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FINAL REPORT AMENDMENT

Study Title

Oral (Gavage) Combined Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFH Ammonium Salt (Ammonium salt of Perfluorinated Hexanoic Acid) in Mice

Author
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(Study Director)

Study Completed On
26 July 2011
(Final Report)

Final Report Amended Date
28 September 2012
(Final Amended Report)

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Laboratory Project ID
20005045

1. STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentially, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA Section 10(d)(1)(A), (B) or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA 10 (g).

Company:

Company Agent:

Title:

Date: _____

Signature: _____

2. GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This final report accurately reflects the raw data obtained during the performance of the study. No deviations from the Good Laboratory Practice (GLP) regulations of the U.S. Environmental Protection Agency [a], the Japanese Ministry of Agriculture, Forestry and Fisheries [b], and the Organisation for Economic Co-operation and Development[c] occurred that affected the quality or integrity of the study, with the following exceptions.

- All reports generated by Charles River Laboratories Preclinical Services Montreal were conducted in accordance with the appropriate OECD Principles of GLP. The OECD regulations were appropriate for these analyses.

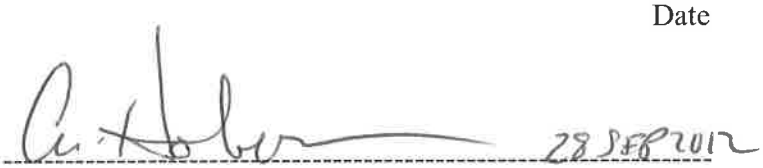
- Health monitoring analysis conducted by Zoologix Inc., for *clostridium perfringens* was conducted non-GLP. The non-GLP conduct of this portion was appropriate for health monitoring.

Submitter:

Date

Sponsor:

Date



28 SEP 2012

Alan M. Hoberman, PhD, DABT, Fellow ATS Date
Executive Director, Site Operations and Toxicology
Study Director

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a. U.S. Environmental Protection Agency. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule. 40 CFR Part 160.

b. Japanese Ministry of Agriculture, Forestry and Fisheries (1999). Notification on the Good Laboratory Practice (GLP) Standards for Agricultural Chemicals. 11 Nousan No. 6283.

c. Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practice [C(97)186/Final].

3. FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Company:

Company Agent:

Title:

Date: _____

Signature: _____

4. QUALITY ASSURANCE STATEMENT

Protocol: 20005045

This Final Report Amendment has been inspected by the Quality Assurance Unit to assure conformance with the applicable Good Laboratory Practice regulations. Reports were submitted in accordance with SOPs as follows.

QAU INSPECTION DATES

		Dates Findings Submitted to:	
Dates of Inspection	Phase(s) Inspected	Study Director	Study Director Management
26 Sep 2012	Report Amendment 1	26 Sep 2012	26 Sep 2012

The Final Report Amendment has been reviewed to assure that it accurately describes the materials and methods, and that the reported results accurately reflect the raw data.

S Wilson

Stacy Wilson
Manager, Regulatory Compliance
Charles River Laboratories
Preclinical Services, Pennsylvania

27 Sep 2012
Date

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5. RATIONALE FOR AMENDMENT

After completion of the final report and review of the data from previously conducted studies with PFH Ammonium Salt, it became clear that the only reported test substance related effect in the 100 mg/kg/day dosage group was the statistically significant ($p \leq 0.05$) reduction in pup weight on postnatal day 0 (PND 0). This statistical significance was not found on any subsequent days of analysis.

Based on this minimal change of 0.1 g in average pup weight, the other potential causes from the difference from the control group value were examined. The average litter size at each weigh day for the 100 mg/kg/day dosage group was the largest; approximately two pups more than the control average (13.2 vs 11.4 on PND 0). The relationship between litter size and fetal/pup weight is a known effect with larger litter sizes tending to have slightly smaller pups. Based on this information, an Analysis of Covariance (ANCOVA) with litter size at the covariant was conducted and the results of these analyses have been added by amendment to the final report.

6. LIST OF CHANGES TO THE FINAL REPORT

Additions are indicated in bold text.

Item 1. Page 37, Section 7.5.7. Data Collection and Statistical Analyses:

Because pup body weights are generally inversely proportional[19] to litter size, pup body weights were analyzed by Analysis of Covariance (ANCOVA) using litter size as the covariate[20].

Item 2. Page 17, Section 5.3. Results and Page 41, Section 8.3. Natural Delivery Observations:

Pup body weights in all treated groups were generally lower in the treated groups compared to the control group values. Pup body weights were significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) on PPD 0 in the 100 mg/kg/day and higher dosage groups compared to the control group value. Statistically significantly reduced pup body weights persisted in the 350 mg/kg/day dosage group through PPD 7 and in the 500 mg/kg/day dosage group through PPD 4. On PPD 20 average pup weights per litter were 89%, 80% and 88% of the control group value. The lack of dosage-dependency can be attributed to the differences in litter size among the groups. **The variance in litter size among the groups also had an effect on the pup body weights. When pup body weights were covaried against the litter size on PND 0, the statistically significant differences among the groups for pup body weights were no longer present (see revised Table 7 attached to this report amendment). This result indicated that the litter size rather than the test substance was affecting the pup weight differences between the groups. The litter size was largest in the 100 mg/kg/day dosage group and therefore the reduced pup weight that occurred in this group was considered related to the litter size. The litter size in the 350 and 500 mg/kg/day dosage groups were affected by increased pup deaths that occurred in these dosage groups. Therefore an effect on pup body weight was related to the test substance in these two dosage groups.**

Item 3. Page 19 and 20, Section 6. Discussion and Conclusion and Page 44 and 45, Section 10. Discussion and Conclusion:

<u>The following paragraph will replace the second paragraph in the referenced sections.</u>

In the F1 generation litters, pup body weights were significantly reduced on PPD 0 in the 100 mg/kg/day and higher dosage groups, but this decrease in body weights in the 100 mg/kg/day dosage group was not statistically significant when covaried against litter size on PPD 0 and persisted only in the 350 and 500 mg/kg/day dosage groups. On PPD 20, average pup weights per litter were 89%, 80% and 88% of the control group value. The lack of dosage dependency can be attributed to the differences in litter size among the groups.

<u>The last paragraph will be revised as follows.</u>

On the basis of these data from this study, the maternal no-observable-adverse-effect-level (NOEL) for PFH Ammonium Salt is 100 mg/kg/day. The NOAEL in the F1 generation **is 100 mg/kg/day**. None of the effects observed in the pups preweaning persisted into the postweaning period.

Item 4. Page 46, Section 11. References:

(19). Kreckmann, K.H., Staples, R.E., Green, J.W., Alvarez, L., Hurtt, M.E. and Murray, S.M. (1994). Use of analysis of covariance to account for litter size and sex ratio effects on pup and fetal body weight. Teratology 49:402.

(20). Snedecor GW, Cochran WG. Analysis of covariance. Statistical methods 6th Ed. Iowa State University Press, Ames; 1967. p. 419-31.

Item 5. Page 45, Section 10. Discussion and Conclusion:

Discussion of amended results:

The additional statistical analysis revealed that all of the reductions in body weight were related to litter size. No statistical significance for average pup weights occurred when litter size was used as a covariant. The biological significance of these results does not impact the conclusion made concerning the 350 or 500 mg/kg/day dosage groups, as other effects on pups (increased mortality) occurred in two dosage groups. The lack of statistical significance in the 100 mg/kg/day dosage group does affect the original conclusion and provides a reasonable explanation for the statistical significance found for the absolute weight analysis. Although the average litter sizes in both the 0 and 100 mg/kg/day dosage groups were within the historical control range for this Testing Facility, the statistically significant difference in average weight on PND 0 between the 100 mg/kg/day dosage group and the 0 mg/kg/day dosage group was clearly related to the larger litter size as demonstrated by the ANCOVA with litter size as the covariant and the absence of any other toxicity in this dosage group.

Justification:

Due to the apparent differences in litter size among the group, it was decided to statistically analyze the pup weights in an analysis of covariance with litter size on day 1 postpartum as the covariant.

7. FINAL REPORT AMENDMENT APPROVAL

Date: 28 SEP 2012

Alan M. Hoberman, PhD, DABT, Fellow ATS
Executive Director, Site Operations and Toxicology
Study Director

8. ATTACHMENTS

The attached page represents the modified Final Report Table 7, page 3.

PROTOCOL 20005045: ORAL (GAVAGE) COMBINED DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF PFH AMMONIUM SALT (AMMONIUM SALT OF PERFLUORINATED HEXANOIC ACID) IN MICE

TABLE 7 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0	100	350	500
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	19	19	19	16
LIVE LITTER SIZE AT WEIGHING					
DAY 0	MEAN±S.D.	11.4 ± 4.5	13.2 ± 1.6	12.0 ± 3.5	9.9 ± 2.9
					[13]b
DAY 4	MEAN±S.D.	11.9 ± 3.8	13.0 ± 1.7	12.0 ± 3.6	9.9 ± 2.0*
		[18]b		[17]b	[11]b
DAY 7	MEAN±S.D.	11.9 ± 3.8	12.9 ± 1.6	11.8 ± 3.6	9.9 ± 2.0
		[18]b		[17]b	[11]b
DAY 14	MEAN±S.D.	11.9 ± 3.8	12.4 ± 1.4	11.6 ± 3.4	9.9 ± 2.0
		[18]b	[15]c	[17]b	[11]b
DAY 20	MEAN±S.D.	11.9 ± 3.8	12.3 ± 1.2	11.6 ± 3.4	9.9 ± 2.0
		[18]b	[15]c	[17]b	[11]b
PUP WEIGHT/LITTER (GRAMS)					
DAY 0	MEAN±S.D.	1.6 ± 0.2	1.5 ± 0.1*d	1.4 ± 0.2**d	1.4 ± 0.2**d
					[13]b
DAY 4	MEAN±S.D.	3.0 ± 0.4	2.8 ± 0.2	2.2 ± 0.6**d	2.4 ± 0.5**d
		[18]b		[17]b	[11]b
DAY 7	MEAN±S.D.	4.4 ± 0.8	4.1 ± 0.4	3.6 ± 1.0**d	3.9 ± 0.8
		[18]b		[17]b	[11]b
DAY 14	MEAN±S.D.	7.4 ± 1.9	6.8 ± 0.8	6.4 ± 1.4	6.8 ± 1.1
		[18]b	[15]c	[17]b	[11]b
DAY 20	MEAN±S.D.	11.0 ± 3.0	9.8 ± 1.5	8.8 ± 2.7	9.7 ± 2.0
		[18]b	[15]c	[17]b	[11]b

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Excludes values for litters that had no surviving pups.
c. Excludes litters with mortality of pups that remained on study after dam was found dead.
d. With pup body weights per litter covaried with litter size per litter, the analyses were not significant.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).