MERQUAT™ 2001 POLYMER - Toxicology Studies

The toxicology studies summarized below were conducted from 1996 to 2001 on polymers with chemical compositions representative of MERQUAT™ 2001 Polymer. Therefore, the toxicology data summarized herein is expected to be predictive of the toxicity of the commercial grades of MERQUAT™ 2001 Polymer.

Eye Irritation

Two GLP compliant studies were performed to assess eye irritation and corrosion in 1997; one to assess the effects of neat (as-produced) substance and another to assess the substance (as used) diluted to a concentration of 5%.

New Zealand White rabbits were received and quarantined for at least five days. Only animals in apparent good health were used in the study. Prior to being selected for this study, both eyes of each animal were examined for any evidence of irritation or abnormalities of the cornea, iris and/or conjunctiva according to the Draize technique. Three rabbits (males), free from evidence of ocular irritation or abnormalities, were assigned to each study.

For each study, the test substances (0.1 ml) were placed by syringe into the conjunctiva! sac which was formed by gently pulling the lower eyelid away from the eye. After instillation, the lids were held together for approximately one second to insure adequate substance distribution. One eye of each rabbit was dosed. The contralateral eye served as a control.

The treated eye of each rabbit was examined for irritation of the cornea, iris and conjunctiva at 1, 24, 48, and 72 hours post dose. Ocular reactions were graded according to the numerical Draize technique.

For animals exposed to neat (as-produced) substance, no corneal opacity or iritis was noted at any observation period. Conjunctiva! irritation, noted in 3/3 eyes, cleared by day 3. No abnormal systemic signs were noted in 2/3 animals. One animal showed signs of diarrhea, few feces and localized alopecia. For animals exposed to a 5% (as-used) solution of substance, no corneal opacity or iritis was noted at any observation period. Conjunctival irritation, noted in 3/3 eyes, cleared by day 1. No abnormal systemic signs were noted during the observation period for the as-used substance.

As-produced and as-used preparations were considered ocular irritants. Neither preparation was considered corrosive.

Skin Irritation and Skin Sensitization

Two studies were performed in accordance with of good clinical practices to assess skin irritation and sensitization in 1997 and 1998 by conducting patch test studies in humans. One study was performed to assess the effects of neat (as-produced) substance and another was performed to assess the substance at an as-used concentration of 5%.

The studies employed an intensified version of the Shelanski and Shelanski (1953)[1] repeated insult patch test. Groups of more than one hundred adult subjects were assigned to each study. During the induction phase, a specific area on the back of each subject was designated to which a patching device pad was infused with approximately 0.2 ml of the test material and affixed to the contact site. The patching device was removed approximately 24 hours later. The exposed skin was examined

[1] Shelanski, H.A. and M.V. Shelanski (1953) A new technique of human patch tests. Proc. Sci. Section, The Toilet Goods Assoc. No. 10, May.

and assigned a score in according to the magnitude of the isible adverse changes that could be visibly detected at the time. During the subsequent challenge phase, an area of the back or arm was chosen as the challenge site.

The application and examination cycle was conducted on each subject on Monday/Tuesday, Tuesday/Wednesday, Wednesday/Thursday, and Thursday/Friday during Weeks #1, #2, and #3 of the induction phase and during Week #5 (i.e., the challenge phase).

For the as-prepared substance during the induction phase, low intensity responses were seen on seventeen subjects but were of negligible clinical significance and did not suffice to characterize the product as an irritant. High intensity responses were seen on seven subjects within eight days after first contact with the product and were consistent with recall responses indicative of sensitization that was induced prior to the initiation of the study (i.e., not likely related to the test substance administered during the test). The high intensity responses of one subject provided an adequate basis to characterize the substance as capable of inducing sensitization in an individual who was apparently naive to the substance until contact was initiated under the study conditions. Although high intensity responses were seen on five other subjects, substance sensitizing activity could not be assessed with confidence since the possibility of cumulative irritation could not be ruled out. Based on these results, exposure to the as-prepared substance was contraindicated.

For the as-used preparation, the low intensity responses obtained on eleven of the subjects during the induction phase were of negligible clinical significance and were insufficient to characterize the product as an irritant under conditions of contact which prevailed during this study. The absence of responses on any of the subjects participating in the challenge phase indicated that the substance was incapable of acting as a sensitizer under conditions of contact which prevailed during this study. Based on these results, exposure to the as-prepared substance was not contraindicated.

Mutagenicity

In 1997, the substance was tested in a GLP compliant bacterial reverse mutation assay[2] using *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and E. coli tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase, the preliminary toxicity assay, was used to establish the dose range for the mutagenicity assay. The second phase, the mutagenicity assay, was used to evaluate the mutagenic potential of the test article.

Water was selected as the solvent of choice based on solubility of the test article and compatibility of the solvent with the target cells. The test article was soluble in water at a maximum concentration of approximately 150 mg/mL.

In the preliminary toxicity assay, the maximum dose tested was 5000 µg per plate. Neither precipitate nor appreciable toxicity was observed. Based on these findings the dosages used in the mutagenicity assay were 6.7, 10, 33, 67, 100, 330, 667. 1000,3333, and 5000 µg per plate.

No positive response was observed in mutagenicity assay in the presence or absence of Aroclor-induced rat liver S9A. Neither precipitate nor appreciable toxicity was observed. All criteria for a valid study were met as described in the protocol. Thus, the results of the bacterial reverse mutation assay indicated that, under the conditions of this study, the substance was considered non-mutagenic.

Acute Oral Toxicity

A study was conducted in 1997 to determine the toxicity of the test article when administered orally to rats. This study was designed to comply with the standards set forth by EPA/TSCA Health Effects Testing Guidelines, 40 CFR Part 798.1175.

Animals were received from Ace Animals, Boyertown, PA. Following a quarantine period of at least one week, five healthy male and five healthy, non-pregnant and nulliparous female Wistar albino rats were randomly assigned to the treatment group. The pretest body weight ranges for males and females were within acceptable limits. The animals were identified by cage notation and indelible body

[2] Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test, Mutation Research, 31:347-364.

marks, and housed 5/sex/cage in suspended wire cages. Bedding was placed beneath the cages and changed at least three times/week. Fresh Purina Rat Chow {Diet #5012) was freely available except for 16-20 hours prior to dosing. Water was freely available throughout the study.

Five male and five female rats were dosed via gavage with the substance at 5000 mg/kg of body weight.

Animals were observed 1, 2 and 4 hours post-dose to assess acute toxicity and once daily for 14 days to assess recovery. The animals were observed twice daily for mortality. Body weights were recorded immediately pretest, weekly, at death and at termination in the survivors. All animals were examined for gross pathology.

All animals survived the 5000 mg/kg oral dose. There were no systemic signs of toxicity noted during the observation period. Body weight changes and necropsy results were normal. The LD50 was determined to be greater than 5000 mg/kg.

Acute Aquatic Toxicity

An acute aquatic toxicity test was performed in 2001 using the cladoceran, *Daphnia magna* per TSCA 797.1300. All organisms used in the test ere less than 24 hours old and in apparent good health. The test was performed under static conditions at 20 ± 2°C at five concentrations of test substance (150, 250, 400, 600, and 1,000 mg/L) and a deionized dilution water control (0 mg/L). Dilution water was adjusted for appropriate hardness and pH. Nominal concentrations of substance were used for all calculations. No insoluble material was observed at any time during the definitive toxicity test. Ten organisms were added to each of two replicate treatments (i.e. controls and substance concentrations). The daphnid loading rate was 40 per liter. The test was performed in loosely covered 300 mL glass beakers containing 250 mL solution. Test vessels were randomly arranged for the 48 hour test and subject to a 16:8 hour light:dark photoperiod providing an approximate light intensity of 22 µEin/m2sec.

The number of surviving organisms, immobilization, and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in behavior) were determined visually, and recorded initially and at 24 and 48 hours. Immobile test organisms were removed when first observed.

Dissolved oxygen, pH, conductivity, and temperature were measured and recorded daily in each discrete test chamber. The temperature in a representative vessel incubated among the test vessels was recorded continuously.

Greater than 50% animals were noted as mobile at all tested concentrations. The in a 48 hour median lethal concentration (LC50) and median effective concentrations (EC50) were greater than 1,000 mg/L. The 48 hour no observed effect concentration (NOEC) was determined to be 250 mg/L.