ATTACHMENT 1

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 10 1987

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of additional data for and the registrant's response to a review of an In Vivo bone marrow chromosome study in rats with Acetochlor and a review of an In Vivo micronucleus assay in mice with Acetochlor. EPA ID #'s 3F2966 and 524-GUI; EPA Record #'s 185604 and 185606; EPA Accession #'s 266002 and 263233; Caswell #3B; Tox Branch Project 7-0375.

TO: Robert Taylor/Vickie Walters (PM #25)
Herbicide - Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/9/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Irving Mauer, Ph.D. 7-8-87
Mutagenicity Secondary Reviewer (Review Section VI)
and
Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 7/9/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 7/10/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Monsanto Company
1101 17th Street, N.W.
Washingotn, D.C. 20036

Action Requested: Review additional data and the registrant's response to an In Vivo bone marrow chromosome study in rats with Acetochlor and review an In Vitro micronucleus assay in mice with Acetochlor.

Recommendations:

For the In Vivo bone marrow chromosome study in rats: under conditions of this study and the additional information provided by the registrant (Ammended Final Report MSL-5724 and the IN Vivo Micronucleus Assay in Mice with Acetochlor, HL-84-405/241-207), this study (Report MSL-5724, R.D. 686) is upgraded to an Acceptable study. Toxicity was demonstrated at the high dose (500 mg/kg) by evidence of a statistically significant body weight loss in both males and females at 48 hours.

For the In Vivo micronucleus assay in mice: under conditions of this test the high dose level of MON 097 (2000 mg/kg) exhibited mortality and signs of clinical toxicity. No evidence of an increase in micronucleated polychromatic erythrocytes was noted at the dose levels tested in this study. This study is classified as Acceptable.

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Discussion:

Each of the deficiencies stated in the Agency DER in reference to the "IN VIVO Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097) [Study No. HL 83-006, Project No. 241-143]" for classifying the study as Unacceptable are addressed by first stating the Agency concern, then giving the registrant's response, followed by the Agency comment on the response.

1. Agency Concern:

From the "Methods" section, the first point:

"1) The tested doses were based on a range-finding study (submitted as an appendix) which demonstrated that 2/2 males and 1/2 females injected i.p. with a high dose of 1000 mg/kg died within 24 hours of treatment, whereas equal numbers of males and females treated with 100 or 300 mg/kg survived without any apparent toxic signs. It was reported that "the test compound produced no apparent effects on the mitotic indices of the animals which survived", however the "normal" range to which the investigators compared these results was not stated."

Registrant's Response:

Monsanto responded by stating that:

"This conclusion, which pertains to the results of the range finding study only, was undoubtedly based on the experience of the Study Director and not a comparison with a normal range of values. A normal range of values for mitotic index was not available for the period during which this study was conducted.

However, the following normal range of values for mitotic index was compiled from studies conducted at Hazleton Laboratories during 1985.

	X ± S.E.	Range	N(# of test groups)
Males	4.7 ± 0.3	1.1 - 8.6	42
Females	5.2 ± 0.4	1.8 - 9.7	26

(supplied by Dr. J.L. Ivett, Hazleton Biotechnologies)

The mean mitotic index calculated for each of the 3 groups in the range finding study fall well within the ranges cited above."

Agency Comment:

The explanation provided by the registrant is acceptable.

2. Agency Concern:

The second point from the "Methods" section:

"2) It was stated in the submitted protocol that rats were to be sacrificed at 48 hours after treatment for analysis of bone marrow cells. Although slides were prepared for these animals, they were not examined."

Registrant's Response:

Monsanto responded by stating that:

"As stated on p. 13 of the original report, under 'Cytogenetic Analysis' the slides from the 48 hour sacrifice were not analyzed because there was no evidence of compound induced mitotic delay at the earlier time points. Table 5 displays the group m mitotic indices and the results of the statistical analyses for the 6, 12 and 24 hour sacrifices. No statistically or biologically significant differences from control values were noted."

Agency Comment:

The explanation provided by the registrant is acceptable. However, it should be noted that the protocol for a study should make reference to changes in procedure when there is evidence that further work is uneccessary.

3. Agency Concern:

The third point from the "Methods" section:

"3) Although pre-test body weights were provided, body weights at study termination were not reported."

Registrant's Response:

Monsanto responded by stating that:

"Terminal body weights were recorded for the animals sacrificed at 24 and 48 hours as per Hazleton standard procedures. However, for reasons unknown, terminal body weight measurements were not required by the protocol for this study and were consequently not included in the final report. These weights are included in the amended report (Section II)."

Agency Comment:

The appended "Summary Table of Body Weight Difference..." from the investigators Amended Final Report shows that at 48 hours both the males and females of the 500 mg/kg dose group exhibited statistically significant lower body weights than that of the control groups.

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4. Agency Concern:

The fourth point from the "Method" section:

"4) Although 6 rats/sex/dose were treated and sacrificed, and bone marrow slides were prepared for all treated rats, generally slides were examined from only 5 rats (or less) of each dose group."

Registrants's Response:

Monsanto responded by stating that:

"The original final report states why only 5 rats/sex group were examined in some cases (p. 9 under 'Chromosome Evaluation'): 'At least sixty cells in metaphase were examined from five of the six rats chosen randomly for each sex and group. In some instances, it was not possible to locate 60 spreads, so as many spreads as could be found were analyzed. If 60 spreads were not found from any of the animals initially analyzed for each sex and group, the sixth animal of that group was then analyzed.'

The design for this study basically incorporated an extra animal of each sex per group as insurance against having an inadequate number of metaphase spreads upon which to make an evaluation.

In a few cases it was not possible to find the desired 5 slides per group which each containing a sufficient number of spreads for analysis."

Agency Comment:

The explanation provided by the registrant is acceptable.

5. Agency Concern:

The first deficiency stated in the "Results" section is as follows:

"A. General Observations: No effects of treatment on physical appearance were apparent. Data for clinical signs were submitted as individual animal data. Data for body weights were submitted as a summary table of pre-treatment weights; no differences between test groups were apparent. The effect of treatment of body weights could not be assessed since post-treatment values were not submitted."

Registrant's Response:

Monsanto responded by stating that:

"Terminal body weights have been included in the amended report (Section II) for this study. A statistical comparison of treated and control group mean body weight change has been conducted by Monsanto (Section III). The results of this analysis indicate a statistically significant reduction in body weight gain in both male and female high dose animals when compared to controls at the 48 hour interval."

Agency Comment:

The Agency comment is discussed in point 3 above.

6. Agency Concerns:

The second deficiency stated in the "Results" section is as follows (under B. Cytogenetic Analyses):

"Numerous discrepancies between the number of animals examined, as reported in the summary tables, and the number of animals for which data were reported in the individual animal data were noted. The number of animals reported as examined in the summary tables in many cases was less than the number of animals with individual data reported. In some cases, data were reported for animals from which "0" cells were examined, e.g. #D77707 (Group 2, 24 hours) and #D77771 (Group 5, 24 hours)."

Registrant's Response:

Monsanto responded to the first part of the Agency concern concern by stating that:

"These apparent deficiencies stem from a possible misinterpretation of the values in column 3 (i.e. Number of animals analyzed per group) of Table 2 (i.e. Summary of Aberration Data). The values in this column represent the number of animals for which slides were prepared and scanned for 60 metaphase spreads. In those cases where 60 metaphase spreads were found in the first 5 animals/sex/group examined, it was not necessary to examine the slide prepared from the sixth animal in that group.

The values in column 3 also include slides from animals that were scanned but in which 60 metaphase spreads were not found. In these cases further chromosomal analysis for aberrations was not conducted."

They further responded to the second part of the Agency concern by stating that:

"These errors have been corrected in the amended report (Section II)."

Agency Comment:

The explanation provided by the registrant is acceptable, however, the "total number of cells analyzed" on Table 5 is incorrect, it should read 540 rather than 480.

7. Agency Concern:

It was stated in the discussion that:

"No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined."

Registrant's Response:

Monsanto responded by stating that:

"Although an effect on mitotic index was not apparent in this study, other appropriate endpoints were available for determining the adequacy of the dose levels tested. The TSCA Health Effects Testing Guidelines 40 CFR Part 798 (FR Vol. 50, No. 188 p. 39445) state under 'Dose Levels' (§798.5385 (d)(5)(ii)) '...the dose being the maximum tolerated dose or that producing some indication of cytotoxicity...' As evidence of having approximated the MTD, a statistically significant decrease in body weight gain was observed at the high dose level. According to a report issued by the U.S. EPA's GeneTox Program on Mammalian in vivo and in vitro cytogenetic assays: (Mutat. Res. 87:143, 1981) '...the doses selected should extend over at least a single-log range, with the maximum dose no less than a factor of 2 less than a dose producing a significant level of toxicity.' The high dose selected for the definitive study (i.e. 500 mg/kg) was within a factor of 2 of the dose that produced 75% mortality in the range finding study (i.e. 1000 mg/kg). It should also be noted that the mitotic index was not reduced in the lone survivor at 1000 mg/kg on the range finding study."

Agency Comment:

The explanation provided by the registrant is acceptable.

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The registrant (Monsanto) went into further discussion by stating:

> "It is Monsanto's opinion that the dose levels employed on this study were appropriate and adequate for assessing the potential of acetochlor to induce structural chromosomal aberrations."

and further:

> "Additional information concerning the clastogenic potential of acetochlor and which supports the adequacy of this study can be found in a report entitled In Vivo Micronucleus Assay in Mice with Acetochlor (HL-84-405/241-207). This micronucleus assay is being submitted concurrently under separate letter and is identified as R.D. No. 685 and Special Report MSL-5723."

Recommendations:

Under conditions of this study and the additional information provided by the registrant (Ammended Final Report MSL-5724 and the IN VIVO Micronucleus Assay in Mice with Acetochlor, HL-84-405/241-207), this study (Report MSL-5724, R.D. 686) is upgraded to an Acceptable study. Toxicity was demonstrated at the high dose (500 mg/kg) by evidence of a statistically significant body weight loss in both males and females at 48 hours.

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Data Evaluation Record

Study Type: In vivo cytogenetics in rats.

Study Identification: "In Vivo Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097)".

Lab. performing study: Hazelton Laboratories America, Inc.
Vienna, VA 22180

Sponsor: Monsanto Agricultural Products Co.
St. Louis, MO. 63167

Study no.: HL 83-006
Project no.: 241-143 (Hazelton)
Accession no.: 071970
Report date: May 24, 1983
Submitted to EPA: 9/22/83
Study director: Michael G. Farrow, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 8/2/85

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

8/2/85

Conclusions: No effect on the incidence of chromosomal abnormalities was apparent. No effect on mitotic index was apparent, therefore there was no evidence that the test material reached the bone marrow in sufficient concentration to produce a toxic effect. Numerous discrepancies in the results reported in the summary tables vs. individual animal data were noted.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemicals: Acetochlor (MON 097), a "brown liquid", 96.3% a.i.

Positive control: Mitomycin C (Sigma Chem. Co.), assumed 100% a.i.

Mitotic arrest- colchicine; supplier, purity not stated.

(2) Doses tested: Acetochlor- 40, 150, and 500 mg/kg by i.p. injection.

vehicle control- corn oil, 5 ml/kg.

positive control- Mitomycin C, 5 mg/kg

mitotic arrest- colchicine 2 mg/kg.

(3) Test animal: Male and female Sprague-Dawley CD albino rats, obtained from Charles River Breeding Laboratories, Kingston, N.Y.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) The tested doses were based on a range-finding study (submitted as an appendix) which demonstrated that 2/2 males and 1/2 females injected i.p. with a high dose of 1000 mg/kg died within 24 hours of treatment, whereas equal numbers of males and females treated with 100 or 300 mg/kg survived without any apparent toxic signs. It was reported that "the test compound produced no apparent effects on the mitotic indices of the animals which survived", however the "normal" range to which the investigators compared these results was not stated.

2) It was stated in the submitted protocol that rats were to be sacrificed at 48 hours after treatment for analysis of bone marrow cells. Although slides were prepared for these animals, they were not examined.

3) Although pre-test body weights were provided, body weights at study termination were not reported.

4) Although 6 rats/sex/dose were treated and sacrificed, and bone marrow slides were prepared for all treated rats, generally slides were examined from only 5 rats (or less) of each dose group.

Results

A. General observations: No effects of treatment on physical appearance were apparent. Data for clinical signs were submitted as individual animal data. Data for body weights were submitted as a summary table of pre-treatment weights; no differences between test groups were apparent. The effect of treatment on body weights could not be assessed since post-treatment values were not submitted.

B. Cytogenetic Analyses: Cells were examined only for 6, 12, and 24 hours after treatment with acetochlor; cells from animals sacrificed 48 hours after treatment were not examined for cytogenetic abnormalities. Data were submitted as summary tables and as individual animal findings.

No effect of treatment on the frequency of chromosomal aberrations, the modal number (i.e. the average number of chromosomes/metaphase), or the mitotic index was apparent at 6, 12 or 24 hours after treatment.

Numerous discrepancies between the number of animals examined, as reported in the summary tables, and the number of animals for which data were reported in the individual animal data were noted. The number of animals reported as examined in the summary tables in many cases was less than the number of animals with individual data reported. In some cases, data were reported for animals from which "0" cells were examined, e.g. #D77707 (Group 2, 24 hours) and #D77771 (Group 5, 24 hours).

These data are tabulated in Table 1 of this review.

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Table 1. Number of Animals Examined for Chromosomal Abnormalities[a]

Group	Test material	Dose	Time of Sacrifice 6 hours	12 hours	24 hours
1	Corn Oil	5 ml/kg	9/12[b]	10/10	10/11
2	Mitomycin C	5 mg/kg	-	-	8/11
3	Acetochlor	40 mg/kg	9/11	10/11	10/11
4	Acetochlor	150 mg/kg	6/12	10/10	10/10
5	Acetochlor	500 mg/kg	11/11	10/10	8/12

[a]data excerpted from submitted study.

[b]number of animals with results reported in individual animal data/ number of animals with results reported in summary table.

Discussion

No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined.

Significant discrepancies were noted by the reviewer between the results of chromosomal examinations as reported in the summary table and the data contained in the individual animal data appendices. For 8/13 reported results, the number of animals with actual data was less than the number of animals reported as examined in the summary table. For two animals, data were reported although the individual data indicated that "0" cells were examined for these animals. These discrepancies are sufficient cause for an audit of the supporting raw data.

Classification: Unacceptable Deficiencies as noted.

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Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/8/87 160
Review Section V, Toxicology Branch/HED (TS-769C)

Mutagenicity Secondary Reviewer: Irving Mauer, Ph.D.
Review Section VI, Toxicology Branch/HED (TS-769C)

Section Head Sign Off: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-769C)

I. Study Type: Mutagenicity - IN VIVO Mouse Micronucleus Test
Guideline §84-2

Study Title: IN VIVO Micronucleus Assay in Mice with Acetochlor

EPA Identification Numbers: EPA ID No. 3F2966 and 534-GUI
EPA Accession No. 266002
EPA Record No. 185604
Shaughnessy No.
Caswell No. 3B
Tox. Branch Project No. 7-0375
Document No.

Sponsor: Monsanto Company
1101 17th Street, N.W.
Washington, D.C. 20036

Testing Laboratory: Hazelton Biotechnologies Corporation
9200 Leesburg Turnpike
Vienna, Virginia 22180

Study Number(s): Report No. HL-84-405
Project No. 241-207
R.D. No. 685
Special Report No. MSL-5723

Study Date(s): June 2, 1986 (March 13, 1985)

Study Author(s): Compiled by F.L. Groya
Joy Cavagnaro, Ph.D.
Thomas Cortina

Test Compound: MON 097 (also known as Acetochlor)
Purity = 96.7%
Description: a dark red liquid
Lot Number: Dayton Batch 18 (BA-18)
Date: June 1, 1983

Vehicle(s): Corn Oil
from C.F. Sauer Co.
Lot No. 52500

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Positive Control(s): Cyclophosphamide
from Sigma
Lot No. 123F-0283

Dose(s): Vehicle Control - Corn Oil at 10 ml/kg
Positive Control (Cyclophosphamide) 40 mg/kg
MON 097 - single doses of 200, 660 or 2000 mg/kg administered by oral gavage at a dosing volume of 10 ml/kg.

Test Animal(s): Male and female CD®-1 mice
received from Charles River Breeding Laboratories, Inc., Kingston, New York, October 17, 1984.
141 animals per sex
approximately 32-40 days old

This study was designed to ev[illegible] the mutagenicity potential of MON 097, administered orally to male and female mice as determined by micronuclei production in polychromatic erythrocytes (PCE).

II. Materials and Methods: A copy of the "Methods and Materials" section from the investigators report is appended. The following comments and highlights on the materials and methods are noted:

Animals were kept under standard animal care conditions. They were acclimated 41 days prior to study initiation. They received Waynes F-6 Rodent Blox and "water" ad libitum.

The animals were "randomized via computer-generated random numbers" to the following groups:

Group	n Male	n Females	Dose
1 - Vehicle Control	27	27	Corn Oil
2 - Positive Control	9	9	Cyclophosphamide 40 mg/kg
3 - Low Dose - MON 097	27	27	200 mg/kg
4 - Mid Dose - MON 097	27	27	660 mg/kg
5 - High Dose - MON 097	27	27	2000 mg/kg

All animals received 10 ml/kg once by oral gavage. Dosing solutions were prepared fresh on day of administration. Dose levels were based on range-finding study (provided in report, see following "Results" section).

Clinical observations for "general appearance, behavior, toxic and pharmacological effects" were noted "twice daily or prior to sacrifice." Body weights were taken prior to treatment and at sacrifice.

Nine animals per sex were sacrified at 24, 48 and 72 hours (except for positive control where all animals were sacrificed at 24 hours).

The cytogenetic techniques used are described in the attached "materials and methods." Six-hundred and twenty-five polychromatic erythrocytes (PCE) were scored for the presence of micronuclei from 8 of the 9 mice, chosen randomly, for each group and sex. The numbers of normochromatic erythrocytes (NCE) were also recorded.

Statistical procedures were described, see attached "materials and methods". Methods appear to be adequate to interpret results.

A Quality Assurance Statement was included.

III. Results

A. IN VIVO Micronucleus Assay Dose Range Finding Study

Six animals per sex were used (CD® -1 mice approximately 32 to 40 days old). They received either 1000, 2000 or 3000 mg/kg of MON 097 as a single oral dose in corn oil.

1. Mortality

One male in the 2000 mg/kg group and both females of the 3000 mg/kg group died.

2. Clinical Observations

The following clinical signs were noted (Table I).

Table I: Clinical Observation Data[a]

Dose (mg/kg):	1000	2000	3000
n =	4	3	3
Observations:			
Urine Stains	4(2)[†]	20(3)	12(3)
Soft Feces	2(2)	4(2)	2(2)
Slightly Depressed	2(1)	14(3)	4(3)
Depressed	--	--	2(1)
Rough Coat	--	5(3)	3(2)
Distended Abdomen	--	10(1)	--
Eyes Squinted	--	--	2(1)
Prostrate	--	--	2(1)
Tremors	--	--	2(1)
Labored Respiration	--	--	1(1)

[†] = # observation (# animals)
[a] = Data extracted from HLA Project No. 241-207 Table 1.

There was an apparent dose-related increase in clinical observations.

3. Body Weight

There was a dose related decrease in body weight gain (Table II).

Table II: Body Weight Gain[a]

Dose (mg/kg)	Body Weight Gain	
	Male	Female
1000	1.0 gm	0.5 gm
2000	0.5 gm	-3.5 gm
3000	-1.5 gm	--*

* = animals died
[a] = Data extracted from HLA Project No. 241-207 Table 2.

4. Conclusions:

The investigators determined that 2000 mg/kg was the MTD, and thus was the highest level to be tested in the primary study.

B. Primary Study

1. Mortality

Eleven males and 12 females of the 2000 mg/kg group (HDT) died.

2. Clinical Observations

No abnormal observations were noted in the control and low dose group at 24, 48 or 72 hours or in the positive control at 24 hours. The mid dose group had a few animals with urine stains or rough coat. The high dose, however, had significantly increased observations involving, urine stains, soft stool, rough coat, depression, labored respiration, red stains on nose and/or eyes, distended abdomen, tremors, and ataxia.

3. Body Weight

The following Table III presents the body weight gain data. The investigators provided both group mean and individual animal data.

Table III: Body Weight Gain (gms)[a]

Dose(mg/kg)	Hours		
	24	48	72
Control	-1.3/-1.3[†]	0.3/-0.7	-0.1/-0.4
Pos. Cont.	-1.6/-1.2	--	--
200	-0.6/-1.3	-0.7/-0.9	-0.1/-0.7
660	-1.3/-0.5	0/1.5	0.9/0.9
2000	-2.9/-0.6	0.9/-3.1	-0.2/-2.4

[†] = Male/Female
[a] = Data extracted from HLA Project No. 241-207 Table 5.

The provided data were variable, and therefore no definite conclusion could be drawn. The investigators stated that: "In general, animals weighed at 24 hours, including control, exhibited slight weight loss following treatment, while 48 and 72 hour animals varied between slight body weight loss and slight gain. The group 5 (2,000 mg/kg) 24 hour males, 48 hour females, and 72 hour females showed a slightly larger loss of body weight".

4. Cytogenetic Analysis

The investigators provided summary and individual animal data. The attached Table 2 from the investigators report presents the summary data. No effect on incidence of micronucleated polychromatic erythrocytes was noted between the 3 MON 097 test groups compared to the solvent control. The positive control, however, showed a statistically significant increase in PCE micronuclei. The ratio of polychromatic (PCE) to normochromatic (NCE) erythrocytes is presented on attached Table 4 (from the investigators report). The investigators noted a decrease in PCE:NCE ratio in the high dose at 72 hours. They attributed this to the onset of stem cell toxicity. They further stated that "According to Schmid (1976), the ratio of PCE:NCE in animals of similar age as used for this study should be about 1.1".

IV. Conclusions

Under conditions of this test the high dose level of MON 097 (2000 mg/kg) exhibited mortality and signs of clinical toxicity. No evidence of an increase in micronucleated polychromatic erythrocytes was noted at the dose levels tested in this study.

V. Core-Classification: Acceptable.