

ROBUST SUMMARY

EVALUATION OF C4 CRUDE BUTADIENE (LOW 1,3-BUTADIENECONTENT) IN THE
MOUSE BONE MARROW MICRONUCLEUS TEST VIA INHALATION EXPOSURE -
MULTIPLE EXPOSURES FOLLOWED BY A SINGLE SAMPLING POINTRECEIVED
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Genetic Toxicity - in Vivo

<u>Test Substance</u>	C4 Crude Butadiene (Low 1,3-Butadiene Content)
Remarks	approx. composition: 10% 1,3-butadiene, 4% isobutane, 4% n-butane, 29% trans-2-butene, 29% 1-butene, 11% isobutylene, 12% cis-2-butene Primary CAS #: 68476-52-8 Other CAS #s in the stream: 25167-67-3, 64742-83-2, 68187-60-0, 68476-44-8, 68955-28-2, and 68956-54-7.
<u>Method</u>	
Method/guideline followed	U.S. EPA OPPTS 870.5395 (1998) and OECD # 474 (1997) guidelines.
Type	Mammalian erythrocyte micronucleus assay.
GLP	Yes.
Year	2001.
Species	Mouse.
Strain	B6C3F1
Sex	Male and Female
Route of administration	Inhalation (gas).
Doses/concentration levels	0, 0.5, 10.0, or 20.0 mg/L.
Exposure period	4 hours/day for 2 days.
Statistical methods	The raw data on the counts of MN-PCE for each animal were first transformed by adding one (1) to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on percent PCE were analyzed separately by a two-way analysis of variance (Winer, 1971). The sex-by-dose interaction in the two-way analysis was reviewed and if significant, a one-way analysis was performed for each sex. Pairwise comparisons of treated vs. control groups were done, if the dose effect was significant, by Dunnett's t-test, one-sided (upper) for MNPCE and two-sided for the percent PCE (Winer 1971). Linear dose-related trend tests were performed only if any of the pairwise comparisons yielded significant differences. The alpha level at which all tests were conducted was 0.05.
Remarks for Test Conditions.	Groups of six male B6C3F1 mice (approximately 26g, 9 weeks old) and six female B6C3F1 mice (approximately 21g, 9 weeks old) were exposed whole-body inhalation to target concentrations of 0, 0.5, 10.0, and 20.0 mg/L of the C4 Crude Butadiene, Low 1,3-Butadiene Content. All inhalation exposures occurred under dynamic airflow conditions and chamber concentrations were monitored by analytical methods. Inhalation exposures occurred on two consecutive days, 4 hours per day. A positive control group was dosed by oral gavage with 120 mg/kg of cyclophosphamide approximately 24 hours before sacrifice. Groups of animals (6/sex/dose) were sacrificed at

<p><u>Results</u></p> <p><u>Conclusions</u> (study author)</p> <p><u>Data Quality</u> <i>Reliabilities</i></p> <p><u>References</u></p> <p><u>Other</u></p>	<p>24 hours after the second treatment for the collection of femoral bone marrow to evaluate the incidence of micronuclei (MN) in polychromatic erythrocytes (2000 PCE/animal) The proportion of PCE among erythrocytes in the bone marrow was estimated by examining 200 erythrocytes/animal.</p> <p>Statistically significant increases in the frequencies of MN-PCE in both sexes of all groups treated with the test material were observed as compared to the negative controls. Although statistical analyses indicated a significant dose response, the difference in MN-PCE incidence at the high- (20 mg/L) and low- (0.5 mg/L) dose was minimal. The positive control treatment induced a significant increase in the frequency of MN-PCE. The mean proportion of PCE among the erythrocytes (200/animal) in the bone marrow was not affected following exposure to the test material while the positive control treatment significantly reduced this value.</p> <p>C4 Crude Butadiene (Low 1,3-Butadiene Content) was positive for the induction of micronuclei in this test system under the experimental conditions used.</p> <p>Reliable without restrictions.</p> <p>Organisation for Economic Co-Operation and Development (OECD) (1997). Guidelines for Testing of Chemicals. #474. Genetic Toxicology: Micronucleus Test, OECD Publication Service, 2 Rue Andre-Pascal, 75775 Paris Cedex 16, France.</p> <p>U.S. EPA (1998). Office of Prevention, Pesticides and Toxic Substances, OPPTS 870.5395. <i>In Vivo Mammalian Bone Marrow Cytogenetic Tests - Micronucleus assay</i></p> <p>Winer, B. J. (1971). <i>Statistical Principles in Experimental Design</i> (2nd Edition). McGraw-Hill, New York, New York.</p> <p>Last updated: Robust summary prepared by contractor to Olefins Panel</p>
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ROBUST SUMMARIES

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C4 CRUDE BUTADIENE, LOW 1,3-BUTADIENE CONTENT: A COMBINED REPEATED EXPOSURE INHALATION TOXICITY STUDY WITH THE REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST IN SPRAGUE DAWLEY RATS

Repeated Dose Toxicity

<p><u>Test Substance</u> Remarks</p>	<p>C4 Crude Butadiene (low 1,3-Butadiene Content), approx. composition: 10% 1,3-butadiene, 4% isobutane, 4% n-butane, 29% trans-2-butene, 29% 1-butene, 11% isobutylene, 12% cis-2-butene Primary CAS#: 68476-52-8; Other CAS #s used to represent this stream : 25167-67-3, 64742-83-2, 68187-60-0, 68476-44-8, 68955-28-2, and 68956-54-7</p>
<p><u>Method</u> Method/guideline followed Test type</p>	<p>OECD 422 Combined repeated exposure inhalation toxicity study with the reproduction / developmental screening test</p>
<p>GLP Year Species Strain Route of administration Duration of test Doses/concentration levels Sex Exposure period Frequency of treatment Control group and treatment Post exposure observation period Statistical methods</p>	<p>Yes. 2001 Rat Crl:CD® (Sprague-Dawley) IGS BR Inhalation (vapor). 36-37 days 0, 2, 10, or 20 mg/L 12 male, 12 female per group. 6 hours/day. 7 days/week 12 male, 12 female, air-only exposed. Not applicable. Adult body weights, body weight gains, feed consumption, organ weights, clinical chemistry data and appropriate hematologic data were evaluated by ANOVA. Detailed clinical observation incidence scores for ranked observations and sensory evaluation scores were statistically analyzed by a z-test of proportions. Rectal temperature and grip performance were analyzed by an analysis of covariance with dose as the factor and time as the covariate. Motor activity was analyzed by a repeated-measure design with treatment as a between-subjects factor and the repeated factor of time.</p>
<p>Test Conditions</p>	<p>Groups of 12 male and 12 female CD rats were exposed to vapors of the test material daily by inhalation for approximately six hours/day at exposure levels of 0, 2, 10, or 20 mg/L. The main study (repeated-exposure general toxicity and neurotoxicity endpoints) males and females were exposed for 36 and 37 days, respectively. Effects on general toxicity, neurobehavioral activity, clinical</p>

<p><u>Data Quality</u> Reliabilities <u>References</u></p> <p><u>Other</u> Last changed</p>	<p>clinical observations, organ weights, gross or histopathology, neurobehavioral activity, clinical chemistry or hematology endpoints. Based on these data, the no-observable-effect level (NOEL) for repeated dose toxicity was 20 mg/L, the highest concentration tested.</p> <p>Klimish value = 1 (Reliable without restrictions). Carney, E.W., Liberacki, A.B., Thomas, J., Houtman, C.E. and Marable, B.R. (2001). C4 Crude butadiene, low 1,3-butadiene content: a combined repeated exposure inhalation toxicity study with the reproduction/developmental screening test in Sprague Dawley rats. Report of The Dow Chemical Company conducted for the American Chemistry Council Olefins Panel.</p> <p>6-Aug-01 Robust summary prepared by contract to Olefins Panel</p>
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<p><u>Results</u> NOAEL (NOEL) LOAEL (LOEL)</p> <p>Remarks</p>	<p>were exposed for two weeks prior to breeding, during breeding (up to two weeks), and continuing through day 19 of gestation. Males from the main study were used to breed these females. The dams were allowed to deliver their litters, which were retained until postnatal day 4. Effects on general toxicity, gonadal function, mating behavior, implantation, and general fertility were evaluated in the satellite group adults, followed by a gross necropsy of the satellite group females on lactation day 5. Litter size, pup survival, sex, body weight, and the presence of gross external malformations was assessed in the offspring. The males were exposed for a total of 36-37 days, and were then necropsied. In addition to the repeated dose toxicity end points assessed (discussed separately), reproductive assessment of the males included mating, conception and fertility indices, reproductive organ weights and gross/histopathology of the reproductive tract. Testis histopathology included a qualitative assessment of stages of the spermatogenic cycle.</p> <p>20 mg/L. Not applicable.</p> <p>Actual time-weighted averages for total olefins for the 2, 10 and 20 mg/L exposure groups were 2.17 ± 0.461, 9.81 ± 1.66, 19.1 ± 2.63 mg/L, respectively, over the 37 exposure days in the study. Owing in part to the nature of the test material, there were technical difficulties in generating vapors from the test material, such that targeted exposure concentrations were not met on one entire day and for brief periods on a few other days. However, the affected instances were limited relative to the total duration of the study and were considered to have no significant impact on study integrity.</p> <p>There were no deaths or treatment-related clinical observations noted. No significant differences in parental body weights, body weight gains or feed consumption were observed at any dose level tested throughout the duration of the study. The only exception to this was a statistically identified increase in feed consumption noted for the 10 mg/L satellite females during the premating period (days 7-14). However, this increase was considered spurious, as feed consumption increases were not noted during subsequent gestation and lactation periods and similar changes in feed consumption were not observed at the highest exposure level of 20 mg/L.</p> <p>There were no treatment-related effects at any dose level on any of the reproductive parameters evaluated in this study. These included measures of reproductive performance (mating, conception and fertility, time to mating, gestation length, litter size), offspring survival (gestation and postnatal survival indices, percent pre- and post-implantation loss), pup body weight and pup sex ratio. The only statistically identified change in any of these parameters was an increase in post-implantation loss occurring only at the low-dose.</p>
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<p><u>Conclusions</u></p> <p><u>Data Quality</u> <u>Reliabilities</u> <u>References</u></p> <p><u>Other</u> Last changed</p>	<p>This was considered a spurious finding, given the lack of a dose response. Of the 12 females mated in each group, the number of viable litters produced was 11, 11, 11, and 12 for the 0, 2, 10 and 20 mg/L exposure level groups, respectively. External morphological alterations observed in the pups were limited to a hernia observed in a single pup from the high dose group. Given the low incidence of this finding, it was considered spurious and unrelated to exposure.</p> <p>Repeated inhalation exposure of C4 Crude Butadiene, Low 1,3-Butadiene to male and female Sprague Dawley rats at levels of 0, 2, 10, or 20 mg/L produced no evidence of adverse effects on any measures of reproductive function. Based on these data, the no-observable-effect level (NOEL) for reproductive toxicity was 20 mg/L, the highest concentration tested.</p> <p>Klimish value = 1 (Reliable without restrictions). Carney, E.W., Liberacki, A.B., Thomas, J., Houtman, C.E. and Marable, B.R. (2001). C4 Crude butadiene, low 1,3-butadiene content: a combined repeated exposure inhalation toxicity study with the reproduction/developmental screening test in Sprague Dawley rats. Report of The Dow Chemical Company conducted for the American Chemistry Council Olefins Panel.</p> <p>6-Aug-01 Robust summary prepared by contractor to Olefins Panel</p>
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Developmental Toxicity/Teratogenicity

<p><u>Test Substance</u> Remarks</p>	<p>C4 Crude Butadiene (low 1,3-Butadiene Content), approx. composition: 10% 1,3-butadiene, 4% isobutane, 4% n-butane, 29% trans-2-butene, 29% 1-butene, 11% isobutylene, 12% cis-2-butene Primary CAS#: 68476-52-8; Other CAS #s used to represent this stream : 25167-67-3, 64742-83-2, 68187-60-0, 68476-44-8, 68955-28-2, and 68956-54-7</p>
<p><u>Method</u> Method/guideline followed</p>	<p>OECD 422</p>
<p>Test type</p>	<p>Combined repeated exposure inhalation toxicity study with the reproduction / developmental screening test</p>
<p>GLP</p>	<p>Yes.</p>
<p>Year</p>	<p>2001</p>
<p>Species</p>	<p>Rat</p>
<p>Strain</p>	<p>Crl:CD[®] (Sprague-Dawley) IGS BR</p>
<p>Route of administration</p>	<p>Inhalation (vapor).</p>
<p>Duration of test</p>	<p>Two weeks prior to breeding, during breeding (up to two weeks), and continuing through day 19 of gestation. The dams were then allowed to deliver their litters, which were retained until postnatal day 4.</p>
<p>Doses/concentration levels</p>	<p>0, 2, 10, or 20 mg/L</p>
<p>Sex</p>	<p>12 male, 12 female per group.</p>
<p>Exposure period</p>	<p>6 hours/day.</p>
<p>Frequency of treatment</p>	<p>7 days/week</p>
<p>Control group and treatment</p>	<p>12 male, 12 female, air-only exposed.</p>
<p>Post exposure observation period</p>	<p>Not applicable.</p>
<p>Statistical methods</p>	<p>Adult body weights and feed consumption, maternal body weight gains, and pup body weights were analyzed by ANOVA. Gestation length, average time to mating (precoital interval) and litter size were analyzed using a nonparametric ANOVA. Pregnancy rates and mating, conception, fertility and gestation indices were analyzed by the Fisher exact probability test. Evaluation of the neonatal sex ratio was performed by the binomial distribution test. Post-implantation loss, pup survival indices, and other incidence data among neonates were analyzed using the litter as the experimental unit by a censored Wilcoxon test.</p>
<p>Test Conditions</p>	<p>Groups of 12 male and 12 female Sprague Dawley rats were exposed to vapors of the test material daily by inhalation for approximately six hours/day at exposure levels of 0, 2, 10, or 20 mg/L. The study design included a main study for repeated dose toxicity end points (summarized separately) and reproductive / developmental toxicity satellite groups of 12 females per exposure level. The reproductive and developmental toxicity satellite groups</p>

