COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council Good Chemistry Makes It Possible

October 14, 2003

Marianne Lamont Horinko
Acting Administrator
U.S. Environmental Protection Agency
P. O. Box 1473
Merrifield, VA 22116

RE: Olefins Panel Robust Summary for Crude Butadiene C4 Category Under the HPV Challenge Program
HPV Registration No.

Dear Ms. Horinko:

On May 4, 2000, the American Chemistry Council Olefins Panel (Panel) submitted a Test Plan under the High Production Volume (HPV) Chemical Challenge Program pertaining to the Crude Butadiene C4 Category. In the test plan, the Panel indicated that screening data for reproductive effects of 1,3-butadiene would become available. This information is now available and a robust summary for this information is attached. With this submission, the Panel has submitted robust summaries for all proposed studies and modeled or calculated parameters.

The Panel plans to submit a final report on the category in 2004.

If you have any questions, please contact Dr. Elizabeth Moran, Manager of the Olefins Panel at 301 924 2006 or Elizabeth_Moran@americanchemistry.com.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Attachments

cc: R. Hefter (EPA)

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***Toxicity to Reproduction***

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| ***Test Substance*** | |
| Remarks | 1,3-Butadiene<br>CAS#: 106-99-0 |
| ***Method*** | |
| Method/guideline followed | OECD 421 and OPPTS 870.3550 |
| Test type | Inhalation reproduction/developmental toxicity screening test |
| GLP | Yes. |
| Year | 2002 |
| Species | Rat; Adults, 12 weeks old and weighing 342-409 grams (males) and 230-283 grams (females) at initiation of exposures |
| Strain | Crl:CD® (Sprague-Dawley) IGS BR |
| Route of administration | Inhalation (vapor). |
| Duration of test | Daily 6-hour exposures, beginning 14 days prior to initiation of the breeding period (15 exposures prior to breeding); $F_0$ males exposed for 83-84 consecutive days; $F_0$ females exposed through gestation day 20 and from lactation day 5 through the day prior to euthanasia (60-70 total days; $F_0$ females which did not deliver were exposed until one day prior to euthanasia (post-mating day 25); selected $F_1$ males and females (one male and one female from each litter) were exposed for 7 consecutive days (Postnatal days [PND] 21-27 or 28-34) |
| Doses/concentration levels | 0, 300, 1500 and 6000 ppm |
| Sex | 12 male, 12 female per group |
| Exposure period | 6 hours/day |
| Frequency of treatment | 7 days/week |
| Control group and treatment | 12 male, 12 female, air-only exposed |
| Post exposure observation period | Daily; one hour following completion of exposure |
| Statistical methods | Parametric one-way analysis of variance (ANOVA) – body weight, body weight gain, food consumption, gestation length, precoital interval, number of pups born, live litter size, pup weights, organ weights (absolute and relative to final body weight), epididymal and testicular sperm numbers and sperm production rate; Chi-square test with Yates correction factor – mating and fertility indices; Kruskal Wallis with Mann-Whitney U test – sex ratios, postnatal survival, percentage of motile sperm with normal morphology |
| Test conditions | Three groups of $F_0$ animals, each consisting of 12 male and 12 female Crl:CD®(SD)IGS BR rats, were exposed to the test article, 1,3-butadiene, via whole body inhalation exposure for six hours daily for 14 days prior to the initiation of the breeding period and continuing throughout the gestation and lactation periods. A control group of identical design was |

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| Test conditions (continued) | exposed to clean, filtered air on a comparable regimen. For $F_0$ dams, the daily inhalation exposures were suspended on gestation day 21 through lactation day 4, inclusively, in an attempt to avoid any confounding effects of exposure on nesting or nursing behavior; exposures were resumed for these dams on lactation day 5. The $F_1$ generation pups were potentially exposed to the test article *in utero*, and through nursing during lactation until weaning. Beginning on postnatal day (PND) 21, one male and one female from each litter were exposed for seven consecutive days to the same concentration of the test article as its dam. Beginning on PND 28, one previously unexposed male and one previously unexposed female per litter were exposed for seven consecutive days to the same concentration of the test article as its dam.<br>Target test article concentrations were 300, 1500, and 6000 ppm (parts per million).<br>All animals were observed twice daily (at least seven hours apart) for moribundity and mortality; weekly detailed physical examination data were collected for $F_0$ animals. Animals were observed for appearance, behavior, and pharmacotoxic signs prior to exposure, during exposure, and within one hour after completion of each daily exposure period. Body weights and food consumption data were recorded for males and females prior to treatment on the first day of exposure (body weights were also recorded at the midpoint of study week 1) and weekly thereafter until study termination for males and until gestation day 0 for females. During gestation, female body weights and food consumption were recorded on gestation days 0, 7, 14 and 20. Dams were monitored for signs of parturition and the day parturition was initiated was considered PND 0. For $F_0$ dams, body weights and food consumption data were collected on lactation days 1, 4, 7, 14, 21 and 28; data were collected weekly for $F_0$ males. Upon completion of delivery, all $F_1$ pups were individually identified; these offspring were observed daily for appearance, behavior and survival during the postnatal period. Detailed physical examinations and body weights were recorded for each pup on PND 1, 4, 7, 14, 21 and 28; food consumption was not recorded for $F_1$ pups. Pups were sexed on PND 0, 4, 7, 14, 21 and 28. Using a random selection process, litters were reduced to 10 pups (5/sex/litter, if possible), on PND 4. $F_0$ males and females received a detailed clinical examination on the day following their last exposure and were then euthanized by isoflurane inhalation. All $F_0$ animals were subjected to a complete macroscopic evaluation and selected organs were weighed. Designated tissues were examined |

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| Test conditions (continued) | microscopically. A complete spermatogenesis evaluation was conducted for all $F_0$ males and included assessments of motility/viability, morphology, and sperm numbers for both the testis and epididymis.<br><br>All $F_1$ offspring were euthanized and discarded without macroscopic pathological evaluation, with the exception of pups that were stillborn or those that died between birth and PND 4 and any pups that were considered moribund and euthanized in *extremis* during the lactation period. Macroscopic pathological evaluations were performed for those animals. |
| ***Results***<br>Actual mean exposure concentrations | 301, 1507 and 6006 ppm |
| NOAEL | NOAEL (no-observed-adverse effect level) for $F_0$ parental and $F_1$ systemic toxicity for males and females directly exposed to the test article for six hours per day via whole-body inhalation: 300 ppm<br><br>NOAEL for $F_0$ reproductive and $F_1$ developmental toxicity for $F_0$ males and females directly exposed to the test article for six hours per day via whole-body inhalation and $F_1$ offspring exposed to the test article *in utero* and directly for six hours per day via whole-body inhalation: 6000 ppm |
| ***Results (continued)*** | |
| LOAEL (LOEL) | Not applicable |
| $F_0$ and $F_1$ data (adverse responses/effects with NOAEL value) | Signs of chromodacryorrhea, chromorhinorrhea and salivation in $F_0$ males and females at 6000 ppm and infrequent occurrences of dried red material (perioral and perinasal regions) for four exposed $F_1$ offspring (three males and one female).<br><br>Persistent reductions in body weight parameters for $F_0$ and $F_1$ males and females in the 1500 and 6000 ppm groups and transient reductions in food consumption (week 0-1) for $F_0$ males and females in these groups |
| Statistical Results (Test Article-Related Results with Statistical Significance [$p<0.05$ or $p<0.01$] Compared to the Control [0 ppm] Group) | 6000 ppm<br>Reduced $F_0$ male body weight Weeks 1 and 3-8 ($p<0.05$), reduced $F_0$ male body weight gain Weeks 0-1 ($p<0.01$) and 3-4 ($p<0.05$) and reduced cumulative $F_0$ male body weight gain Weeks 0-1 through 0-9 ($p<0.01$) and Weeks 0-10 and 0-11 ($p<0.05$)<br><br>Transient reduced $F_0$ male g/animal/day and g/kg/day food consumption and food efficiency Week 0-1 ($p<0.01$); |

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| Statistical Results (Test Article-Related Results with Statistical Significance [p<0.05 or p<0.01] Compared to the Control [0 ppm] Group) (continued) | increased $F_0$ male g/kg/day food consumption Weeks 5-6 through 8-9, 10-11 and 11-12 (p<0.01) due to lower body weights<br><br>Reduced $F_0$ female g/kg/day food consumption Week 0-1 (p<0.05)<br><br>Reduced $F_0$ female g/kg/day food consumption gestation days 0-7 (p<0.05)<br><br>Reduced $F_1$ male body weight gain PND 23-24 (p<0.05)<br><br>Reduced $F_1$ female body weight PND 25-28 (p<0.05), reduced $F_1$ female body weight gain PND 26-27 (p<0.05)and reduced $F_1$ female body weight gain PND 21-28 (p<0.01)<br><br>Reduced $F_1$ male body weight gain PND 29-30 and 32-33 (p<0.01), reduced $F_1$ male body weight gain PND 28-35 (p<0.05) and reduced $F_1$ female body weight PND 33-35 (p<0.05) and body weight gain PND 28-35 (p<0.01)<br><br>Increased $F_0$ male brain weight relative to final body weight (p<0.05); reduced $F_0$ male seminal vesicle/coagulating gland weight relative to brain weight (p<0.05)<br><br>1500 ppm<br><br>$F_0$ male body weight gain Weeks 0-1<br><br>Reduced cumulative $F_0$ male body weight gain Weeks 0-7 (p<0.05 or p<0.01)<br><br>Reduced $F_0$ male g/animal/day and g/kg/day food consumption Week 0-1 (p<0.05 and p<0.01, respectively); reduced $F_0$ male food efficiency Week 0-1 (p<0.01); increased $F_0$ male g/kg/day food consumption Weeks 6-7 through 11-12 (p<0.01)<br><br>Reduced $F_0$ female g/kg/day food consumption Week 0-1 (p<0.05)<br><br>Reduced $F_0$ female g/kg/day food consumption gestation days 0-7 (p<0.05)<br><br>Reduced $F_1$ male body weight gain PND 29-30 and 32-33 (p<0.05)<br><br>Reduced $F_1$ female body weight PND 27-28 (p<0.05) |

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| Statistical Results (Test Article-Related Results with Statistical Significance [$p<0.05$ or $p<0.01$] Compared to the Control [0 ppm] Group) (continued) | Reduced $F_1$ female body weight gain PND 26-27 ($p<0.01$) and reduced $F_1$ female body weight gain PND 21-28 ($p<0.05$)<br><br>Reduced $F_1$ female body weight PND 31 ($p<0.05$) and PND 32-35 ($p<0.01$)<br><br>Reduced $F_0$ male seminal vesicle/coagulating gland weight relative to brain weight ($p<0.05$) |
| Remarks | Under the conditions of the current study, there were no adverse, test article-related effects on any parameter measured in either the $F_0$ or $F_1$ animals at the exposure level of 300 ppm. There was no test article-related mortality nor were there any apparent effects on gonadal function, mating behavior, conception, gestation, parturition, lactation of the $F_0$ generation at exposure levels up to 6000 ppm. There were no test article-related effects on the development of $F_1$ offspring from conception through weaning. In addition, no test article-related clinical findings were noted for $F_1$ animals directly exposed to the test article (via inhalation) at 300 or 1500 ppm.<br><br>There were no effects on body weight parameters for $F_1$ males and females directly exposed to the test article at 300 ppm.<br><br>There was an exposure-related increase in ejaculatory plugs, which had no apparent biological significance in this study.<br><br>Test article-related effects that were considered adverse that were noted exclusively at 6000 ppm consisted of:<br>Clinical observations indicative of chromodacryorrhea, chromorhinorrhea, and salivation in $F_0$ males and females.<br>Occasional occurrences of dried red material (perioral and perinasal regions) in $F_1$ pups.<br>Test article-related effects that were considered adverse that were noted at 1500 and 6000 ppm consisted of:<br>Persistent reductions in body weight parameters in $F_0$ and $F_1$ males and females.<br>Transient reductions in food consumption (week 0-1) for $F_0$ males and females.<br><br>There were other test article-related observations in the $F_0$ generation that were not considered adverse. Clinical observations consistent with, but less severe than, those reported at 6000 ppm were also reported at 300 and 1500 ppm. These observations were not considered adverse at these lower levels because the signs were always transient and only |

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| | reported during the one-hour post-exposure observations. |
| ***Conclusions*** | Based on the results of this study, an exposure level of 300 ppm was considered to be the NOAEL (no-observed-adverse-effect level) for $F_0$ parental systemic toxicity of 1,3-butadiene when rats were directly exposed to the test article for 6 hours per day via whole-body inhalation. The NOAEL for effects on gonadal function, mating behavior, conception, gestation, parturition, lactation of the $F_0$ generation, and the development of $F_1$ offspring from conception through weaning was considered to be 6000 ppm. The NOAEL for systemic toxicity for $F_1$ animals following postweaning 6-hour daily exposures (PND 21-27 or PND 28-34) was considered to be 300 ppm. There were no measurable differences between animals exposed from PND 21-27 and those exposed from PND 28-34. |
| ***References*** | NA |
| ***Other*** | NA |
| Last changed | 12-Sept-03<br>Robust summary prepared by WIL Research Laboratories, Inc. |