose of 5 mg/kg or less, no measurable interaction of TGIC with testis DNA occurred. While in liver DNA at the same dose and in testis DNA and liver DNA at higher doses, measurable DNA adduct formation were identified.

Species Differences in Epoxide Hydrolase Activity

The data provided in the TGIC Toxicology Review at the end of the document summarize species differences in epoxide hydrolase activity. The conclusions from the literature review and the studies described above are:

- humans metabolize TGIC and other epoxides much faster than mice,
- the mouse is the standard animal model for the Dominant Lethal, Mammalian Spot Test and chromosomal aberration studies, and
- the mouse is the common laboratory species with the lowest level of epoxide hydrolase.

Therefore, the mouse is the most sensitive species with respect to the toxic effects of epoxides.

Conclusions of the Toxicology Summary

The significance of these data in establishing occupational health standards is as follows:

- The mouse is the most sensitive species with respect to the toxic effects of epoxides and molecules with epoxide groups;
- Inhalation is the most likely and most toxicologically significant route of occupational exposure;
- The inhalation NOELs for TGIC in chromosome aberrations and dominant lethal studies were 2.5 mg/m^3;
- The acceptable threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists (ACGIH) for TGIC is 0.05 mg/m^3, (50 times lower than the lowest NOEL in an animal bioassay); and
- The lack of findings in the 13-week reproductive toxicity screening study provides sufficient data to address any concerns on reproductive and developmental toxicity endpoints. This data is supported by the negative

results in the Dominant Lethal Assay and Mammalian Spot Test and the lack of any significant histopathological findings in the reproductive organs of the test animals in the two year chronic exposure study.

- In a two year cancer bioassay, TGIC was non-carcinogenic at the maximum tolerated dose (MTD) of 100 ppm in rats (a species which is approximately 2 times more sensitive to the toxic effects of TGIC than humans.
- Finally, these data suggest that if appropriate engineering controls and industrial hygiene practices are followed, TGIC can be safely used in commerce without undesirable health effects.

USE AND EXPOSURE INFORMATION

Recently, in a letter to EPA, the two largest importers of TGIC into the United States (US) provided the following information:

- There are no TGIC manufacturers in the US and only three major importers.

- Two importers account for approximately 80% of the TGIC used in the US.

- There are essentially no exposures to TGIC during the import/distribution process because TGIC is transported directly from the overseas manufacturer to the powder coating manufacturer.

- The use, and therefore exposure, to TGIC is limited almost entirely (greater than 95%) to the powder coating industry.

- There are a relatively small number (perhaps 50 to 60) of powder coating manufacturers in the US and a very small number of workers are potentially exposed to TGIC at these manufacturing locations. The number of custom coaters, which use the powder coating formulations in the US, has been estimated to be approximately 1000, with a potentially exposed population of perhaps 10,000 employees.

- Powder coatings containing TGIC represent less than 20% of the total powder coating materials manufactured in the US.

- TGIC is typically used at a concentration of 4.5 to 5% in powder coatings as a hardener for polyester resins. Upon blending and extrusion of the TGIC into the powder coating by the manufacturer, there is essentially no free TGIC available for exposure to downstream users of the finished powder coating.

- At the custom coaters' facilities, powder coatings are applied to metal parts and cured at temperatures of approximately 200° C. Under these conditions, TGIC reacts completely with the polyester, and no free TGIC exists.

- Therefore, potential exposure exists almost exclusively within the powder coating manufacturing operations, and users/consumers of powder coating products are rarely, if ever exposed to free TGIC.

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APPENDIX A

COMPREHENSIVE SUMMARY OF TGIC TOXICOLOGY DATA

Acute Toxicity Studies in Rodents

Acute toxicity (oral, dermal and intravenous (i.v.) injection) is expressed as the dose killing 50 percent of the animals under test (= LD_{50}). The same applies for inhalation studies with the exception that the LC_{50} (lethal concentration) instead of the LD_{50} (lethal dose) is measured.

a) Oral

Six different acute oral toxicity studies with rats were performed. Individual values varied between 1450 and 188 mg/kg bodyweight with an average value of 391 mg/kg (1,2,3,5,6,21). Males were slightly more sensitive than females (294 mg/kg versus 439 mg/kg) but no obvious sex specificity was observed. In case of death all animals died within 6 days. At necropsy hemorrhagic lungs and dilated intestinal tract regions were the only substance-related effects observed.

The acute oral toxicity in Chinese Hamsters was 1672 mg/kg, the females (800 mg/kg) being more susceptible than the males (2200 mg/kg) (7).

b) Dermal

The acute dermal toxicity was measured in rats in 4 different assays. The values ranged from >2000 to >3000 mg/kg (8,9,10), whereby in an additional study of doubtful quality and without any methodological information a value of 185 mg/kg was reported (21). In all three studies no mortality occurred and no substance-related effects were noticed.

c) Inhalation

Two acute inhalation studies with rats were performed applying TGIC dust of an average particle size of 2-3 microns. LC_{50} values of 650 mg/m^3 (18) and >309 mg/m^3 (19) were measured. In a similar test using mice instead of rats a LC_{50}

of 2000 mg/m^3 was measured (57). In this study, the mean diameter of the dust particles was 3.2 - 3.9 microns. In all three studies, discoloration of the lung and swollen periocular tissues were the only substance-related effects observed.

d) Intravenous

The acute toxicity of TGIC in mice after intravenous injection was calculated to be 148.7 mg/kg (4). All animals died within 6 days following injection. Substance-related toxic effects were recorded in the surviving animals after 29 days. Besides dose-dependent loss of body weight, hematopoietic hypoplasia of the bone marrow, lymphoid depletion of the thymus and ovarian atrophy was observed. No sex difference in the toxicity was recorded.

e) Eye Irritation

Four different eye irritation studies in rabbits were performed with TGIC. While one study (15) showed no reaction after rinsing the eye and only minimal irritation without rinsing the eye, the three remaining studies (16, 17, 58) showed marked to severe irritation of cornea, iris and conjunctiva, independent of whether the eye was rinsed or not. Therefore, it can be concluded that TGIC is extremely irritating, but not corrosive.

f) Skin Irritation

In five different rabbit skin irritation tests, using either 0.25 or 0.5 g of TGIC in suspension, results varied from no reaction to minimal irritation. (3,11,12,13,14). It can therefore be concluded that TGIC has only a minimal skin irritation potential.

g) Skin Sensitization

While one sensitization study in Guinea pigs showed strong sensitization (59), two more recent maximization studies in Guinea pigs resulted only in weak sensitization, e.g. only 20 to 25% of the animals were sensitized (3, 20).

Subacute Toxicity

Groups of 10 rats dosed orally with 54 / 43 mg/kg/day and 216 / 172 mg/kg/day (male / female) for seven consecutive days and terminated on day 8 showed no overt signs of toxicity (21). Upon histopathological examination, necrosis of the distal convoluted kidney tubules as well as hemorrhage of the gastric and duodenal mucosa was seen in several high dose animals.

In a percutaneous toxicity study in rats (21), groups of 10 rats (5 males and 5 females each) were dosed with 26 and 130 mg/kg/day by dermal application of the wet substance for 7 consecutive days. No overt signs of toxicity were observed. At necropsy on day 8, moderate skin ulceration and necrosis at the application site and gastrointestinal and kidney lesions (hemorrhage) were observed in the high dose animals.

A single-dose toxicity study in mice was performed using the intravenous route (4). Doses of 60, 120 and 149 mg/kg were injected to groups of 20 animals (10 males and 10 females each). After 33 days the animals were sacrificed and prepared for histopathological investigations. No mortality occurred, but body weight loss was observed most prominent between day 4 and 8 at all doses. A decrease of the number of reticulocytes, white blood cells and platelets as well as hematopoietic depletion of the bone marrow returned to normal by day 29. Lymphoid depletion of the thymus, spleen and lymph nodes as well as necrosis of the gastric mucosa and atrophy of the ovary was observed over 33 days.

In a repeated-dose study in mice (5 consecutive daily injections) doses of 27, 53 and 62 mg/kg were intravenously injected to groups of 20 animals (10 males and 10 females each) (4). No mortality occurred, but decreased body weights, reduced numbers of red and white blood cells and platelets, as well as lymphopenia and neutropenia were recorded in all dose groups, but returned to

normal by the end of the observation period (day 65). Histopathological findings were similar to those found in the single-dose toxicity study.

Two studies with Beagle dogs, a single-dose toxicity study with dose-levels of 1.8, 18 and 36 mg/kg and a repeated-dose study (5 consecutive daily doses) with dose-levels of 0.8, 8 and 16 mg/kg/day, were performed by the intravenous route on groups of 4 dogs (2 males and 2 females each). No deaths were observed until the final necropsy on day 65. A dose-dependent reduction of red and white blood cells as well as platelets, and moderate to severe leucopenia and neutropenia recovered in the low and mid-dose and partially recovered in the high dose. Renal impairment (blood creatine and blood urea nitrogen) completely recovered by day 65. Hematopoietic hypoplasia of the bone-marrow and lymphoid depletion were still marked at day 65, and necrosis of the mesenteric lymph nodes, tonsils, spleen, liver, lung and gastric tract were frequently observed in a dose dependent manner.

In a repeated-dose local tissue reaction assay in guinea pigs (4), 5 daily doses of 0.5 ml of either 1.8 or 3.6 mg/kg were subcutaneously injected. Besides moderate necrosis at the injection site no other substance related signs of toxicity were observed.

Subchronic Toxicity

19-Day Oral (Dietary Admixture) Range-Finding Study in Rats with TGIC (66)

In this study groups of 6 male and 6 female Sprague-Dawley rats were exposed to diets containing 0, 10, 40, 160, and 640 ppm of TGIC for 19 days (equivalent to 0, 1.06, 4.4, 15.9, and 48.6 mg/kg per day). Clinical signs were piloerection and curved body position in most of the rats of the high dose group, only. No mortalities occurred, but lower food consumption was observed in high dose

animals. Body weight gain was significantly reduced in high dose animals and females of the intermediate dose group. Lower leucocyte counts and higher erythrocyte counts were observed in the high dose group animals. Enlarged mesenteric lymph nodes, lower absolute spleen and thymus weight, and lower weights of ovaries, uterus, prostate and seminal vesicles were recorded. Microscopic examination revealed marked sinusoidal hemorrhage in the mesenteric lymph nodes at 160 and more pronounced at 640 ppm animals. In addition, at 640 ppm lymphoid deletion of the spleen and thymus was observed. No effects were observed in 10 and 40 ppm exposed animals.

13-Week Oral (Dietary Administration) Toxicity and Fertility Study in Rats with TGIC (67)

In this study groups of 10 male Sprague-Dawley rats were exposed to diets containing 0, 10, 30, and 100 ppm of TGIC for 13 weeks, corresponding to 0, 0.72, 2.08, and 7.32 mg/ kg/day. In parallel, 4 groups of 20 female rats received a control diet containing no TGIC. After 64 days, male rats were placed with two female rats in mating cages. One subgroup of the pregnant females were kept isolated and on day 19 of pregnancy they were hysterectomized and the content of the uteri was analyzed. Another subgroup of the pregnant females was allowed to litter and pups were examined during their entire lactating period until weaning. No treatment-related symptoms were observed during the entire treatment period and no mortality occurred. Slightly lower body weights were recorded for the high dose group male rats, and slightly lower leucocyte counts were observed at 100 ppm. Reddish discoloration in combination with hemosiderosis of the mesenteric lymph nodes was found at 100 ppm. Slightly lower sperm counts were recorded for 100 ppm male rats, but sperm viability was not affected. This effect may be secondary to the reduced body weights observed in this group. All fertility parameters were comparable to that of the control animals. Pregnancy rates, litter data in hysterectomized rats, and litter data of delivered pups were comparable to controls, and no change in any fertility

parameter was observed. 30 ppm was considered to be the overall NOEL in this study.

Chronic Toxicity / Carcinogenicity

Carcinogenicity Study in Male Rats with TGIC (68)

In this study groups of 50 male Sprague-Dawley rats were exposed to diets containing 0, 10, 30, 100, and 300 ppm of TGIC, corresponding to 0, 0.43, 1.30, 4.36 and 13.6 mg/kg per day, for 63 weeks (high dose) and 99 weeks for all other doses. Additionally, groups of 30 male rats were exposed to diets containing 0, 100 and 300 ppm of TGIC for 26 weeks. These groups were used for the analysis of subchronic effects.

26-week Study: No mortality occurred, however, reduced food consumption and corresponding lower weight gain were observed in both treated groups in a dose dependent manner. The efficiency of food conversion was markedly reduced at 300 ppm. Lower leucocyte counts and lower lymphocyte counts were recorded in the high dose group, and lower globulin as well as protein concentrations were measured at 300 ppm. Enlarged mesenteric lymph nodes, associated with hemosiderosis, plasmocytosis, mastocytosis and hemorrhage were found at 300 ppm. Slight to moderate lymphoid depletion of spleen and thymus was also observed at 300 ppm but not at 100 ppm. 100 ppm was considered to represent the NOAEL, while no NOEL was determined.

Two year study: Due to the high rate of mortality occurring at 300 ppm (44% at week 62) and due to the poor health condition of the remaining animals exposed to 300 ppm, it was decided to terminate this group at an early stage. The MTD was definitively exceeded with 300 ppm. The cause of death remains still unsolved, but it is suggested that histamine-related hypotension might be a major cause of sudden death. At 100 ppm and below, no difference in mortality as

compared to the control group occurred. At termination of the study (week 99) a slight reduction of body weight at 100 ppm was recorded which correlates well with the noted reduced food consumption. Blood pressure was identical between control and dosed animals. In the groups exposed to 10, 30 and 100 ppm there was no difference in hematology and blood chemistry parameters observed. There was no statistical difference in tumor formation among the treated groups versus control animals, and there was no reduction of the latency period of tumor formation. Differences observed were a high incidence of mastocytosis, hemosiderosis, and sinusoidal hemorrhages in the mesenteric lymph nodes combined with high incidence of lymphoid depletion in the spleen at 300 ppm at 63 weeks. It can be concluded that under the conditions of the assay TGIC is not carcinogenic and a NOEL was determined at 30 ppm corresponding to 1.3 mg/kg/day.

In order to investigate the carcinogenic potential of TGIC, a 30-week dermal initiation-promotion study in mice was performed (22). After an initial dermal application of 150 micrograms of Dimethyl-benzanthracene (DMBA) to the abraded skin of groups of 48 mice (24 males and females each), which is intended to have a tumor initiation effect, all animals were caged for 3 weeks without any treatment followed by 26 week treatment period. During this treatment period one group of animals was treated twice weekly topically (to the abraded skin) with a 2.5% w/v solution of TGIC in acetone. No skin or other tumors were found at the end of the treatment period whereas a positive control group treated with a known carcinogen developed a significant number of tumors during the same period of time.

In Vitro Bacterial and Mammalian Mutagenicity Studies

a) Bacterial Systems

Two Ames Tests using Salmonella and Escherichia coli strains were performed using rat-liver microsomal extracts as metabolizing systems (28, 25). In both studies, TGIC induced increased numbers of revertant colonies (with and without metabolic enzymes) at doses of 1.2 to 5000 micrograms/ml in strains TA98, TA100 and TA1535 but not in strain TA1537, TA1538 and E. coli. These results are observed for molecules containing epoxide groups and are not unexpected.

b) Mammalian Systems (in vitro)

Balb 3T3 Mouse fibroblast cells were tested in two transformation assays with (43) and without (42) metabolic activation for the generation of transformed cells which are no longer contact inhibited and therefore form multi-layered colonies. At dosed of 8.75 to 140 micrograms/ml (without metabolic activation) and 0.3 to 5.0 micrograms/ml (with metabolic activation) no increase of transformed cells was recorded. This finding reduces the likelihood of a carcinogenic potential of TGIC.

In a forward mutation assay using mouse lymphoma cells (41) dosed at 0.175 to 6 micrograms/ml in the presence and absence of metabolic enzymes showed a slight increase of the mutation frequency as compared to control cells, but only at the highest dose tested. Under both conditions (with or without metabolic activation) the high dose was characterized by a low relative growths rate of no more than 10% of control cells which may render the results equivocal, especially since no dose-effect relationship was observed.

A chromosome aberration study on isolated human lymphocytes in vitro (40) did not indicate a mutagenic or clastogenic potential of TGIC. At doses of 0.0625 to 1.0 micrograms/ml (the highest non-toxic concentration applicable without metabolic activation) and 0.625 to 10 micrograms/ml with metabolic enzymes, no increased aberration frequencies were detected. This test indicates the different sensitivities between human and rodent cells, which should be kept in mind for risk assessments.

Another example of the species differences is shown below by comparing two DNA-repair tests performed in human fibroblasts and rat hepatocytes the latter representing in addition a cell type with metabolic activation.
The test in Human fibroblasts (37) dosed at 2.7 to 400 micrograms/ml (the highest dose applicable) showed no signs of extra DNA-synthesis measured by incorporation of 3H-Thymidine into DNA. On the other hand, using rat hepatocytes (38) dosed at 0.2 to 20 micrograms/ml an increase of 3H-Thimidine incorporation was measured at doses of 10 and 20 micrograms/ml, indicating some unscheduled DNA-synthesis or, DNA-repair.

This comparison clearly demonstrates that the rat cells are more sensitive to TGIC, expressed by the much lower dosing regimen, and that human cells, even at 20 times higher doses, did not show any signs of mutagenicity, whereas rat cells did show DNA damage already at much lower doses. This could well be directly correlated with the much higher levels of epoxide hydrolase in human tissue as compared to rat tissue.

c) In Vivo Mammalian Genotoxicity Studies

During the past eight years numerous in vivo studies with mice and hamsters have been performed in order to evaluate the mutagenic and clastogenic potential of TGIC.

Four different types of studies have been conducted measuring different endpoints within the general frame of what is called genotoxicity:

a) Tests designed to evaluate the potential to induce structural chromosomal aberrations in mammalian somatic cells (clastogenic effects);
b) Tests developed to measure the clastogenic effects in male germ cells, e.g. in the reproductive system;
c) Tests designed to measure heritable changes of the genetic material during spermatogenesis and transmitted to the offspring; and
d) Tests designed to detect the potential to induce somatic mutations in early embryos.

(a) Chromosomal aberrations in somatic cells

Two Sister Chromatid Exchange (SCE) studies in somatic Chinese Hamster Cells were performed. Single doses of 35 and 70 mg/kg (30) and 140, 280 and 560 mg/kg (29) were administered by gavage to male and female animals and 24 hours later bone-marrow preparations were analyzed for SCEs.

Doses of 35 and 70 mg/kg (30) were clearly negative with respect to SCE induction, whereas doses 0f 140, 280 and 560 mg/kg (29) significantly increased the number of SCE although no clear-cut dose response relationship could be established. It can therefore be concluded that TGIC induced SCE is NOT clastogenic in mouse spermatocytes at doses of up to 96 mg/kg.

In addition, a Nucleus Anomaly Test was performed in Chinese Hamsters (39). Male and female Hamsters were dosed once with 140, 280 and 560 mg/kg by gavage. 24 hours later bone-marrow cells (as a model for somatic interphase cells) were prepared and analyzed for nuclear anomalies. While no differences were observed between control and low-dose animals, a significant increase of nuclear anomalies, mainly single Jolly bodies, was observed in the intermediate

and high-dose group. Therefore, it has to be concluded that TGIC induced nuclear anomalies at doses of 280 and 560 mg/kg.

(b) Chromosomal Aberrations in Mouse Spermatogonial Cells

These studies are designed to evaluate the potential of a chemical to induce structural aberrations in spermatogonial cells, an early stage of sperm cell development. It is not designed to detect functional mutations transmitted to the next generation, although certain types of chromosomal aberrations such as inversions, deletions and other rearrangements can lead to heritable mutations. Yet, most of the structural abnormalities detectable in such an assay lead to infertile sperm.

Four studies were performed by dosing male mice for five consecutive days with TGIC with doses of 29 to 350 mg/kg (oral gavage application) or 2.5 to 50 mg/m^3 (inhalation study), and analyzing the metaphase chromosomes of spermatogonial cells one day after the last dosing.

In two of the studies (32, 33) doses of 30 and 43 mg/kg did not impair spermatogonial development whereas doses of 125, 128 and 350 mg/kg caused increased numbers of chromosomal aberrations apart from dose-dependent cytotoxic effects. In the third study (54) at all three dose-levels tested (29, 57 and 115 mg/kg) a dose dependent increase of chromosomal aberrations was observed including rare chromosomal structural aberrations normally not observed in control preparations. A NOEL of 5 to 10 mg/kg was calculated.

A fourth study (55) was performed according to the same protocol except that the test substance was applied as a fine powder via inhalation at doses of 2.5, 10 and 50 mg/m^3 air for five consecutive days (during 6 hours per day). While 10 and 50 mg/m^3 air caused extensive cytotoxic effects to male germinal cells, expressed by a marked reduction of dividing cells, rendering it impossible to evaluate the observed increase of chromosomal aberrations, the dose of 2.5 mg/m^3 air showed no increase in chromosomal aberrations and no cytotoxic effects at all.

It can therefore be concluded that TGIC at concentrations of 28 mg/kg and higher (application by gavage) as well as 10 mg/m^3 air and higher (application by inhalation) caused structural chromosomal aberrations in pre-meiotic male germinal cells.

An additional chromosomal aberration study in mice (31), designed to measure the effects on primary and secondary spermatocytes (meiotic and post-meiotic stages of sperm development) was performed by applying TGIC orally at 32 and 96 mg/kg to groups of male mice five times over a period of ten days. Three days later metaphase chromosomes were spread and analyzed for aberration. Neither in primary nor in secondary spermatocytes an increase of chromosomal aberrations was found, indicating that TGIC is clastogenic in mouse spermatocytes at doses of up to 96 mg/kg.

(c) Dominant-lethal effects in mice

In two dominant-lethal tests groups of male mice received a single dose of 137, 275 and 550 mg/kg (34) and 160 and 480 mg/kg (35), respectively, by oral gavage. One day later, they were mated weekly to virgin female mice for three consecutive weeks, covering the post-meiotic phase of sperm cell development. The uteri of all the females were analyzed for lethal mutants expressed as embryonic death.

While in the first study (34), no effects on fertility and embryonic development were observed, a slightly increased number of embryonic death resulting from the first mating period of the high dose group was observed in the second study (35). This increase, however, has to be judged as incidental since it occurred only in females mated during the first mating period, e.g. with sperm already fully developed, dehydrated, with condensed chromatin and therefore almost inaccessible for chemical intereaction. Since mature sperm are more resistant towards chemical attack than earlier stages of sperm development, one would expect to see a more pronounced effect in subsequent mating periods.

A third study followed a slightly different dosing regimen. TGIC was applied to male mice at concentrations of 2.5, 10 and 50 mg/m^3 air via inhalation for 5 consecutive days during 6 hours per day (56). Altogether eight weekly mating periods, covering the whole spermatogonial life-cycle, were analyzed for possible lethal mutants, e.g. embryonic death. Apart from general toxicity (10% death among males of the high dose group) and reduced mating performance during the first three weeks in the high and occasionally in the intermediate dose groups there was no indication of dominant-lethal effects. The reduced and transient fertility probably reflects cytotoxic and clastogenic effects leading to reduced viable sperm numbers expressed by the observed reduced fertility, although general toxicity may have contributed also the reduced mating activity of the males.

Of prime importance is the fact that, despite the toxic effects on mature sperm, spermatids and spermatogonia, there was no increase in embryonic death, e.g. no sign of induction of dominant-lethal mutations. This may support the hypothesis that any damage to the genetic material by TGIC during spermatogonial development may lead only to dead sperm but not to heritable genetic damage.

(d) Mammalian Spot Test in mice

A mammalian spot test in mice designed to detect recessive somatic mutations in melanocytes induced in the early embryological stages of development and expressed after birth. Doses of 13.5, 27 and 54 mg/kg were injected intraperitoneally into female mice pregnant for 7 to 9 days. Neither litter size nor survival of the pups was affected by the applied doses and no increase in mutant spots was observed. Up to 54 mg/kg TGIC did not induce recessive somatic mutations in mice.

Biochemical Studies

In a study to measure the pH-dependent alkylation potency of TGIC (44) through its reaction with the model nucleophile p-nitrobenzyl-pyridine (NBP), TGIC was

hydrolyzed at different pHs and subsequently analyzed for its mutagenic potential, e.g. its alkylating potential, in a Salmonella mutagenicity test. Preincubation at pH 0.1, 1 and 2 caused 100%, 82% and 0% reduction of its alkylating and mutagenic potential, indicating that TGIC can be hydrolyzed or inactivated with respect to its mutagenic potential when passing through the stomach.

In a study to measure the DNA-binding potential of TGIC (60), ^{14}C-labeled TGIC was applied orally to rats at doses of 5, 17 and 200 mg/kg. The animals were killed after 8 hours and DNA from liver and testis was analyzed for DNA-adducts. The results indicate that at a dose of 5 mg/kg there were less than 1 adduct per 10^8 nucleotides, e.g. no measurable interaction with testis-DNA was observed while in liver DNA at the same dose and testis and liver DNA at higher doses measurable amounts of DNA-adducts were found. This experiment shows that at a dose of 5 mg/kg TGIC is not or only to a negligible extent interacting with testis DNA and therefore unlikely to induce heritable damage to the male reproductive cells.

Species differences of Epoxide Hydrolase

TGIC was hydrolized by microsomal Epoxide Hydrolase (mEH) of mouse liver homogenate to the corresponding diol. The rate was 15 nmole/mg protein within 30 min, which is equivalent to 0.5 nmole/mg protein/minute. (61)

Seidegard et al (1986) compared the mEH activity of different species (mouse, rat, rabbit, and human) with different substrates, but without TGIC. The substrates were styrene oxide (SO), benzo(a)pyrene-4,5-oxide (BPO) and estroxide (EO). The results can be summarized as follows (62):

	activity (nmol/mg protein. min)		
Species	**SO**	**BPO**	**EO**
Mouse	4.31 +/- 0.7	1.08 +/- 0.2	1.76 +/- 0.3
Rat	12.7 +/- 1.3	6.9 +/- 0.4	12.8 +/- 0.6
Rabbit	17.1 +/- 2.7	9.11 +/- 1.0	21.3 +/- 1.8
Human	48.3 +/- 3.3	14.0 +/- 1.0	23.1 +/- 1.6

The mouse is shown to be the species with the lowest epoxide hydrolase (EH) activity and therefore, is the most sensitive species with respect to the toxic effects of the epoxides.
For all three substrates the activity in Humans was at least 12 x higher than that of mice. That means that humans can oxidize epoxides about 12 times faster than mice, the species most frequently used for genetic in-vivo experiments.

Interspecies differences are much smaller as shown by Oesch et. al.(1983) with 22 strains of rats yielding only a 3-fold difference between the most extreme rat strains (Fischer F344 and DA/Han) (63). Differences between organs within the same species were observed in rats (63). The highest levels of EH were recorded in liver, followed by testis, kidney, lung, spleen and cutis. The difference between the two extremes was about 100-fold. The half-life of ^{14}C-labeled TGIC, administered intravenously to rabbits (64), was measured to be smaller than 5 minutes, whereas in humans the half-life of TGIC was in the order of 1.4 minutes and larger than 2 minutes in mice (65).

From the above data it can be concluded that humans metabolize TGIC (and other epoxides) much faster than mice do. The mouse as the standard animal species for dominant-lethal, spermatogonial and spermatocyte tests has been shown to be the species with the lowest level of epoxide hydrolases and therefore being the most sensitive species with respect to the toxic effects of epoxides. This will ultimately lead to higher cytotoxicity and greater chance for chromosomal aberrations in mice than in man. With this knowledge in mind, it

would be appropriate to use smaller than usual safety or uncertainty factors to establish an OEL [part of the safety factor accounts for the possible higher sensitivity of humans versus the animal species tested].

Ecotoxicology

The LC_{50}, measured during a time period of 96 hours, for Zebrafish is >77 mg/L (46), 77 mg/L being the saturation concentration. The EC_{50}, measured during a 24-hour period, for Daphnia magna is >77 mg/L, the EC_0 value being 58 mg/L (47). Therefore TGIC has a low toxic profile for aquatic organisms.

In the Modified Sturm Test (48), performed over 28 days, only 9.1% of the test substance was degraded at an initial concentration of 10 mg/L, whereas 48% was degraded at an initial concentration of 20 mg/L. Therefore TGIC is not readily biodegradable.

The melting point of TGIC is around 95°C, and the vapor pressure is as low as 9.1×10^{-4} Pascal at ambient temperature (49).

Summary prepared by Dr. H.J.Weideli and R.J. Papciak.

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