Appendix B

Robust Summaries for Selected Studies on Acetone and Isopropanol

ACETONE
(CAS No. 67-64-1)

VCCEP SUBMISSION
Robust Summary Document

American Chemistry Council Acetone Panel

June 30, 2003

Acetone-RS-1

Test Substance	
CAS #	CAS# 67-64-1
Chemical Name	Acetone
Remarks	Purity: 100% (determined by gas chromatography)
Method	
Test Type	Inhalation developmental toxicology: teratology in rats
GLP (Y/N)	Not Specified
Year (Study Reported)	1988
Species	Rat
Strain	Sprague-Dawley
Route of exposure	Inhalation; whole body exposure chambers
Exposure concentration levels	0, 440, 2,200 and 11,000 ppm High exposure level set at 50% of lower explosion limit. Low exposure concentration set to approximate recommended TLV level.
Exposure generation	Acetone was supplied to a vaporizer located at the fresh air inlet of each animal exposure chamber. Two vapor generators were used on the high exposure chamber. Operating temperatures of the vaporizer were adjusted (93-137^{o}F) to completely vaporize the supplied test material.
Chamber T_{90} and air flow rate	12 minutes at 15 cfm
Chamber analysis	Uniformity of vapor concentration: prior to and once during the study for each chamber using on-line GC. Chamber concentration during exposure: determined by online gas chromatography system equipped with an 8-port stream select valve. Exposure chambers, holding chamber and room were continuously monitored. Acetone degradation and stability studies: yes
Duration and frequency of exposure	6 hours/day; 7days/week for 14 days (gestation days 6-19).
Control and treatment groups	4 groups each consisting of 10 virgin females, 30-31 sperm positive female rats for developmental toxicity evaluation and 7 sperm positive female rats for ketone blood sampling.
Sentinel Animals	5/sex examined prestudy for bacterial pathogens and gross and microscopic pathology.
Animal acclimation period	30 days
Animal feed	NIH-07 diet, ad libitum except during exposures during which feed was removed from the exposure chambers.
Post exposure observation period	None
Mating ratio	One male to two or three females
Duration of mating period	Five consecutive nights
Statistical methods	Analysis of variance, Tukey's t-test, if appropriate an orthogonal trend test. In the case of proportional data the t-test and trend analysis were performed on transformed variables. The litter was used as the basis for analysis of fetal variables.

Remarks field for test conditions	Female rats were weighed and individually identified by ear tag 1-2 weeks prior to mating. At this time 40 virgin females were randomly selected using body weight as the blocking variable. The remaining females were bred by caging two or three females overnight with each male. Mating was confirmed on the following morning by a sperm positive vaginal smear. If evidence of mating was detected this day was designated day 0 of gestation. The positively mated females were weighed and randomly assigned to exposure groups using body weight as the blocking variable. Mating was conducted for 5 consecutive nights in order to obtain 122 positive mated animals (31-32/group). Seven additional mated females were assigned to each exposure group for monitoring of ketone body levels in plasma. Mated females were individually caged in exposure chambers on day 0 of gestation. Mated animals were exposed from gestation days 6 –19 and sacrificed on gestation day 20. Virgin females were weighed and assigned to exposure groups 4 days prior to the start of exposures. They were exposed for 14 consecutive days concurrently with the mated animals and were sacrificed on the day after the last day of exposures. Mated rats were weighed on gestation days 0, 6, 10, 14, 17 and 20. Virgin rats were weighed 2 and 6 days prior to exposure initiation and on exposure days 1, 5, and 10, and at termination. On the day of sacrifice urine from each female rat was evaluated (dip stick) for pH, protein, glucose, ketones, bilirubin, blood and urobilinogen. At necropsy animals were examined grossly for signs of maternal toxicity. Maternal liver and kidney weights were obtained. Ovaries were saved for sectioning and quantitative follicle counts (reported separately). The number, position and status of uterine implants were recorded. Placentas were examined and discarded unless abnormal. Live fetuses were weighed, examined for gross defects and sexed internally. Fifty percent of the live fetuses from each litter, randomly selected, and any fetuses with gross external abnormalities were examined for visceral defects by dissection of fresh tissue. The heads of fifty percent of the live fetuses were placed in Bouin's solution and then serially sectioned and examined for craniofacial abnormalities. All fetal carcasses were stained for skeletal evaluation. Plasma was collected from seven mated females in each exposure group to access the level of ketone bodies (acetone, acetoacetic acid and beta-hydroxybutyrate) in the blood and to determine post-exposure blood levels of acetone. Blood was collected 30 minutes post exposure on gestation days 7, 14 and 19 and one hour prior to the start of exposure on the following day. Fetuses from these females were not evaluated for developmental effects except for gross examination.

Results	Acetone Stability and Chamber Concentration Levels Test material stability studies confirmed that acetone was stable in both the vapor generator reservoir and in the exposure chamber with and without animals. Test material concentration uniformity data for each chamber was acceptable for all chambers prestudy and after animals were in chamber. The daily mean concentrations for all chambers were between 95 and 102% of target At least 97% of individual concentration determinations were within 10% of each target concentration. Fo Females There were no maternal deaths. Pregnant females in the 11,000 ppm group exhibited a significant reduction in body weight, uterine weight and extra-gestational weight gain (maternal body weight at sacrifice minus gravid uterine weight minus day 0 maternal body weight). No other symptoms of maternal toxicity were evident. Mean body weights of the virgin females were not statistically significantly affected at any time during the 14 day exposure period, although there was a 6% reduction in mean body weight of virgin animals in the 11,000 pm group compared to control. This was the same relative reduction in body weight observed in the pregnant animals. Mean liver and kidney weights of the pregnant dams were not affected by exposure to acetone vapors. No exposure related effects were observed on urine pH, protein, glucose, ketones, bilirubin, blood or urobilinogen from pregnant or virgin females. F_1 Litter Data Gestational exposure of rats to acetone vapors on days 6-19 of gestation had no effect on the number of implantations, the mean percent live pups/litter or the mean percent of resorptions/litter. However the percent of litters with resorptions was greater (not statistically significant) for the 11,000 ppm group than for control (77 vs 50%). The number of live fetuses/litter and the percent intrauterine deaths/litter for all groups were within the range of controls. Male and female fetal weights were slightly reduced (approximately 15%) at 11,000 ppm compared to control. Fetal weights at 440 and 2,200 ppm were unremarkable. Fetal sex ratios were not affected by gestational exposure to acetone. Fetal Malformation Data The incidence of fetal malformations was not significantly increased in the acetone exposed groups compared to control. However the percent of litters with at least one pup with malformations was greater at 11,000 ppm than for control (11.5 vs 3.8%). None of the major malformations observed at 11,000 ppm (cleft sternum, ectopic heart, major vessel malformations, edema, unilateral arhinia, and microstomia. Vertebral agenesis, or a missing tail) were observed in control litters. Several fetuses at 11,000 ppm had multiple malformations. Fetal Variation Data Fetal variation data were unremarkable. The types and numbers of variations were comparable in the acetone exposed and control groups. Plasma Ketone Analysis Analysis of plasma samples 30 minutes post exposure showed an increase in plasma acetone levels which correlated with increasing exposure concentration. Acetone levels dropped to control levels by 17 hours post exposure for all exposure groups except 11,000 ppm. The 11,000 ppm group was still slightly elevated compared to control at 17

	hours post exposure. Within exposure groups acetone levels did not increase over the gestation period. Neither exposure to acetone vapor, nor advancing gestation resulted in alterations in the plasma levels of acetoacetic acid or beta-hydroxybutyrate.
Remarks	
Conclusions	The Study Director concluded that the 2,200 ppm acetone level was the no observed effect level (NOEL) in the Sprague Dawley rat for developmental toxicity. The NOEL for maternal toxicity was also 2,200 ppm.
Data Quality	Reliable without restriction (Klimisch Code)
References	NIH Final Report No.: NIH-Y01-ES-70153 (November 1988)
Other	Updated: 4/17/03

Acetone-RS-2

Test Substance	
CAS #	CAS# 67-63-0
Chemical Name	Isopropanol
Remarks	Purity: 99.95%
Method	
Test Type	Developmental neurotoxicity evaluation in time mated rats treated on gestational day 6 through postnatal day 21
GLP (Y/N)	Y
Year (Study Performed)	1990
Species	Rat
Strain	CD(SD)BR (VAF)
Sex	Females
Route of administration	Oral gavage
Duration of treatment	Fo females- Treated from Gestation Day 6 through Postnatal Day 21
Doses/concentration levels	0, 200, 700, and 1200 mg/kg/day
Vehicle control	Deionized/distilled water
Dose volume	5 mL/kg (adjusted based on most recent body weight)
Frequency of treatment	Once/day, treated from gestation day 6 through postnatal day 21
Analytical confirmation of concentration.	Test substance purity, dosing solution homogeneity, stability and dose concentration confirmation.
Control and treatment groups	64 Fo female sperm-positive rats/group
Post exposure observation period	None
Statistical methods	A combination of categorical analysis, survival time and repeated measures techniques were utilized. Analysis included: analysis of variance, Pearson's chi-square test, Fishers exact test, Cochran-Armitage chi-square, Bartlett's test or Shapiro-Wilks', Dunnett's t-test, Fligner's test, Jonckheere's test and survival analysis.
Dose rangefinding study	Several prior studies conducted at this and other testing facilities.
Remarks field for test conditions	Each group in this study was divided into four subgroups or replicates. The breeding dates between replicates were separated by at least 14 days. Each replicate consisted of 16 time mated females/dose level (64 animals/group total). Sperm positive female rats were assigned to treatment groups by a stratified randomization method designed to provide uniform body weights across dose groups at study initiation. Fo females were treated from gestation days 6 through postnatal day 21. All animals were examined twice daily for clinical observations during the dosing period and daily otherwise. Maternal body weights were recorded on days 0, 6, 9, 12, 15, 18, and 20 of gestation and on postnatal days 0, 4, 7, 13, 17, and 21. Food consumption was recorded for corresponding intervals during gestation and for postnatal days 0-3, 3-6, 6-9 and 9-12. Maternal animals were allowed to deliver and rear their young. Pups were counted, examined externally, weighed and sexed on postnatal days 0 and 4. Following examination on postnatal day 4, litter size was standardized by random culling at either a 4:4 or 5:3 sex ratio. Litters with insufficient numbers of pups were removed from study after culling. The remaining litters were examined and weighed on postnatal days 7, 13, 17, 21, 36, 49 and

	68. Male pups were examined daily starting at postnatal day 20 for testicular descent. Female pups were examined daily starting at postnatal day 30 for vaginal opening. One male and one female pup from each litter were assigned to each of three behavioral tests. Motor activity was assessed for one hour in a figure 8 maze on postnatal days 13, 17, 21, 47 and 58. Auditory startle response was assessed on postnatal days 22 and 60. Learning and memory were assessed with an active avoidance paradigm run on postnatal days 60-64. Three sacrifices occurred during the study. The first took place after culling and involved those dams that had failed to deliver as well as the dams and pups from litters of insufficient size or sex ratio. The second sacrifice took place on postnatal day 22 and the third sacrifice occurred on postnatal day 68. On postnatal day 22 the dams were evaluated for body weight, liver and kidney weight and the number of uterine implants. On postnatal days 22 and 68 one male and one female pup from each litter were weighed and killed. A total of 24 of these pups were perfused *in situ* at each sacrifice (postnatal days 22 and 68) and were examined for histopathological lesions of the central and peripheral nervous system. The brains of the remaining animals at each sacrifice were removed and separated into the telencephalon, diencephalons, medulla oblongata/pons and cerebellum. These sections were all weighed separately.

Results	Dosing formulations were homogeneous and stable under refrigerated conditions for at least 49 days. Dosing formulations were 94-103% of target based on predosing analysis. One dam in the 1200 mg/kg/day group died on postnatal day 15. No other maternal deaths occurred. Maternal body weights were equivalent across all groups at all time points. No statistically significant effects on maternal body weight gain were observed. The mean food consumption of the dames in the 700-mg/kg/day group was significantly increased during the postnatal, days 0-3, time interval. No other maternal food consumption effects were noted. There were no treatment related clinical signs observed in the dams at any dose level. The length of gestation was equivalent across all of the groups. The number of live pups born, the perinatal pup death rate, the pup survival rate and the sex ratio at birth were not affected by treatment at any dose level. The test material exposed animals attained sexual maturation at essentially the same time as the control animals. No significant effects were noted on pup weight, nor were any abnormalities or external malformations observed. Of the 64 mated animals/group on this study 27 (control), 27 (low dose), 26 (mid dose) and 31 (high dose) litters had the appropriate litter size and sex ratio to be kept on study after culling on postnatal day 4. Pup behavioral data exhibited no differences in the total motor activity performance for any exposed group compared to control. A significant increase in auditory startle response (mean startle amplitude) was observed in male pups in the second replicate on postnatal day 60 at 700 mg/kg/day. This effect was considered spurious as it only occurred in one replicate of four on only one day of testing. Active avoidance testing yielded one significantly altered variable. Adaptation period crossings were significantly increased in males from the 700 mg/kg/day group on the second day of testing. Since this finding did not reoccur and was not dose related it was not considered biologically significant. No organ weights effects were observed for the dams or for the pups on postnatal days 22 or 68.
Conclusions	The Study Director concluded that isopropanol administered by gavage to pregnant and lactating CD rats resulted in one maternal death out of 35 pregnant animals at 1200 mg/kg/day and no exposure related clinical signs of toxicity in the remaining animals. The no observed adverse effect level (NOAEL) for maternal toxicity/lethality in this study was 700 mg/kg/day. There was no evidence of developmental neurotoxicity at any dose tested. The developmental neurotoxicity NOAEL was greater than 1200 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information (CMA Reference No: IPA-3.0-DNEU-RTI)
Other	Updated: 5/6/2003

Acetone-RS-3

Test Substance	
CAS #	CAS# 67-63-0
Chemical Name	Isopropanol
Remarks	99.9% (determined by gas chromatography)
Method	
Test Type	90 Day Inhalation Motor Activity Study in Female Fisher 344 Rats
GLP (Y/N)	Y
Year (Study Published)	1998
Species	Rat
Strain	Fisher 344
Sex	Female
Route of exposure	Inhalation; whole body exposure chambers
Exposure concentration levels	0 and 5,000 ppm of isopropanol vapor
Exposure generation	Isopropanol was supplied into a heated, spiral-grooved glass evaporator with a countercurrent air stream.
Chamber analysis	Chamber concentrations during exposure were determined at 30-minute intervals by GC.
Duration and frequency of exposure	6 hours/day; 5days/week; One half of the animals were exposed for 9 weeks (9 week subgroup) and the remaining animals were exposed for 13 weeks (13week subgroup).
Control and treatment groups	2 groups each consisting of 30 females (15 females/subgroup/group).
Pretest health screen	Conducted on randomly selected animals
Animal acclimation period	3 weeks
Animal feed and water	Purina Mills certified Rodent Chow® 5002 diet and municipal water, available ad libitum except during exposures and neurobehavioral evaluations.
Motor activity evaluations	Motor activity was assessed for both subgroups prior to the initiation of exposures and following 4, 7 and 9 weeks of exposure. Motor activity was also evaluated in the 13-week subgroup following 11 and 13 weeks of exposure. These evaluations were made 18-20 hours following the end of the last exposure for that week.
Motor activity reversibility evaluations	Evaluations made on days 2, 4 and 7 during the week following the final exposure for rats in both subgroups and weekly thereafter (days 14, 21, 28, 35 and 42 days following the last exposure) for 5 weeks for the 13 week subgroup.
Statistical methods	Continuous parametric variables compared by Levene's test for homogeneity of variances and by t-tests. Motor activity versus test session time curves (habituation curves) analyzed using repeated measures analysis. Repeated measures analysis performed for ambulatory activity, fine movements, rearing activity, and total activity. Cumulative test session motor activity data were analyzed for exposure related changes if the results of repeated measures analysis indicated an effect of treatment. These analyses were performed using the methods described above for continuous parametric variables.

Remarks field for test conditions	Animals were observed individually on a daily basis for clinical signs of toxicity. Clinical signs were recorded on a group basis during exposures. Motor activity evaluations were performed on the schedule outlined above. Motor activity measurements were conducted in an isolated room modified to control sound and light levels and environmental odors. Light levels in the motor activity enclosures ranged from 0.9 to 1.1 ft candles. White noise (59-62 decibels) was generated in the motor activity room during each test session to minimize the potential impact of extraneous noise. The rats were tested individually using an automated photocell recording apparatus designed to measure activity in a novel environment. The length of the test session was 90 minutes. Data for ambulatory activity, fine motor activity, rearing and the sum of these individual types of activity (sum of all counters or total activity) were collected automatically in nine consecutive 10 minute intervals for analysis. All animals were weighed prior to the start of the exposures, weekly during the study, on the days of motor activity testing and immediately preceding sacrifice. Following the final motor activity evaluation the animals were sacrificed. Necropsies were not performed.
Results	
Remarks	The mean isopropanol concentration measured over the exposure was 5011 (±) 105 ppm. No exposure related mortality occurred during the study. Clinical signs noted during exposure included an apparent decrease in movement within the animal cage and a diminished startle response to tapping on the inhalation chamber wall. The only exposure related clinical sign observed during the study during nonexposure periods was swollen periocular tissue. Mean body weight and body weight gain was reduced in the isopropanol exposed animals after one week of exposure. However by week 3 statistically significant increases, compared to control, were observed in these parameters. At the end of week 9, body weight and body weight gains were increased 6% and 17% compared to control. At the end of week 13, body weight and body weight gains were increased 5% and 13% compared to control. Forty-two days following the discontinuation of exposures mean body weights and body weight gains were increased 3% and 9% respectively, compared to control. Female rats exposed to 5,000 ppm exhibited increases in mean cumulative motor activity at all evaluation time points during exposure. These increases reflected increases in ambulation, fine motor activity and rearing activity. The 9-week subgroup exhibited increases at week 4 (41%), week 7 (79%) and week 9 (76%). During recovery the 9-week subgroup recovery values were comparable to control. The 13-week subgroup exhibited increases at week 4 (41%), week 7 (53%), week 9 (144%), week 11 (144%) and week 13 (116%). Recovery was complete by day 14-post exposure. Minor changes were observed in the shape of the motor activity habituation curves for isopropanol exposed animals in each subgroup at approximately 50% of the measurement intervals beginning at week 4. No changes were observed in these curves at 42 days post exposure indicating that complete recovery of motor activity effects had occurred.

Conclusions	The Study Director concluded that the repeated exposure of female rats to 5000 ppm of isopropanol produced reversible increases in motor activity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Journal of Applied Toxicology 18, 373-381 (1998).
Other	Updated: 4/23/03

Acetone-RS-4

Test Substance	
CAS #	CAS# 67-63-0
Chemical Name	Isopropanol
Remarks	Purity: 99.9%
Method	
Test Type	Multi-generation (2-generation) rat reproduction study
GLP (Y/N)	Y
Year (Study Performed)	1990-1991
Species	Rat
Strain	Sprague-Dawley CD VAF/Plus
Sex	Males and females
Route of administration	Oral intubation
Treatment Schedule	P1 animals: dosed daily for 10 weeks prior to mating and throughout the mating period for the F1 (P2) animals and until the day prior to euthanasia. P2 animals: dosed daily for 10 weeks prior to mating and throughout the mating period for the F2 animals and until the day prior to euthanasia. F1 (P2) litters were dosed beginning on day 21 postpartum. P1 and P2 females were dosed during gestation, lactation, and through weaning on Day 21 postpartum of the F1 and F2 litters.
Doses/concentration levels	0, 100, 500 and 1000 mg/kg/day
Vehicle control	Reverse osmosis water
Dose volume	5 mL/kg (adjusted based on most recent body weight)
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and dose concentration confirmation.
Control and treatment groups	30 P1 and P2 rats/sex/group in the control, low, mid and high dose groups.
Post exposure observation period	None
Mating ratio	One male to one female from the same treatment group.
Duration of mating period	Up to 7 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second male from another unmated pair from the same dose group for up to seven additional days. If necessary this process was repeated a third time for a maximum number of 21 mating days total.
Statistical methods	Body weights, food consumption, organ weights and relative organ weights were evaluated using Bartlett's test and a one-way analysis of variance followed by Dunnett's test. A linear regression for dose response was also performed and tested for lack of fit to the regression line. Male and female fertility index, male mating index, female fecundity index, gestational index, live birth and lactation indices, survival indices and the incidence of P2 male centrilobular hepatocyte hypertrophy were evaluated using the Chi-square test and Fisher Exact test. Armitage's test for linear trend was also performed. Pup weight was analyzed by a standard nested analysis of covariance with pups nested within dams and with dams nested within doses with litter size as the covariate. Differences between groups were evaluated

	using the Least Significant Difference technique. Males and females were tested separately.
Dose rangefinding study	None
Remarks field for test conditions	*P1 Generation* Viability checks were performed at least once daily. Clinical examinations were performed and body weights obtained for each male prior to P1 selection, on the first day of dosing and weekly thereafter. Clinical examinations were performed and body weights obtained for each female prior to P1 selection, on the first day of dosing and weekly thereafter until mating was confirmed, then on Days 0, 7, 14 and 21 of gestation and on Days 0, 4, 7, 14 and 21 postpartum. Food consumption was determined in the males and females weekly except during mating and in the females on Days 0, 7, 14 and 21 of gestation and on Days 0, 4, 7, 14 and 21 postpartum. The P1 mating period began after 10 weeks of dosing and ended when all females were confirmed mated or 3 weeks of mating had elapsed. Sibling matings were avoided. Females were housed for up to 7 days with the initial male; if positive evidence of mating was not present (sperm or copulatory plug) then the female was paired with a second male from another unmated pair from the same dose group for up to seven additional days. If necessary this process was repeated a third time for a maximum number of 21 mating days total. The day of mating confirmation was considered Day 0 of gestation. Mated females were returned to individual housing. On gestation Day 21 mated females were provided bedding and were examined twice daily for signs of parturition. Parental males were euthanized and necropsied following delivery of their last litter. Parental females were euthanized and necropsied following weaning of their litters. Mated females that failed to deliver by Day 26 were necropsied. Dams whose entire litter died prior to weaning were euthanized and necropsied. Organ weights (liver and kidney) were collected for all P1 animals that survived to scheduled termination. The reproductive tissues, pituitary and gross lesions from the control and high dose groups and the liver and kidneys from all P1 animals that survived to scheduled termination were examined microscopically. *Postnatal Evaluations – F1 Litters* The date of birth was considered postnatal day 0. Litters were checked daily during the postnatal period for deaths and unusual conditions. Dams were observed for viability, nesting behavior and nursing behavior. Dead pups were examined externally and internally depending on the extent of autolysis. Offspring were counted; live animals were sexed, weighed and examined for anomalies on postnatal Days 0, 1, 4, 7, 14, and 21. On Day 4 postnatal litters were reduced in size to 4 male and 4 female pups. Litters of less then 8 were not adjusted. Culled pups were euthanized. Culled pups that appeared normal received an external examination and tissues were not saved. Culled pups that appeared abnormal were given a cursory gross necropsy and tissues were

preserved as deemed necessary for possible microscopic examination.

On postnatal Day 21, surviving pups were examined and 5 randomly selected pups/sex/group were euthanized and a gross necropsy performed. In addition 30 pups/sex from the control, low and mid dose groups and 26 pups/sex from the high dose group were randomly selected for the P2 parental pool. These animals were group housed by sex until postnatal Day 28. Treatment of these animals began on postnatal day 21. All high dose pups were selected due to deaths among the high dose weanlings prior to selection of the P2 generation. Runts were not excluded.

P2 Generation

Procedures for the treatment, evaluation and mating of the P2 (F1) generation and for the F2 litters were identical to the procedures described above. However, one high dose sibling pairing occurred in the P2 generation. The P2 mating period was as described above for the P1 mating period. Necropsy and microscopic evaluation of P2 animals were as described above for the P1 generation.

Postnatal Evaluations – F2 Litters

All observations specified above for the F1 litters were made for the F2 litters. At postnatal Day 21 gross necropsies were performed on 5 pups/sex/group.

Results	*P1 and P2 Generations* No treatment related clinical signs of toxicity were observed in any parental animals during the study. In addition no treatment related trends were evident in the parental mortality data of either generation. Statistically significant increases were observed in mean absolute and/or relative liver and/or kidney weights of the parental animals. These increases occurred primarily in the high dose animals compared to concurrent controls. Some effects were also observed at the mid dose level. No treatment related microscopic alterations were observed in the liver or kidney from treated female animals from either generation. Microscopic alterations characteristic of male rat hyaline droplet nephropathy were observed in the kidneys from treated male rats of both parental generations. Male rat hyaline droplet nephropathy has been shown to be a rat specific phenomenon and is not considered relevant to human risk assessment. The increase observed in male rat kidney weights was considered related to male rat hyaline droplet nephropathy. In addition, centrilobular hepatocyte hypertrophy was observed in a few high dose P2 male rats and was considered treatment related. Increased male and female liver weights and female kidney weights from both parental generations were considered adaptive changes typical of increased metabolic load from the test material. There were no significant postmortem findings in either parental generation and no treatment related microscopic changes were observed in the reproductive organs or other tissues. No statistically or biologically significant differences were observed in the reproductive data of the treated or control animals of either parental generation. *F1 and F2 Generations* Increased mortality was observed in the high dose F1 offspring compared with control from postnatal Days 0-2. However no clinical signs of toxicity were observed in treated or control offspring of either generation. High dose male F1 body weights were statistically lower than control on postnatal days 0 and 1 and F2 high dose male and female body weights were statistically significantly lower than control on postnatal Days 0, 1 and 4 compared with control. Several F1 weanlings died or were euthanized prior to P2 selection, one each in the low and mid dose groups and 18 in the high dose group. No treatment related post mortem findings were observed in the offspring of either generation. Stability and concentration analysis of dosing solutions confirmed that dosing solutions were stable for up to 28 days under refrigerated conditions and that dosing solutions were within 8% of the intended nominal concentrations.
Conclusions	The Study Director concluded that a dose of 1000 mg/kg/day of isopropanol produced evidence of parental effects as indicated by increases in absolute and/or relative liver and/or kidney weights compared to control. In addition, increased absolute or relative liver weights were observed in parental animals dosed at 500 mg/kg/day. These effects were not considered to be an adverse effect.

	Centrilobular hepatocyte hypertrophy was observed in a few high dose P2 male rats. Increased mortality was observed in high dose F1 offspring compared to controls. In addition high dose offspring of both generations were lighter than controls at several intervals. Therefore the parenteral NOAEL (No Observed Adverse Effect level) was established at 500 mg/kg/day, while the reproductive NOAEL was established at >1000 mg/kg/day. The offspring toxicity NOAEL was 500 mg/kg/day based on reduced offspring body weights and increased mortality observed at 1000 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Confidential business information
Other	Revised 5/12/2003

Acetone-RS-5

Test Substance	
CAS #	CAS# 67-63-0
Chemical Name	Isopropanol
Remarks	Purity: 99.9 % by gas-liquid chromatography
Method	
Test Type	An inhalation oncogenicity study in rats
GLP (Y/N)	Y
Year	1991-1993
Species	Rat
Strain	Fisher 344
Sex	Males and females
Route of exposure	Inhalation; whole body exposure chambers
Duration of test	104 weeks
Exposure period	72 or 104 weeks
Post exposure observation period	None
Duration and frequency of exposure	6 hours/day; 5 days/week for at least 104 weeks
Exposure concentration levels	0, 500, 2,500 and 5,000 ppm Target concentrations selected based on prior studies conducted in Fisher 344 rats.
Exposure generation	Liquid isopropanol was metered from a container by a pump into a heated glass evaporator. Two or three generators were connected together in series in order to generate the 2500 and 5000 ppm target concentrations. Evaporator temperature was maintained at the lowest level sufficient to vaporize the test substance.
Chamber T_{99} and air flow rate	T_{99}: 20-22 minutes; air flow: 900-1000 L/min
Chamber analysis	Uniformity of vapor concentration: determined prior to and during the sixth, twelfth, eighteenth and twenty fourth months of the exposure regimen. Chamber concentration during exposure: determined twice/hour by flame ionization gas chromatography. Chamber temperature and humidity: determined twice/hour during exposures.
Control and treated groups	65/sex/group exposed for 104 weeks (core study animals) 10/sex/group exposed for 72 weeks (interim sacrifice animals)
Statistical methods	Data for quantitative continuous variables were evaluated using Levene's test for equality of variances, analysis of variance, and t-tests. Nonparametric data were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Mortality was analyzed by life table analysis. Incidence data were compared using Fisher's Exact test.
Dose rangefinding study	Prior 9 day and 13 week inhalation studies were conducted.
Remarks field for test conditions	All animals were individually observed for signs of toxicity except during exposures when observations were recorded on a group basis. On nonexposure days animals were examined once/day for overt clinical signs and twice daily for mortality. Detailed observations were recorded on each animal weekly. Body weights were recorded weekly for the first 14 weeks and every other week thereafter. Indirect ophthalmoscopic and slit lamp examinations were performed on all animals prestudy and during weeks 71, 80, 104(males), and 107 (females). Blood smears were obtained for the core study animals at approximately 13,

19, and 25 months. Differential leukocyte counts were evaluated for all surviving core animals from the control and high concentration group at these time points. Hematology measurements were made for all surviving core animals at the terminal sacrifice. Animals were not fasted prior to bleeding. The hematology parameters evaluated included: hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte count, differential leukocyte count and platelet count.

Samples for urinalysis and urine chemistry were collected from 10/sex/group at Study week 57. Evaluated parameters included protein, glucose, renal epithelial cells and volume. Osmolality was determined for a separate group of 10/sex/group. Samples were also collected at weeks 74 (males and females), 104 (males) and 108 (females) for the evaluation of protein, glucose, osmolality and volume.

At scheduled sacrifices animals were anesthetized and exsanguanated. Gross necropsies were performed. Organ weights were obtained for the liver, kidneys, brain, heart, lungs, testes and spleen. A complete list of tissues was preserved for all animals. These tissues were examined microscopically for all control and high exposure group animals from the interim sacrifice and terminal sacrifice. The kidneys and gross lesions were examined for all animals.

Results

Remarks

The mean analytical and nominal isopropanol concentrations calculated over the duration of the exposure period were as follows:

Target Concentration (ppm)	Analytical Concentration (ppm)	Nominal Concentration (ppm)
500	504 ± 14	493 ± 14
2,500	2509 ±58	2522 ± 53
5,000	5037 ±115	5012 ± 108

No daily mean concentration of isopropanol vapor above the estimated minimum detection limit was measured in the control chamber.

Appropriate chamber vapor distribution was demonstrated repeatedly throughout the study (coefficient of variation less than 3%). Plots of the number and mass concentration of particles confirmed no differences between the 5000 ppm and 0 ppm exposure atmospheres indicating that an aerosol was not present in the 5000 ppm chamber.

Mortality rates for the core, 24 month sacrifice animals, were as follows:

Target Concentration (ppm)	Male	Female
0	82%	54%
500	83%	48%
2,500	91%	55%
5,000	100%	69%

The last male from the 5000 ppm group died during week 100. The mean survival time of the 5000 ppm males was significantly less than control.

Clinical signs noted in some male and female mice during exposures to 5000 ppm included hypoactivity, lack of startle response and narcosis. Hypoactivity and lack of startle response were also noted for some males and females during exposure to 2500 ppm. No clinical signs were noted during exposure of animals at 500 ppm. Clinical signs noted during non-exposure

periods included emaciation and dehydration for male rats from the 5000 ppm group. In addition a slight increase was observed in the number of male and female rats at 5000 ppm with urine stains and in the number of female rats with swollen periocular tissue. Urine stains were also observed more frequently in the females at 2500 ppm than in control. No clinical signs were noted during nonexposure periods that were considered exposure related in the male rats at 2500 ppm or in the males or females at 500 ppm.

Decreased body weight and/or body weight gain were noted for the male and female rats from the 5000 ppm group at the end of the first and second weeks of exposure. Following this time point, increased body weight and body weight gain were noted. For male rats from the 2500 and 5000 ppm groups, increased body weights and body weight gain were observed for the duration of the study. For the females concentrated-related increases in body weight and body weight gain were observed throughout most of the study, although the increases observed for the 500 ppm group were slight and probably not statistically significant.

No exposure related ophthalmoscopic findings were noted for any of the treated animals. No exposure related changes in hematology parameters were observed for male or female rats from any exposure group at any time period. At the one-year evaluation urinalysis and urine chemistry for male and female rats from the 5000 ppm group revealed a decrease in osmolality and an increase in total protein (males only) and total volume. At 74 weeks the males and females from the 5000 ppm group exhibited a decrease in osmolality as well as increases in total protein, total glucose (females only) and/or total volume. Similar findings were observed in the 5000 ppm females at termination. None of the 5000 ppm males were alive at the time of this evaluation. Similar changes were observed for males from the 2500 ppm group at weeks 74 and 104.

Absolute and relative (to body and brain) liver and kidney weights were increased for male rats from the 5000 ppm group at the interim sacrifice and for male rats from the 2500 ppm group at terminal sacrifice. Relative liver weight (as a percentage of brain weight) was also increased for male rats from the 2500 ppm group at the interim sacrifice. The 5000 ppm females exhibited an increase in absolute and relative (to body and brain) liver and kidney weight. Other organ weight changes included a concentration related increase in testes weight (absolute and relative to body and brain) observed in male rats at the interim sacrifice. In addition, an increase in lung weight (absolute and relative to body and brain) was observed in the 5000 ppm females at the interim sacrifice but not at termination.

At the interim sacrifice, the only exposure related gross lesion was an increase in granular kidneys for males from the 2500 and 5000 ppm groups. At the terminal sacrifice this finding was also observed for the 2500 ppm males. Male rats in the 2500 and 5000 ppm groups that died or were sacrificed moribund exhibited an increase incidence of thickened stomachs, granular kidneys and color changes of the kidneys. No exposure related gross lesions were observed for female rats that were sacrificed at the interim or terminal sacrifice. For female rats that died or were sacrificed moribund an increased incidence of thickened stomachs (5000 ppm) and granular kidneys (2500 and 5000 ppm) were observed.

Microscopic evaluation revealed that the kidney was a target for nonneoplastic effects in rats exposed repeatedly to isopropanol vapor. Male rats at 2500 and 5000 ppm that died or were sacrificed moribund exhibited mineralization, tubular dilation, glomerulosclerosis, interstitial nephritis, interstitial fibrosis, hydronephrosis and transitional cell hyperplasia. In addition an increase in the severity of many of these lesions was observed for surviving male and female rats from the 2500 and 5000 ppm groups. Increased frequencies of other lesions which were believed to be a result of the renal lesions or increased soft tissue mineralization included cellular hyperplasia of the parathyroid glands (females only), myocardial degeneration/fibrosis, glandular ectasia within the gastric mucosa (females only), and fibrous osteodystrophy in male and female rats from the 5000 ppm group that died or were sacrificed moribund. Other nonneoplastic lesions observed with increased frequency in male rats at 5000 ppm that died or were sacrificed moribund included basophilic cell foci within the liver, splenic hemosiderosis, rhinitis and squamous metaplasia of the respiratory epithelium within the nasal cavity and iridocyclitis. Other nonneoplastic lesions observed with increased frequency in female rats at 5000 ppm that died or were sacrificed moribund included atrial thrombosis, splenic hemosiderosis, ocular keratitis, rhinitis, dacryosolenitis, and squamous metaplasia of the respiratory epithelium within the nasal cavity.

The only neoplastic lesion observed during the study was interstitial cell adenomas of the testes. An increased frequency of testicular seminiferous tubule atrophy and interstitial cell adenomas of the testes was observed for male rats at 5000 ppm at the one-year sacrifice. A concentration related increase in interstitial cell adenomas of the testes was also observed for male rats found dead or sacrificed moribund. The overall frequencies of interstitial cell adenomas of the testes were as follows:

Target Concentration (ppm)	%
0	64.9
500	77.3
2,500	86.7
5,000	94.7

Independent consulting pathologists indicated that the frequency of this finding in the control group was unusually low contributing to the appearance of a significant effect in the isopropanol exposed groups.
A decrease in pituitary adenomas and granular lymphocyte leukemia was observed for male rats at 5000 ppm, which died or were sacrificed moribund. This decrease was attributed to the early mortality observed in this group. There were no increased frequencies of neoplastic lesions for female rats. However, an exposure related decrease in the frequency of large granular lymphocyte leukemia was observed for isopropanol exposed females.

The main cause of death for the males and females at 5000 ppm was chronic renal disease. This also contributed to many of the deaths observed in the males at 2500 ppm. The main cause of death in the male and female controls was large granular lymphocyte leukemia.

Conclusions	The Study Director concluded that exposure of rats to isopropanol vapor for 24 months produced clinical signs of toxicity (hypoactivity, lack of startle response or narcosis) during exposures at 2500 and 5000 ppm as well increased body weight and body weight gain. A number of nonneoplastic lesions were observed, with the most significant lesions being in the kidney. The only neoplastic lesion observed for male rats was an increase in interstitial cell adenomas of the testis, which was considered to represent marked hyperplasia and not autonomous growth. In addition the increased incidence of testicular tumors in the isopropanol exposed groups appeared to be reflective of the lower incidence observed in the control group. No increased frequencies of neoplastic lesions were noted in the females exposed to isopropanol. Thus the no observed effect level (NOEL) for toxic effects was 500 ppm and the NOEL for oncogenicity effects was determined to be greater than 5000 ppm.
Data Quality	Reliable without restriction (Klimisch Code)
References	Confidential Business Information
Other	Updated: 5/20/03

Acetone RS-6

Test Substance	
CAS #	CAS# 67-64-1
Chemical Name	Acetone
Remarks	Purity: 99% by gas-liquid chromatography
Method	
Method/Guideline followed	NTP Protocol
Test Type	A 13 week drinking water toxicity study in mice
GLP (Y/N)	Not Specified
Peer Review	Yes, NTP Board of Scientific Counselors Technical Reports Review Subcommittee and associated Panel of Experts and NTP Pathology Working Group
Year (Study Reported)	1991
Species	Mouse
Strain	$B6C3F_1$
Sex	Males and females
Route of administration	Oral (drinking water)
Duration of test	13 weeks
Doses/concentration levels	Males: 0, 1,250, 2,500, 5,000, 10,000 and 20,000 ppm Females: 0, 2,500, 5,000, 10,000, 20,000 and 50,000 ppm
Exposure period	13 weeks
Frequency of treatment	Acetone treated drinking water available *ad libitum*.
Control group and treatment	10 animals/sex/group
Post exposure observation period	None
Drinking Water Analysis	Yes, for stability and confirmation of acetone concentration.
Statistical methods	Tests included non-parametric multiple comparison procedures of Dunn or Shirley. Jonckheere's test was used to assess the significance of the dose response trends and to determine whether Dunn's or Shirley's test was more appropriate for pairwise comparisons.
Dose rangefinding study	Prior 28 day study.
Remarks field for test conditions	The animals were exposed to the test material through the drinking water, *ad libitum,* throughout the 13 week study duration. Clinical observations were made twice daily. Body weights were recorded prior to initiation, weekly during the treatment period and prior to necropsy. Water consumption was recorded twice weekly. Hematology was evaluated at necropsy. Sperm morphology and vaginal cytology examinations were performed. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and high dose and select tissues were examined at lower dose levels.
Results	
Remarks	Acetone consumption expressed as a time weighted average (mg/kg/day) over the 13 weeks of study was as follows:

Dose Concentration (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
1,250	380	-
2,500	611	892
5,000	1,353	2,007
10,000	2,258	4,156
20,000	4,858	5,945
50,000	-	11,298

	All animals survived the 13-week duration of the study. No clinical signs of toxicity were reported. Treated male and female mean body weights and body weight gains were comparable to control throughout the study. Water consumption was reduced in all treated females groups compared to control. The greatest difference from control (-52%) was noted in the females treated at 50,000 ppm. Hematology results revealed no consistent effects other than minimal increases in hematocrit and/or hemoglobin, which could be attributed to slight dehydration. These increases were observed in the males at concentrations of 5000 ppm and greater and in the females at 20,000 and 50,000 ppm. Absolute and relative liver weights were increased and absolute and relative spleen weights were decreased in the females only at 50,000 ppm. Sperm morphology and vaginal cytology were unremarkable in all treated groups. The only histopathological change associated with acetone exposure in this 13-week study was centrilobular hepatocellular hypertrophy. This finding was characterized by cells with abundant eosinophilic cytoplasm and slightly enlarged nuclei. These changes were zonal, involving all liver lobes with a minimal degree of severity in 2 of 10 females at 50,000 ppm.
Conclusions	The Study Director concluded that acetone was mildly toxic to female mice when administered in drinking water for 13 weeks. The minimal toxic dose was estimated to be 50,000 ppm for female mice. The liver was identified as a female mouse target organ. Significant toxicity was not observed in male mice. Target organs were not identified in the male.
Data Quality	Reliable without restriction (Klimisch Code)
References	NIH Publication No.: 91-3122; January 1991
Other	Updated: 5/15/03

Acetone-RS-7

<table>
<tr><td><u>Test Substance</u></td><td></td></tr>
<tr><td>CAS #</td><td>CAS# 67-64-1</td></tr>
<tr><td>Chemical Name</td><td>Acetone</td></tr>
<tr><td>Remarks</td><td>Purity: 99% by gas-liquid chromatography</td></tr>
<tr><td><u>Method</u></td><td></td></tr>
<tr><td>Method/Guideline followed</td><td>NTP Protocol</td></tr>
<tr><td>Test Type</td><td>A 13 week drinking water toxicity study in rats</td></tr>
<tr><td>GLP (Y/N)</td><td>Not Specified</td></tr>
<tr><td>Peer Review</td><td>Yes, NTP Board of Scientific Counselors Technical Reports Review Subcommittee and associated Panel of Experts and NTP Pathology Working Group</td></tr>
<tr><td>Year (Study Reported)</td><td>1991</td></tr>
<tr><td>Species</td><td>Rat</td></tr>
<tr><td>Strain</td><td>Fisher 344 rat</td></tr>
<tr><td>Sex</td><td>Males and females</td></tr>
<tr><td>Route of administration</td><td>Oral (drinking water)</td></tr>
<tr><td>Duration of test</td><td>13 weeks</td></tr>
<tr><td>Doses/concentration levels</td><td>0, 2500, 5000, 10,000, 20,000 and 50,000 ppm</td></tr>
<tr><td>Exposure period</td><td>13 weeks</td></tr>
<tr><td>Frequency of treatment</td><td>Acetone treated drinking water available ad libitum.</td></tr>
<tr><td>Control group and treatment</td><td>10 animals/sex/group</td></tr>
<tr><td>Post exposure observation period</td><td>None</td></tr>
<tr><td>Drinking Water Analysis</td><td>Yes, for stability and confirmation of acetone concentration.</td></tr>
<tr><td>Statistical methods</td><td>Tests included non-parametric multiple comparison procedures of Dunn or Shirley. Jonckheere’s test was used to assess the significance of the dose response trends and to determine whether Dunn’s or Shirley’s test was more appropriate for pairwise comparisons.</td></tr>
<tr><td>Dose rangefinding study</td><td>Prior 28 day study.</td></tr>
<tr><td>Remarks field for test conditions</td><td>The animals were exposed to the test material through the drinking water, ad libitum, throughout the 13 week study duration. Clinical observations were made twice daily. Body weights were recorded prior to initiation, weekly during the treatment period and prior to necropsy. Water consumption was recorded twice weekly. Hematology was evaluated at necropsy. Sperm morphology and vaginal cytology examinations were performed. Macroscopic examinations were performed on all animals. Select organs were weighed. A range of tissues was examined microscopically in the control and high dose and select tissues were examined at lower dose levels.</td></tr>
<tr><td><u>Results</u></td><td></td></tr>
<tr><td>Remarks</td><td>Acetone consumption expressed as a time weighted average (mg/kg/day) over the 13 weeks of study was as follows:
<table>
<tr><th>Dose Concentration (ppm)</th><th>Males (mg/kg/day)</th><th>Females (mg/kg/day)</th></tr>
<tr><td>2,500</td><td>200</td><td>300</td></tr>
<tr><td>5,000</td><td>400</td><td>600</td></tr>
<tr><td>10,000</td><td>900</td><td>1,200</td></tr>
<tr><td>20,000</td><td>1,700</td><td>1,600</td></tr>
<tr><td>50,000</td><td>3,400</td><td>3,100</td></tr>
</table>
All animals survived the 13-week duration of the study. No clinical signs of toxicity were reported. Body weight gains were depressed in the males (28%) and females (13.5%) treated at 50,000 ppm compared to control. Water</td></tr>
</table>

	consumption was reduced in males and females at 50,000 and in females only at 20,000 ppm. Male and female rats at 50,000 ppm and male rats at 20,000 ppm exhibited a mild but statistically significant leukocytosis, produced by an absolute increase in lymphocytes. A mild, statistically significant, depression in erythrocyte count was observed in the males at 20,000 and 50,000 ppm. Males exposed at 5,000 ppm or higher also exhibited depressed reticulocyte counts and slightly depressed hemoglobin levels. In addition, marginal, statistically significant, increases in mean corpuscular hemoglobin and mean cell volume were observed. These findings were considered consistent with a mild macrocytic normochromic anemia with a depressed regenerative response. Platelet counts were marginally depressed in males and females at 50,000 ppm. Alterations in relative (to body weight) organ weights were observed in the kidney, liver and testis as follows: Relative kidney weights increased at 20,000 ppm and greater for females and 50,000 ppm for males. Relative liver weights increased at 20,000 ppm and higher in males and females. Relative testes weight increased at 50,000 ppm. Decreased sperm motility, caudal weight and epididymal weight and an increased incidence of abnormal sperm were seen in males at 50,000 ppm only. No testicular effects were evident microscopically. Vaginal cytology was unremarkable in all treated groups. Upon microscopic examination minimal to mild hemosiderosis was observed in the spleens of males treated at 20,000 and 50,000 ppm. Hematoxylin and eosin stained bone marrow sections were unremarkable. The incidence and severity of nephropathy, observed in male rats only, increased with increasing dose and appeared to be treatment related at 20,000 and 50,000 ppm.
Conclusions	The Study Director concluded that acetone was mildly toxic to rats when administered in drinking water for 13 weeks. The minimal toxic dose was estimated to be 20,000 ppm for male rats. The testis, kidney and hematopoietic system were identified as male rat target organs. Target organs were not identified in the female. The incidence and severity of nephropathy observed in treated males were considered the most prominent treatment related findings in this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	NIH Publication No.: 91-3122; January 1991
Other	Updated: 5/15/03

Acetone-RS-8

Test Substance	
CAS #	CAS# 67-64-1
Chemical Name	Acetone
Remarks	Purity: 99+ % by gas-liquid chromatography
Method	
Test Type	A drinking water immunotoxicity study via antibody response to sheep red blood cells (SRBC)
GLP (Y/N)	Y
Year	2003
Species	Mouse
Strain	CD-1
Sex	Males
Route of exposure	Drinking water
Duration of test	29 days
Exposure period	28 days
Post exposure observation period	0 days; evaluation on day 29 following 28-day exposure period.
Duration and frequency of exposure	Continuous (*ad libitum* in drinking water) for 28 days
Exposure concentration levels	0, 100, 500, or 1,000 mg/kg/day Target concentrations selected based on prior studies conducted in mice. 1000 mg/kg/day equals guideline limit dose. Time-weighted average doses based on water consumption and actual body weights were 0, 121, 621, 1144 mg/kg/day.
Exposure generation	Acetone mixed with municipal water once per week at concentrations of 0.6, 3 or 6 mg/ml.
Concentration analysis	Target concentrations verified one time analytically following defined mixing instructions. All solutions were verified to be within 5% of the target concentrations.
Control and treated groups	8/group administered water only for 28 days (vehicle control) 8/group administered water only for 28 days, and cyclophosphamide (positive control for immunosuppression) 8/group administered 100 mg acetone/kg/day for 28 days 8/group administered 500 mg acetone/kg/day for 28 days 8/group administered 1000 mg acetone/kg/day for 28 days
Statistical methods	Means and standard deviations for body and organ weights, antibody response and most hematology were analyzed using a Bartlett's test followed by ANOVA. WBC differentials and RBC indices were not statistically compared. If analyses indicated a significant difference by the ANOVA, the treatment groups were compared to the drinking water control using a Dunnett's test.
Dose range-finding study	None
Remarks field for test conditions	Twice each day a cage-side examination was conducted and to the extent possible the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, morbundity, mortality and the availability of feed and water. Body weights were obtained prior to study start and weekly thereafter. Water consumption was determined weekly for each mouse. Test material intake was calculated using solution concentrations, body weights and water consumption data.

	Four days prior to sacrifice, all mice were immunized with a single intravenous injection of 1 x 10^8 SRBC via the lateral tail vein. Approximately 24 hours after the last dosing, serum was collected via retro-orbital bleeds and spleens and thymuses were removed and weighed. A single cell suspension was prepared for each spleen and the number of splenocytes enumerated. Splenocytes were combined with SRBC, guinea pig compliment and agar to conduct an Antibody, Plaque Forming Cell (AFC) assay. In addition, hematology profiles (hematocrit, RBC count, platelet count, RBC indices [MCH, MCV and MCHC], hemoglobin concentration, total WBC count and WBC differential were evaluated.
Results	
Remarks	The time-weighted average acetone doses calculated over the duration of the exposure period were as follows. Vehicle Control (0 mg/kg/day) Positive Control (0 mg/kg/day) 100 mg/kg/day (121 mg/kg/day) 500 mg/kg/day (621 mg/kg/day) 1,000 mg/kg/day (1144 mg/kg/day) Consumption values of acetone-supplemented water were not different from municipal drinking water with one exception. During the first week, the mice provided water containing approximately 6 mg/ml acetone (1,000 mg/kg/day) drank an average of 1.1 gram less water than did mice from the vehicle control group. However, this level of water consumption was not appreciably less than that measured over days -5 to 1 and did not compromise the targeted acetone exposure of 1,000 mg/kg/day (average acetone consumption for days 1-8 = 1,179 mg/kg/day). Body weights were unremarkable throughout the 28 day study and no mortalities occurred in any group. There were no signs of toxicity noted during daily health observations. Hematology parameters were unaffected by acetone consumption and showed no treatment-related effects. Eosinophil percentages appeared to be lower than controls in some groups of mice treated with acetone but the results were not dose-related, with all values within the range of historical controls. Pair wise comparisons following acetone treatment for 28 consecutive days did not identify statistical differences from controls for spleen or thymus weights from acetone-treated animals. The mean thymus weights of mice administered acetone at 1,144 mg/kg/day were about 25% lower than controls but were not statistically different from control thymus weights. Any suggestion of a treatment-related effect on thymus weights was not supported by a treatment-related effect for

	WBC counts or lymphocyte differentials. Any change in thymus weights and selected hematology parameters at the highest dose of acetone were also not consistent with previous studies. Dietz *et al.* evaluated similar end points (including thymus weights) in B6C3F1 mice following higher acetone exposures for an extended exposure period *via* drinking water and reported no effects on thymus weights, eosinophils or WBC counts. It is possible that the reduced thymus weights merely reflect the beginning of an overt stress response as aside from MHC II expression, the thymus is known to be the most sensitive marker in the immune system for stress conditions. No effects were noted for spleen cellularity or AFC response following acetone exposures. The AFC responses ranged from 1088 – 1401 AFC/10^6 splenocytes following water or acetone administration, and were not statistically different from control values.
Conclusions	*Ex vivo* tests that challenge immune cells to respond functionally (*e.g.*, AFC response) are generally more convincing indicators of immunotoxicity and in this study, no treatment related effect of acetone on the SRBC antibody response was observed. The eosinophils in two acetone treated groups appeared to be lower than the controls but demonstrated no dose-related trend, hence this observation does not appear to be treatment-related. Furthermore, these data were within the range of historical values for negative controls and were not consistent with hematology data reported in a previous study. Lymphoid organ weights are useful adjunct measures for the assessment of immunotoxicity potential. In this study, acetone-treatment did not produce any alterations in spleen or thymus weights that were statistically different from controls. In conclusion, acetone administration under the conditions of this study did not produce immunotoxicity in CD-1 mice.
Data Quality	Reliable without restriction (Klimisch Code 1a)
References	Woolhiser, M.R., Andersen, P.K. and Waechter, J.M., Jr. (2003) Acetone: 4-Week Drinking Water Immunotoxicity Study in CD-1 Mice. Report of The Dow Chemical Company.
Other	

Acetone-RS-9

Test Substance	
CAS #	CAS# 67-64-1
Chemical Name	Acetone and 2,5-Hexanedione, alone and in combination
Remarks	Purity: Not Specified
Method	
Test Type	6 Week drinking water fertility study in male rats
GLP (Y/N)	Not Specified
Year (Study Published)	1991
Species	Rat
Strain	Mol/Wistar, SPF
Sex	Males and females
Route of administration	Orally by drinking water (Males treated only)
Duration of test	6 week treatment period (Animal Set A) 6 week treatment period plus 10 week recovery period (Animal Set B)
Doses/concentration levels	Animal Sets A and B (Males treated only): 2,5-Hexanedione: 0.13, 0.25, 0.50% Acetone: 0.5% Acetone: 0.5% plus 0.13% 2,5-Hexanedione Acetone: 0.5% plus 0.25% 2,5-Hexanedione Acetone: 0.5% plus 0.50% 2,5-Hexanedione
Vehicle control	Water
Frequency of treatment	*Ad libitum*
Control and treatment groups	Set A: 8 groups 10/sex/group (treatment) Set B: 8 groups 10/sex/group (treatment and recovery)
Mating	1 treated male mated to 1 untreated female until evidence of mating (presence of copulatory plug) was observed.
Post dosing period	10 weeks
Statistical methods	The number of matings and the number of pregnancies were evaluated using the Chi-square test. Body weight, food and water intake, number of fetuses, testis weight and tubular diameter were evaluated by Student's t-test.
Dose rangefinding study	None
Remarks field for test conditions	This study consisted of two sets of animals. The animals in Set A were exposed to the test materials for 6 weeks and then sacrificed. The animals in Set B were exposed to the test materials for 6 weeks and were then held for a 10-week nontreatment period. Only the male animals were treated with the test materials. Body weights, food and water consumption were evaluated for the males weekly. At the end of the six-week dosing period the fertility of the Set A males was evaluated. Following the 10-week recovery period the fertility of the Set B males was evaluated. Each Set A male was introduced daily during the last week of dosing to a non-dosed virgin female. Each Set B male was introduced daily during the last week of recovery to a non-dosed virgin female. On day 20 of gestation the females were sacrificed and the number of pregnant females and the number of fetuses were determined. At termination of dosing (Set A) and recovery (Set B) the testes were examined microscopically. Cross sections of the left testes

	were used for a semi-quantitative scoring of the pathological changes. Slides were scored for the number of vacuoles and the severity of other changes. Seminiferous tubular diameter was determined in the recovery animals only. Thirty tubules were measured on each slide for each male in each group following the termination of the recovery period.
Results	No treatment related effects were observed in the 0.5% acetone only treatment group. The water intake of the 0.5% acetone exposed rats did not differ from the control rats during dosing. The water intake of the rats exposed to 2,5-hexanedione was dose dependently decreased and slightly further decreased when this treatment was combined with acetone. Based on average water intake the calculated intake of 2,5-hexanedione in the three dose groups was 170, 270, and 440 mg/kg/day. Food consumption and weight gain were also decreased dose dependently in animals treated with 2,5-hexanedione alone and were further decreased when treated with both 2,5-hexanedione and acetone. During recovery the water intake, food consumption and body weight gain of the treated animals were comparable to control except in the 0.5% 2,5-hexanedione and 0.5% 2,5-hexanedione plus 0.5% acetone groups which continued to exhibit reduced food consumption. The number of males that mated, as evidenced by a copulatory plug, was not affected by treatment in any group. Treatment with 0.5% 2,5-hexanedione alone caused a significant decrease in the number of pregnancies and the number of fetuses and a reduction in testes weight. All of these effects were potentiated by acetone. The highest combined treatment resulted in infertility in the rats. After the 10-week recovery period the effects on the testes were still evident. Minor effects were reduced during recovery but infertility and other severe effects were not reversible within the 10-week recovery period. Tubular diameter was the most sensitive parameter to 2,5-hexanedione. It was reduced dose dependently and significantly ($P<0.01$) at all dose levels and further reduced when combined with acetone. No pathological changes could be seen by light microscopy in rats dosed for six weeks with 0.13% 2,5-hexanedione. Minor changes were seen in some rats dosed with 0.25% 2,5-hexanedione. Acetone slightly potentiated the effects of 2,5-hexanedione. Thus an increased number of vacuoles and the margination of chromatin in the tubular cells were seen in rats dosed with 0.13% 2,5-hexanedione and 0.5% acetone. The minor changes observed in rats treated with 0.25% 2,5-hexanedione were partly reversible during the 10 week recovery period. However, the atrophy observed after 6 weeks in rats treated with 0.5% 2,5-hexanedione further developed during the following 10-week recovery period. Acetone enhanced this atrophy resulting in almost total atrophy of the testis at the end of the recovery period.

Conclusions	The Study Director concluded that no treatment related effects were observed in the 0.5% acetone only treatment group. 2,5-Hexanedione has a testes injuring effect that at high dose levels and in combination with acetone lead to nonreversible infertility before there was any influence on mating. The no effect level for acetone alone was 0.5%. No effect levels were not established for 2,5-hexanedione when administered alone or in combination with 0.5% acetone.
Data Quality	Reliable without restriction (Klimisch Code)
References	Pharmacology and Toxicology 1991, 69, 43-46.
Other	Updated: 5/5/2003

Acetone-RS-10

Test Substance	
CAS #	CAS# 67-64-1
Chemical Name	Acetone
Remarks	Purity: 100% (determined by gas chromatography)
Method	
Test Type	Inhalation developmental toxicology: teratology in mice
GLP (Y/N)	Not Specified
Year (Study Reported)	1988
Species	Mouse
Strain	Swiss (CD-1)
Route of exposure	Inhalation; whole body exposure chambers
Exposure concentration levels	0, 440, 2,200 and 6,600 (reduced from 11,000 ppm on Day 1 based on clinical observations) High exposure level of 11,000 ppm originally set at 50% of lower explosion limit. Low exposure concentration set to approximate recommended TLV level.
Exposure generation	Acetone was supplied to a vaporizer located at the fresh air inlet of each animal exposure chamber. Two vapor generators were used on the high exposure chamber. Operating temperatures of the vaporizer were adjusted (93-137^{o}F) to completely vaporize the supplied test material.
Chamber T_{90} and air flow rate	12 minutes at 15 cfm
Chamber analysis	Uniformity of vapor concentration: prior to and once during the study for each chamber using on-line GC. Chamber concentration during exposure: determined by online gas chromatography system equipped with an 8-port stream select valve. Exposure chambers, holding chamber and room were continuously monitored. Acetone degradation and stability studies: yes
Duration and frequency of exposure	6 hours/day; 7days/week for 12 days (gestation days 6-17)
Control and treatment groups	4 groups each consisting of 10 virgin females and 33 plug-positive female mice for developmental toxicity evaluation
Sentinel Animals	5/sex examined prestudy for bacterial pathogens and gross and microscopic pathology.
Animal acclimation period	30 days
Animal feed	NIH-07 diet, ad libitum except during exposures during which feed was removed from the exposure chambers.
Post exposure observation period	None
Mating ratio	One male to two or three females
Duration of mating period	Five consecutive nights
Statistical methods	Analysis of variance, Tukey's t-test, if appropriate an orthogonal trend test. In the case of proportional data the t-test and trend analysis were performed on transformed variables. The litter was used as the basis for analysis of fetal variables.

Remarks field for test conditions	Female mice were weighed and individually identified by ear tag 1-2 weeks prior to mating. At this time 40 virgin females were randomly selected using body weight as the blocking variable. The remaining females were bred by caging two or three females overnight with each male. Mating was confirmed on the following morning by the presence of a vaginal plug. If evidence of mating was detected this day was designated day 0 of gestation. The positively mated females were weighed and randomly assigned to exposure groups using body weight as the blocking variable. Mating was conducted for 5 consecutive nights in order to obtain 132 positive mated animals (33/group). Mated females were individually caged in exposure chambers on day 0 of gestation. Mated animals were exposed from gestation days 6–17 and sacrificed on gestation day 18. Virgin females were weighed and assigned to exposure groups 2 days prior to the start of exposures. They were exposed for 12 consecutive days concurrently with the mated animals and were sacrificed on the day after the last day of exposures. Mated animals were weighed on gestation days 0, 6, 9, 12, 15 and 18. Virgin mice were weighed 2 and 6 days prior to exposure initiation and on exposure days 1, 5, and 10, and at termination. At necropsy animals were examined grossly for signs of maternal toxicity. Maternal liver and kidney weights were obtained. Ovaries were saved for sectioning and quantitative follicle counts (reported separately). The number, position and status of uterine implants were recorded. Placentas were examined and discarded unless abnormal. Live fetuses were weighed, examined for gross defects and sexed internally. Fifty percent of the live fetuses from each litter, randomly selected, and any fetuses with gross external abnormalities were examined for visceral defects by dissection of fresh tissue. The heads of fifty percent of the live fetuses were placed in Bouin's solution and then serially sectioned and examined for craniofacial abnormalities. All fetal carcasses were stained for skeletal evaluation.

Results	Acetone Stability and Chamber Concentration Levels Test material stability studies confirmed that acetone was stable in both the vapor generator reservoir and in the exposure chamber with and without animals. Test material concentration uniformity data for each chamber was acceptable for all chambers prestudy and after animals were in chamber. The daily mean concentrations for all chambers were between 96 and 102% of target At least 98% of individual concentration determinations were within 10% of each target concentration. On the last day of study one of the two generators used on the high exposure chamber failed. This exposure was discontinued after 5 hours and 9 minutes rather then at 6 hours. Fo Females Mice in the 11,000 ppm chamber exhibited severe narcosis at the end of the first day of exposure. Therefore acetone concentration was reduced in the high level to 6,600 ppm for the remainder of the exposure period. As a result 10 virgins and 5 dams were exposed to the higher concentration for 1 exposure day. The remaining high exposure dams were exposed to 6,600 ppm throughout the exposure period. There were no maternal deaths and no overt signs of toxicity evident in any of the groups after the highest exposure level was reduced to 6,600 ppm. There were no effects evident on maternal body weight, uterine weight or extra-gestational weight gain (maternal body weight at sacrifice minus gravid uterine weight minus day 0 maternal body weight) in any of the exposure groups. Mean kidney weights of the pregnant dams were not affected by exposure to acetone vapors. Mean liver weights and liver to body weight ratios in the high exposure group were significantly greater than control. F_1 Litter Data Gestational exposure of mice to acetone vapors on days 6-17of gestation resulted in a slight, but significant, increase in the percent of late resorptions at 6,600 ppm. Acetone exposure had no effect on the number of implantations/litter, the mean percent live pups/litter or the mean percent of total intrauterine deaths. Male and female fetal weights were significantly reduced (approximately 8%) at 6,600 ppm compared to control. Fetal weights at 440 and 2,200 ppm were unremarkable. Fetal sex ratios were not affected by gestational exposure to acetone. Fetal Malformation Data The incidence of fetal malformations was not significantly increased in the acetone exposed groups compared to control. No fetal malformations were observed that had not previously been found in control fetuses. Fetal Variation Data There was no increase in the incidence of fetal variations when all types were combined. However, when examined individually there was a statistically significant increase in the incidence of reduced sternal ossifications in the high exposure group. Data in the low and mid exposure groups were unremarkable.
Remarks	

Conclusions	The Study Director concluded that the 2,200 ppm acetone level was the no observed effect level (NOEL) in the Swiss (CD-1) mouse for developmental toxicity. Minimal maternal toxicity was observed at 6,600 ppm. The NOEL for maternal toxicity was also 2,200 ppm.
Data Quality	Reliable without restriction (Klimisch Code)
References	NIH Final Report No.: NIH-Y01-ES-70153 (November 1988)
Other	Updated: 4/17/03

Acetone-RS-11

Test Substance	
CAS #	CAS# 67-63-0
Chemical Name	Isopropanol
Remarks	Purity: 99.9 % by gas-liquid chromatography
Method	
Test Type	An inhalation oncogenicity study in mice
GLP (Y/N)	Y
Year	1991-1993
Species	Mouse
Strain	CD-1
Sex	Males and females
Route of exposure	Inhalation; whole body exposure chambers
Duration of test	78 weeks
Exposure period	54 or 78 weeks
Post exposure observation period	24 weeks following the 54-week exposure period.
Duration and frequency of exposure	6 hours/day; 5 days/week for 54 or 78 weeks
Exposure concentration levels	0, 500, 2,500 and 5,000 ppm Target concentrations selected based on prior studies conducted in CD-1 mice.
Exposure generation	Liquid isopropanol was metered from a container by a pump into a heated glass evaporator. Two or three generators were connected together in series in order to generate the 2500 and 5000 ppm target concentrations. Evaporator temperature was maintained at the lowest level sufficient to vaporize the test substance.
Chamber T_{99} and air flow rate	T_{99}: 20-22 minutes; air flow: 900-1000 L/min
Chamber analysis	Uniformity of vapor concentration: determined prior to and during the sixth, twelfth and eighteenth months of the exposure regimen. Chamber concentration during exposure: determined twice/hour by flame ionization gas chromatography. Chamber temperature and humidity: determined twice/hour during exposures.
Control and treated groups	55/sex/group exposed for 78 weeks (core study animals) 10/sex/group exposed for 54 weeks (interim sacrifice animals) 10/sex/group exposed for 54 weeks followed by a 24 week recovery period.
Statistical methods	Data for quantitative continuous variables were evaluated using Levene's test for equality of variances, analysis of variance, and t-tests. Nonparametric data were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Mortality was analyzed by life table analysis. Incidence data were compared using Fisher's Exact test.
Dose rangefinding study	Prior 9 day and 13 week inhalation studies conducted.
Remarks field for test conditions	All animals were individually observed for signs of toxicity except during exposures when observations were recorded on a group basis. On nonexposure days animals were examined once/day for overt clinical signs and twice daily for mortality. Detailed observations were recorded on each animal at each body weight weighing interval. Body weights were recorded weekly for the first 14 weeks and every other week thereafter.

Blood smears were obtained for the core study animals at 12 and 18 months. Differential leukocyte counts were evaluated for all surviving core animals from the control and high concentration group at these time points. Hematology measurements were made for all surviving core animals at sacrifice. Animals were not fasted prior to bleeding. The hematology parameters evaluated included: hematocrit, hemoglobin, erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte count, differential leukocyte count and platelet count.

At scheduled sacrifices animals were anesthetized and exsanguanated. Gross necropsies were performed. Organ weights were obtained for the liver, kidneys, brain, heart, lungs, testes and spleen. A complete list of tissues was preserved for all animals. These tissues were examined microscopically for all control and high exposure group animals from the core, interim sacrifice and recovery animals.

Results

Remarks

The mean analytical and nominal isopropanol concentrations calculated over the duration of the exposure period were as follows.

Target Concentration (ppm)	Analytical Concentration (ppm)	Nominal Concentration (ppm)
500	502 ± 13	490 ± 15
2,500	2504 ±54	2513 ± 58
5,000	5020 ±105	4986 ± 114

No daily mean concentration of isopropanol vapor above the estimated minimum detection limit was measured in the control chamber.

Appropriate chamber vapor distribution was demonstrated repeatedly throughout the study (coefficient of variation less than 2%). Plots of the number and mass concentration of particles confirmed no differences between the 5000 ppm and 0 ppm exposure atmospheres indicating that an aerosol was not present in the 5000 ppm chamber.

Mortality rates for the core, 18 month sacrifice animals, were as follows:

Target Concentration (ppm)	Male	Female
0	35%	24%
500	40%	35%
2,500	47%	22%
5,000	44%	33%

Mortality rates for the recovery sacrifice animals, were as follows:

Target Concentration (ppm)	Male	Female
0	30%	30%
500	30%	50%
2,500	20%	50%
5,000	40%	20%

No statistically significant differences from control were observed in mean survival time for any of the isopropanol 18-month sacrifice or 12 month sacrifice/recovery groups of animals.

Clinical signs noted in some male and female mice during exposures to 5000 ppm included hypoactivity, lack of startle response, ataxia, prostration and narcosis.
Hypoactivity, lack of startle response and narcosis were also noted for some males and females during exposure to 2500 ppm. No clinical signs were noted during exposure of animals at 500 ppm. The only clinical sign noted during non-exposure periods (including recovery), believed to be exposure related, was ataxia. This was noted in some male and all female mice at 5000 ppm immediately following exposure. This finding was generally absent the following morning.

Concentration related percent increases in core animal body weights and body weight gains compared to control were observed as follows:

Target Concentration (ppm)	Male		Female	
	Body Weight	Body Weight Gain	Body Weight	Body Weight Gain
500	2%	6%	-	-
2,500	5%	23%	-	15%
5,000	7%	30%	5%	30%

Increases from control were generally statistically significant for the 2500 and 5000 ppm males and were observed beginning weeks 6 and 3 respectively. Occasional small increases in body weight gain were observed for core females at 500 ppm. However, these increases were inconsistent and not clearly related to exposure. The increases noted in the females at the two highest exposure levels were consistent throughout the study. The mean body weight and body weight gain data of the recovery animals were generally comparable to those of the core animals during the exposure and recovery phases of the study.

No exposure related changes in hematologic parameters were observed for male or female mice from any of the isopropanol exposed groups that were examined at 12 months or at study termination.

At the 12-month sacrifice, absolute and relative (to body and brain weight) liver weights were increased for male mice from the 5000 ppm group. These increases were considered exposure related. No other organ weight effects were observed at 12 months.

At the terminal sacrifice, slight, but not statistically significantly, increased liver/body weight ratios were noted in the core males at 2500 (+7%) and 5000 ppm (+6%). A concentration related increase in absolute liver weight and liver/body weight ratio was noted for core females at the terminal sacrifice, however, statistical significance was only observed at 5000 ppm. The increases in liver/body weight ratios were 1 and 5% at 500 and 2500 ppm. The only organ weight change noted in recovery animals was a concentration related increase in absolute and relative (to body and brain) liver weight in male mice. Differences from control were generally statistically significant. The

	exposure related increases observed in liver weights were not associated with any microscopic findings and were most likely attributed to microsomal enzyme induction. A decreased absolute brain weight and brain/body weight were noted in the females at 5000 ppm. There was no microscopic finding associated with this organ weight change. Increased frequencies of abnormal stomach content were observed for male mice from all isopropanol exposure groups at the interim sacrifice. This finding was not believed to be of biological significance. The only gross lesion noted at terminal sacrifice was an increased frequency of seminal vesicle enlargement for male mice at 2500 and 5000 ppm. Microscopic evaluation revealed an increased incidence of ectasia (dilation) of the seminal vesicles for males at 2500 and 5000 ppm. No associated inflammatory or degenerative changes were present. Microscopic examination also revealed an increased incidence of tubular proteinosis in the kidney of male and female mice from all isopropanol exposure groups. Female mice from the high exposure group were also noted to have renal tubular dilation. These findings were not considered biologically significant due to their minimal degree and lack of an increased frequency of other more serious renal lesions. There were a few other microscopic lesions noted only in female mice and included mucosal cell hyperplasia within the glandular portion of the stomach, congestion of the adrenal gland, and extramedullary hematopoiesis and hemosiderosis of the spleen. None of these findings were believed to be biologically significant due to the degree (minimal-mild) and nature of the findings. There were no increased frequencies of neoplastic lesions noted for the core male or female mice from any exposure group. No exposure related differences in microscopic frequencies of nonneoplastic or neoplastic lesions were seen for the recovery male or female mice.
Conclusions	The Study Director concluded that exposure of mice to isopropanol vapor for 18 months produced clinical signs of toxicity (hypoactivity, lack of startle response, narcosis, ataxia or prostration) during exposures at 2500 and 5000 ppm as well increased body weight and body weight gain. The few nonneoplastic lesions observed were minimal in degree and probably not biologically significant. There were no increased frequencies of neoplastic lesions noted for male or female mice from any isopropanol exposure group. Thus the no observed effect level (NOEL) for toxic effects was 500 ppm and the NOEL for oncogenicity effects was determined to be greater than 5000 ppm.
Data Quality	Reliable without restriction (Klimisch Code)
References	Confidential Business Information
Other	Updated: 5/15/03