211(b) Toxicology Research Group
Gas+ETOH Rat Developmental Toxicity Study Report
Reviewer Checklist

Comments	Action/Response	Page #
EPA		
ORD finds that., in general, the six developmental toxicity studies were conducted according to the EPA test guidelines. At this time, however, we cannot recommend approval of the study reports as submitted, because we have identified several deficiencies in the statistical analyses and reporting of data. The attached memoranda provide specific comments on each of the study reports.	No action required	
Additionally, in our review of the developmental toxicity study reports, we noticed that certain data on dams that delivered early were omitted from the appendices because the data were excluded from the statistical analyses. Please note that all of the data generated as part of the21 l(b) testing program must be provided to EPA for review, even if they are negative or are not used in the statistical analyses.	Data added to report	C-4, C-8, AND D-3
We strongly encourage the RG to address our comments before revised versions of the reports are submitted to EPA. The RG should also adequately respond to the comments raised byte independent peer reviewers and provide the additional information requested. To help our reviewers complete the reviews of the revised study reports, we also request that a summary of the changes or marked-up copies be provided.	Checklist prepared	
In general the NHEERL reviewers concluded that the studies were conducted in accordance with the testing guidelines; however, several issues were raised that warrant further attention. Although not essential, it would be preferable to include tables into the text that accurately summarize developmental toxicity data, showing some of the key endpoints, e.g., corpora lutea, implantations, live fetuses, preimplantation loss, post implantation loss, and total malformations, variations, and affected implants (from pages F-1 and F-2).	All tables removed from text at Sponsor's request	

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The study authors concluded that the maternal body weight data did not indicate maternal toxicity, and regarded the significant decrease in weight gain on GD 20-21 to be an apparent spurious effect. However, the report text failed to mention that, according to page C-2, there were also significant linear responses for reduced weight gains on GD 8- 1 1, 5-2 1, and 0-2 1 as well as for the extrauterine weight gain on GD 0-21. Furthermore, the high-exposure group showed consistent, albeit nonsignificant, reduced weight gains at all other intervals examined. Collectively, we regard these weight gain data to be evidence of slight maternal toxicity at 20,000 mg/m3.	Revised abstract, summary, results, and discussion	i, I-1, I-2, 4-1, 4-2, 4-5
The presentation of data in Tables 1-1 and 4-1 show the N value as the number of fetuses, implying that the fetus, not the litter, is being used (inappropriately) as the experimental unit of analysis. This coinflicts with the summary table on page G-1, where N is the number of litters; the means and SD values are identical in all three tables. The Methods indicate that the analysis was appropriate in that a nested design was used (fetal weights were nested in litters). To reduce concenns of inappropriate statistics, the Tables 1 - 1,4- 1, and on page G- 1 should indicate that a nested analysis was used. The number of litters as well as fetuses should be shown be shown in all three tables.	Tables 1-1 and 4-1 removed. Table G-1 revised to reflect the revised statistical analyses	1-1, 4-2, G-1
The statistical analyses of the fetal examination data are inappropriate and should be revised. In the analyses (chi-square and Fisher's exact test), the study authors used the fetus, rather than the litter, as the experimental unit. Since fetuses from the same litter are not independent of each other, a fetus-based analysis inappropriately inflates the degrees of freedom. (The study authors also did chi-square and Fisher's exact test on litter incidences; this is statistically valid, but is inadequate without additional statistics because it doesn't consider within-litter incidences and can lead to false negatives.)	Data reanalyzed	3-11, Appendix K

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For dams that delivered early, the individual body weight and food consumption data are omitted from Appendices C and D. It is acceptable for these animals to remain excluded from statistical analyses. However, because treatment could affect gestation length and premature delivery, it is important that the data from these animals still be included in the appendices and available for review.	Data added to report	C-4, C-8, AND D-3
Peer Reviewer – Schlesinger		
Overall, the study was conducted in a scientifically sound manner and followed the appropriate protocols. There were no significant deviations from these protocols that would have affected the outcome of the study. The conclusions as presented are sound.	No action required	
There are some concerns about a number of statistical issues that have been previously raised in regards to related reports. These are indicated below.	Data reanalyzed	
Specific comments:		
Exposure Schedule: It is stated that the exposure period was "at least" 6 hours per day. What was the range of actual daily exposure durations.	Revised to six hours	3-7
Page 3-8. Statistical Analysis: When the Bartlett's test indicated nonhomogeneity of variance the investigators used a nonparametric analysis of variance. An alternative approach would be to use some transformation that would have resulted in variance homogeneity, and then a parametric analysis of variance could have been used. This apparently has been used for some of the analyses are reported. This uniformity would have made the statistical analysis of all data sets much more internally consistent and would not present any potential for Merent types of tests having different degrees of conservativeness in detecting an significant changes from air control. In any case, the investigators should indicate which specific statistical tests were used for which datasets.	No change; the footnotes with the mean data indicate which tests were performed	

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The investigators also indicate that they performed statistical testing with both transformed and nontransformed percentage data but that the former where not reported since they were not statistically significant. This implies that whichever procedure resulted in significance would be reported. This is an improper use of statistical techniques. If data need to be transformed because they are not normally distributed as raw data then the statistical tests with these transformed data are the ones that should be reported. There cannot be a "picking and choosing" of data sets to report.	Results of the transformed data added to the report	F-1, F-2
It is also stated that statistical tests were conducted at both the 5% and 1% significance levels. The justification for this is not apparent. One of the a priori decisions that should to be made before the study is conducted involve choice of the appropriate level of significance. Only one should be selected based upon whatever criteria the study director chooses and only this level should be reported.	Clarified in the text	3-10
Page 4-1 Gestation Body Weight: The Appendix L is initially confusing, since each summary table is not individually labeled as to whether it is actual weight were in the body of the main text rather than in the appendix. or weight change. Furthermore, it would be better if all of the summary tables	Appendix B revised to clarify data types presented. No change in the location of the summary tables	Appendix B
Table 4-2. There does seem to be somewhat of an exposure level-related increase in stunted growth, although the numbers are indeed small.	Table 4-2 removed from report	4-2
Page 4-6. Exposure Data: It is stated that the particle levels were 1 and 1.5 mg/m^3 and that this represents a "minimal" aerosol component. Firstly, what was the composition of the aerosol; were there HEPA filters on the exposure units? Secondly, in the opinion of this reviewer, these reported levels of particles are not minimal but are high for a system in which there should be total vapor.	No change. The source of the dust is the make-up air from the animal room which was not filtered.	

Comments	Action/Response	Page #
Page 4-7. Discussion: There clearly were changes in the skeletal variations at the mid level of exposure. While there was no exposure concentration related pattern, the investigators should provide some possible explanation as to why these changes may have occurred before discounting them in the determination of the no effect level.	No change	
Appendix H (p. H-1): The statistical notations on this table are not clear. For example, the total fetuses with skeletal abnormalities seem to be significant with the chi square test and the Fisher test. This needs some clarification.	Statistical notations removed from Appendix H. Statistical details are in Appendix K	H-1 – H-5 and Appendix K
Peer Reviewer – Goldsworthy		
It is interesting that gasoline with MTBE, but not BGVC only, also noted a high incidence of bifid centra of the thoracic vertebrae. Historical control data range presentation for these findings should be noted if possible.	Historical control data presented in the Discussion	4-5
I agree with the report's conclusion that these decreases in the present study are likely not to be treatment related or biologically significant. These rare findings may need to be further assessed in the context of the entire database API is generating in this testing initiative.	No action required	
Results section should present (in table format) the total skeletal variations data and total visceral variations data, especially in light of the significant increases in the 10,000 mg/m^3 group for total skeletal variations.	No change	
211 (b) Research Group Reviewer		
General Comments		
The final report should have sequential numbering in addition to the A-1, A-2, etc. system so that we can be certain that we have all report pages.	Sequential numbering added	
There is no Appendix K, Historical Control Data, included with the report. This should be reviewed prior to report finalization.	Historical control data added	Appendix K

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The QA Statement is blank. Before the report is finalized, we should receive a revised QA statement that lists all audited phases of the study and the audit dates. The report should not be finalized without sponsor review of the QA statement.	QA statement added	ix
Tables with nested analysis (fetal data) need to list the number of litters per exposure level.	Number of litters presented	G-1
The statistical programs include linear trend analysis but no criteria for ignoring statistically significant linear regression, which appears to be the procedure in place when the intergroup comparisons is not statistically significant. There is also a test for linear lack of fit, but it isn't clear if this is routinely performed or only when the intergroup comparisons are negative, or some other rationale. The decision tree for the statistical programs needs to be explained more fully, and the criteria for dismissing statistically significant findings should be described in more detail also.	Linear trend analyses results added to the results	4-1, 4-2
There are a number of statistically significant linear regression analyses for gestation body weight change and food consumption that could be considered justification for a alternate interpretations of maternal data. These are not addressed in the text, which I believe will need to be done to appropriately justify the data interpretation.	Abstract, summary, results, and discussion revised	i, I-1, I-2, 4-1, 4-2, 4-5
Specific Comments (by page and section)		
Page ii, Table of Contents: Justification of Dosing Route and Justification of Dose Selection both appear on page 2-2, not 2-1.	Corrected	Ii
Page iv, Table of Contents: Appendix K is missing.	Appendix K added	V
Page viii, QA Statement: As noted above, this should list the phases and dates of inspection.	QA statement added	ix

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Comments	Action/Response	Page #
Page 1-1, Summary: First paragraph, Lines 6-9: There should be more of a connection between "The Sponsor selected the doses." and the next lines. Something like "based upon the following considerations" would be helpful. Also, there is no justification provided for why 2K was expected to be a NOAEL. Comment regarding the overall testing program might be appropriate.	Revised to the statement supplied by the Sponsor	1-1
Third paragraph, line 5: Cesarian should be spelled out, as in the next paragraph.	Revised	1-1
Page 1-2, Summary: There is no reason to include a table of fetal weights in the summary when this is not a parameter that is interpreted as being affected by exposure, and there are no unusual findings to clarify. This table should be deleted.	Table removed	1-1
First paragraph, lines 1-2: The sentence should be: "...and fetal growth resulted& no signs...".	Revised	1-2
First paragraph, lines 3-6: There's no need to repeat the information presented on the previous page regarding bifid thoracic vertebral centra. The last two sentences in this paragraph should be deleted. The final sentence of the summary, regarding the NOAEL, could be moved into the same paragraph as the "In conclusion" sentence.	Removed	1-2
Page 2-1, Introduction: First paragraph, first sentence: "...administered& whole-body...".	Revised	2-1
Justification for Selection of Test System: I believe the reference should be 1994 (for the 21 1 b program), not 1996 (the testing guideline).	Reference corrected	2-1
Page 2-2, Justification of Dose Selection: Adequate justification for dose selection would provide the reasoning for why 2K was expected to produce no effects. Since the mid-dose was probably not expected to cause effects, it wasn't selected to produce a dose response.	Added the statement supplied by the Sponsor	2-2

Comments	Action/Response	Page #
Page 3-2, Characterization of the Test Substance: All of this information as described in the first paragraph should be included in the report as an appendix. It (MRD-00-714?) could be included withhear and compared to the data in Appendix J.	No change. There is a separate report for this data	
Page 3-3, Analytical Concentration: Line 5: "...analyses showed major components of the test atmosphere and were used to assess ..."	Revised	3-3
Page 3-3, Particle size analysis: Line 2: There should be a space between "the" and "control"	Added	3-3
Page 3-4, Feed and Water: Both the feed and water analyses should be included in the report as appendices.	Analyses added in Appendix M	Appendix M
Page 3-6, Administration of Test Substance and Exposure Schedule: Line 1: There should be a space between "M3" and "inhalation."	Added	3-7
Page 3-7, Euthanasia: The method of euthanasia for viable fetuses must be included.	Added	3-8
Page 3-8, Tissue Preservation: The method of storage/preservation for the skeletal specimens needs to be included also.	Added	3-8
Page 3-8, Statistical Analysis: Second paragraph: Transformations need to be reported when performed, whether statistically significant or not.	Transformations reported	F-2
Third paragraph: The lab needs to provide explanations for: (a) why linear regression is performed when there isn't a difference between groups; and (b) what criteria is used to ignore statistically significant linear trend performed in such situations. What was the criteria for performing a lack of fit test?	Linear regression and the lack of fit test run automatically with the statistical analyses. The results have been reported.	4-1
Page 3-9, Statistical Analysis, continued: Second paragraph: Was Armitage's test for linear trend always performed, or only if the Fisher exact was statistically significant?	No longer applicable	

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Page 4-1, Clinical lnlife Observations: Second paragraph: Please revise the third sentence to indicate that red ocular discharge and dry red ocular discharge were observed in the same female.	Added	4-1
Page 4-1, Gestation Body Weight: The statistical significances (linear regression) for GD 8-1 1, 5-21, 0-21, and 0-21 C need to be addressed. The data for these intervals suggest a slight effect upon the 20K group, which is not considered biologically significant by the authors when statistically significant for the GD 20-21 interval; therefore it is appropriate that the authors note the reason(s) why the signficant regressions should not be considered biologically important.	Linear regression results added to the results.	4-1
Page 4-1, Gestation Food Consumption: The statistical significances (linear regression) for GD 5-8,8-11, 11 -14, 20-1 1, and 5-20 need to be addressed. As with body weight change, the data suggest a slight effect upon the 20K group. Since this has not been considered biologically significant by the authors, there should be some justification provided here.	Linear regression results added to the results.	4-2
Page 4-2, Gross Postmortem Observations: "Dark red material" should be identified by location. This observatation and the observations of "placentas," "dark red material in the uterus," "dark brown material in the stomach and intestines," and "fetus in the cervix" do not appear as these descriptions on the summation of observations on page E-1.	Added to results and Appendix	4-2, E-1
Page 4-2, Uterine Implantation Data: Referring to page F-2, total variations: Why was a "lack of fit" test performed for the linear response, when the linear regression was not significant?	Lack of fit test runs automatically. In this case it indicates that the linear gression was not an appropriate regression model for the data.	
Page 4-2, Fetal Body Weight: Table 4-1 should be deleted; it repeats information presented in Appendix G and is unnecessary for study interpretation.	Table 4-1 deleted	4-2

Comments	Action/Response	Page #
Page 4-3, Fetal Observations: Table 4-2 should be deleted; it repeats information presented in Appendix H and is unnecessary for study interpretation.	Table 4-3 deleted	4-3
Page 4-4, Visceral Observations: It isn't clear if visceral observations are considered to be developmental toxicity findings, artifactual, or something else. This needs some clarification.	Clarified	4-3
"Abnormal abdominal contents" is not an informative description.	Revised	4-3
Table 4-3 should be deleted; it repeats information presented in Appendix H and is unnecessary for study interpretation.	Table 4-3 deleted	4-3
Page 4-5, Skeletal Observations: Second sentence: There is no explanation (in this section) of the reasoning for why the statistically elevated total skeletal variations and bifid thoracic vertebral centra are not considered effects related to exposure. Please include (noted in summary).	Explanation added	4-3
Table 4-4 should be deleted; it repeats information presented in Appendix H and is unnecessary for study interpretation.	Table 4-4 deleted	4-3
My count for bifid thoracic centra for the 10K.group was 34 rather than 33, and for 20K was 17 rather than 16. Please check these numbers.	Numbers checked twice. Replaced 16 with 17 for 20K	H-4
Page 4-6, Exposure Data: Appendix J is not referred to in the report; shouldn't it be referred to in this section?	Added	4-4
Final paragraph, final sentence: room^"	Added the “s”	4-4
Page 4-7, Discussion: Third paragraph, second and third paragraphs: Please delete these sentences, since they repeat information provided in the previous paragraph.	Deleted	4-5
Page 4-8, Protocol Exceptions: Chamber temperature and humidity: "protocol-defined	Corrected	4-6
The temperatures and humidity values outside of the protocol-defined range should be provided here, as well as a summation sentence that provides the range of values.	The range of values is presented earlier in the report. The section was revised to reference Appendix I	4-6, I-23 – I 30

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Page 5-2, Fetal External and Visceral Examinations: Observations are defined as not malformations and not variations, but not defined by whatlhow they differ from normal, nor if they are considered to be developmental toxicity endpoints. Please clarify.	Revised section	4-8
Appendices:		
Appendix C: Page C-1, C-2: Delete "Run 1 ."	Deleted	C-1, C-2
Appendix D: Page D-1 : Delete "Run 1 ."	Deleted	D-1
Appendix E: Page E-1: The descriptions summarized here are not consistently described as noted on the individual observations and/or in the text on page 4-2.	Revised	E-1
The observations associated with the early delivery should be noted as such and probably reported in separate rows of the table.	Revised	E-1
Appendix F: F-2, total variations: Why was a "lack of fit" test performed for the linear response, when the linear regression was not significant?	No change. The lack of fit test runs with every analyses	
Appendix G: The group sizes based upon the number of litters needs to be presented. The individual data need to present the mean male and mean female fetal weights for each litter.	Number of litters presented	G-1
Appendix H: Page H-2: "Abnormal abdominal contents:" what is this?	Revised	H-2
Page H-4: My count for bifid thoracic centra for the 1 OK group was 34 rather than 33, and for 20K was 17 rather than 16. Please check these numbers.	Numbers checked twice. Rvised 16 to 17	H-4
Pages H-26, H-28, H-30, H-37, H-39, H-47, H-48, H-53, H-64, H-71, H-74, H-87: Please provide the reason(s) that some fetal specimens needed to be arbitrarily assigned fetal numbers.	Reason added	H-26, H-28, H-30, H-37, H-39, H-47, H-48, H-53, H-64, H-71, H-74, H-87
211 (b) Research Group QA/QC Reviewer		
The following items require further consideration:		

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Comments	Action/Response	Page #
Page i, Abstract and throughout the report: when indicating concentrations of test article, indicate target concentrations (unless they are the actual ones measured).	Target added	Throughout the report
Page vii, Compliance Statement: The sponsor also needs to sign a compliance statement. It can be a separate one from the Testing Facility's, but there must be one signed by the sponsor.	Space added on com0pliance statement for Sponsor's signature	vii
Compliance Statement: Since it was the sponsor's responsibility to maintain the method of synthesis, fabrication, or derivation of the test fuel, and this has not been completed, it must be included in the sponsor's compliance statement.	Added to the test substance section of the report. We do not consider this to be a compliance issue as it is now available.	3-1
Page viii, Personnel: Is a compound preparation supervisor an appropriate person to have listed under personnel? Did this person actually work on this study?	No change. Required by protocol	
Page ix, QA Statement: The QA statement needs to be completed.	QA statement added	ix
Page 2-1, Experimental Date: This date should be March 21 or later as the skeletal re-evaluation was done on March 21 (Experimental termination should be the last date that data are collected)	Date revised	2-1
Page 3-1: During the audit, no real "test material receipt record" could be found. It appears that dispensing received the material on 4/10/01 and on 6/14/01, but none was available for when the test material arrived at EMBSI. All records should be searched to find the original receipt record to determine the exact date on which this test material was received at EMBSI.	The test substance was logged into our data and this is the documentation of receipt.	
Page 3-1, The container numbers: The container numbers for the 6/14/01 receipt date are confused. Container 9A is correct, but the next container should be 9B(2b, large container) as should containers 10A, 11A and 12 A were all dispensed from large container 2. Please clarify.	Section revised	3-1

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Data sheets for containers 9A-12A should indicate manufacturer's number 2, the large tank number.	Added to data	
Page 3-1, Materials and Methods, Test Material: The expiration dates given are for five years. Are there data to support these expiration dates? It should be kept with study data.	Expiration dates were not supplied by the Sponsor. The expiration dates used were per EMBSI SOP	
Page 3-2, Test Material, Analytical Concentration: It is indicated that chromatographic analyses showed major components of the test atmosphere and was used to assess the stability of the test substance over the duration of the study. The number of components and their identities should probably be indicated for reproducibility purposes.	Added the number of components.	3-2
The next paragraph should indicate that this analysis was done to determine component proportions of the test material atmosphere compared to the liquid test material.	Added	3-2
Page 3-5, Environmental Conditions: These are the protocol-required ranges for temperature and humidity. The actual measured ranges should be given.	The actual measured ranged were added for the chambers. The animals rooms were monitored to determine that they were within the acceptable range, but values were not recorded.	3-5
Page 3-7, Test Atmosphere: The daily mean exposure concentrations were "intended" to be within +10% of the target exposure levels.	Revised statement to include the protocol deviation	3-8
Page 4-1, Gestation Body Weight: One mid-dose and one high-dose animal lost between 22 and 27 grams between GD 20 and 21. This may have affected the means at this interval, particularly the high dose group.	Body weight data and food consumption data for GD 21 and other affedted intervals not used for the statistical analyses for IGK757 (high dose animal). This data was excluded because the animal was attempting to deliver its litter on GD 21.	i, I-1, I-2, 4-1, 4-2, 4-5, C-1, C-2, C-6, C-10, D-1, D-5
Page 4-2, Gross Postmortem Observations, first sentence: The dark red material and placentas were in the "stomach of the" 2000 mg/m3 dam…….	Revised	4-2
Page 4-4, Table 4-3: Aneurysm is misspelled.	Table 4-3 deleted	4-2
Page 4-6, last sentence: The light intensity in the chamber room ranged from 4.6 to 40.0 footcandles.	The light intensity in the chamber room was 32 to 40 foot-candles. The low range for the animal room was 4.6 foot-candles	4-3

Comments	Action/Response	Page #
Page 4-12, Protocol Exceptions: Having no analytical data (hydrocarbon distribution) for the 20,000 mg/m3 group for the week 3 interval should be included as a protocol deviation. This was required data.	Added	4-6
Page 4-12, Protocol Exceptions: The extent of the temperature and humidity excursions needs to be given. Some sense of how frequently, for how long, and what temperature and humidity were reached should appear.	Section revised to refer reader to Appendix I where all excursions are identified	4-6
Page C-1: The standard deviation for the GD 11 weight for the 10,000 mg/m3 group should be 19.66. Shouldn't this round to 19.7?	We calculate 19.55 which rounds to 19.6. No change	
Page C-8: The column heading next to the GD 20-21 should indicate GD 5-21 not 0-5.	Corrected	C-8
Page E-1: There is an entry of Cervix, abnormal contents. The one animal in the high dose that had a finding in the cervix, had a fetus in the cervix, not really abnormal contents.	Added that a fetus was the abnormal content	E-1
Page E-4, animal number 741: It should probably be noted that 17 live pups were delivered.	Added	E-4
Page F-2: It is not clear why there are only 21 litters considered in the low-dose group for % Preimplantation Loss. The mean using 22 liters is 2.4.	Corrected	F-2
Page F-2, % Postimplantation Loss, Low Dose: It is unclear where the value of 6.3 comes from. The audit found the mean to be 2.6+4.3. Please verify.	Corrected	F-2
Page G-2, the first fetus in each litter is only report to one decimal place where all others have two. It appears that the column is just missing the digit, as they are not rounded.	Corrected	G-2

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Page H-4, please verify the incidence of "Vertebrae, Thoracic Centra Bifid," as it appears there are 21, 16, 34, and 17 affected in the control, low, mid, and high dose, respectively. Please verify and change corresponding tables within the text if necessary.	Data verified twice. Values are 21, 16, 33, and 17.	H-4
Page I-4, Particle size, second paragraph: A particle size determination ……. from the control and 20,000 mg/m3 concentration.	Revised sentence	I-4
Page I-4, Particle size, second paragraph. Is there any way to make the explanation of the particle size correspond to the data reported? Can it be simplified at all?	Second paragraph revised. Added a third paragraph	I-4
Page I-10: There are two GC calibrations labeled with the date, 10/31/01. Please clarify in the data, which one is used and verify that the one reported is the correct one.	Clarified in data and reported calibration verified	I-9
Page I-11, 20,000 mg/m3 data: The mean concentration minimum should be 19,913 mg/m3. The nominal for the last day, should be 19,486. This changes the mean to 20,127 with a standard deviation of 479. Please verify these changes .	Corrected The nominal for the last day is 19556 as reported. No change	I-10
Appendix J: Several pages are labeled as J-1.	Pagination corrected	Appendix J
Appendix J, Page J-1: The procedure for collecting the charcoal tubes needs to be described somewhere in the report (either in Appendix I or J). A description of how they were stored prior to receipt in the Analytical Chemistry Lab is needed as well.	Collection and storage procedure added to the Test Substance section of the report.	3-2
Appendix J, Page J-1, Sample Preparation, next to last sentence: charcoal tube sections were ……..for at least 60 minutes according to the data.	Corrected	J-1
Page J-1, Characterization: MTBE in the first sentence should be ETOH.	Corrected	J-1
Table J-1: Data from the analysis of the neat material would be helpful in showing that the test material is stable in vapor phase.	No change. This is in the characterization report	

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Page J-2, Results: There should be some indication of what the data show. There was no change in test material composition for the duration of the test, at different dose levels, etc.	Added to the last sentence of the first paragraph	J-2
Table J-1: For the Dec. 3, 2001 sample in the 2000 mg/m3 group, the n-butane value should be 12.2. Please verify.	Corrected	J-3
Table J-1: The footnote indicating that there was no breakthrough in any of the samples except sample 9 is not true. There was breakthrough apparently for sample number 7. It appears from the data that this sample was repeated and no breakthrough was seen in the repeat sample. There are two problems with this. First, it is unclear how a repeat sample could have been run, as all of the samples were collected and stored until analysis. No repeat sampling could have occurred by the time the breakthrough was noted. Second, the data in Table J-1 should be footnoted as a repeat sample with an explanation (if this was indeed the case).	The statement is true. The apparent breakthrough on sample number 7 was carryover in the column from the previous analysis. The sample was re-injected and the results reported. A footnote was added to the data and Table J-1	J-3
Historical Control: No historical control data are reported. These data need to be reported.	Historical control data added	Appendix L
Data for the animals that delivered early or those that were not pregnant need to be reported somewhere in the report. A separate table for those animals needs to be created.	Data for animals that delivered early were added to Appendices C and D	C-4, C-8, AND D-3
Data Issues:		
Animal number 800 had 18 Corpora Lutea and only 14 implantation sites. This should have been verified (per SOP) by an independent technician, but was not. This would be considered an SOP deviation and should be documented as such.	Documented and acknowledged by the Study Director as SOP deviation	
Analytical data: The GC print-out for Analytical Reference 171434-001B should have been 171434-002. The rest of the samples were also designated incorrectly (001-C should have been 003, 002A should have been 004, etc.)	The printouts are designated correctly. No change	

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All chamber trial data (GC print-outs) indicate study number 169534R and the incorrect test material number. Please verify and correct.	Corrected	
The data trail from collection of the charcoal tube samples in the inhalation chambers to the freezer before being transferred to the analytical lab is not clear. There is no documentation to verify that these samples were taken and frozen until submission to the analytical lab.	Memo added to data describing how samples were stored.	
Since charcoal tube samples from chambers were apparently frozen until all samples could be analyzed, there needs to be a stability study done to verify that these samples are stable in the freezer for this period of time.	No change. This is the standard method for storing volatile samples on charcoal tubes. Once the substance is on the charcoal, only high heat or a solvent will remove it. Additionally, the data reported in Appendix J shows that the samples are stable.	
There needs to be a clear indication of what the analytical lab considers raw data. When data are not printed out until a week after the analysis, it is not appropriate to consider the paper print-out to be data. Since the GC program is storing data, the computer system must be completely validated and follow all of the requirements of an on-line data collection system (including change-control procedures, limited access, complete maintenance of a data trail, etc.). Please verify that this is the case.	The printout is the raw data. We have tested the security of the chromatogram in the system and it is secure. All other operations around the chromatogram are documented.	
There is a notation in the room log that extra animals from this study were moved from PE103 to 104 on Dec. 3. There is another notation, however, that extra animals from PE105 (Study 117150C) were moved to PE103 on Dec. 7. Please clarify and verify that another study's animals were not in this study's animal room.	No change. These were untreated animals of the same health profile as the Study 171434 animals.	
Rack use/change log: There are no study numbers on these sheets beginning on Nov. 6. Please clarify.	Receipt date was recorded, which was acceptable.	
Skeletal evaluation data: The late entry of NOA for those animals that were re-evaluated was not always error-coded. Please correct.	Corrected	

Comments	Action/Response	Page #
Test material use log: It appears that there was a sizeable amount of test material (approximately 1,135g) used between chamber trials and the first exposure that is not accounted for. Please explain.	Memo added to the data. It was used for sorbent tube sampling method development	
All of the Analytical Method Validation data (from before the samples were run and after the samples were run) need to be compiled in a report to show which solvents were best at extracting ETOH and that the 10% 2-propanol did not interfere with the other components during the analyses (It is assumed that that is the reason for analyzing the 10% 2-propanol and test material samples after the study was completed).	No change. This activity is part of the characterization study.	
It appears that the only identifier on the individual GC print-outs on the daily chamber analyses is "Inhalation Staff". The responsible technician has initialed the cover sheet, but on the actual data print-out, this person is not identified. Now that more than one technician runs the inhalation exposures, the initials of the responsible technician should appear on the individual data print-outs.	Memo added to data	
The GC print-out of the butane standard checks are not identified as such. Please label these data with appropriate identification.	Memo added to the data. All butane standard checks are in their own section of the data.	
Analytical Sampling Record in the Inhalation Lab only includes study intervals 11/27, 12/3,12/10,12/17 in order of collection. The method development samples were recorded out of order. Please explain.	The method development samples were separated from the study samples to make the data less confusing.	