MEMORANDUM

SUBJECT: Acephate Technical - Mouse Somatic Cell Mutation Assay and Range-Finding Study

EPA ID No.: 239-2471
Record No.: 199425
Accession No.: 40209101
Caswell No.: 2A
Proj. No.: 7-0944

FROM: Krystyna K. Locke, Toxicologist
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: William H. Miller, PM 16
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Toxicology Branch/HED completed an evaluation of the following studies:


Study No. 1 was classified as Acceptable. Acephate was not mutagenic under the conditions of this study.

Study No. 3, was classified as Acceptable as a range-finding study, whereas the related studies (No. 2 and 4) were classified as Supplementary Data. Acephate was not assayed for mutagenic potential in these studies.
DATA EVALUATION REPORT

Study Type: Mutagenic (Mouse Somatic Cell Mutation Assay/Spot Test)

TOX Chem No.: 2A
MRID No.: None
Project No.: 7-0944

Accession No.: 40209101


Record No.: 199425

EPA ID No.: 239-2471

Synonyms: ORTHENE

Study No.: 2107-141

Sponsor: Chevron Environmental Health Center, Inc.
Richmond, CA

Testing Facility: Hazleton Laboratories America, Inc.
Rockville, MD


Author: M.R. Moore

Report Issued: October 1986

Conclusions:

Technical acephate was not mutagenic under the conditions of this assay, but positive results were obtained with ethynitrosourea (ENU), a known mutagen. At the 50 ppm level (LDT), no maternal and developmental toxic effects were observed, and no evidence of toxicity in nonpregnant mice was noted. Significant decreases in pregnancy rate and in percentage of live pups born occurred in the 200 ppm group. Maternal toxic effects were observed mostly in the 600 and 800 ppm (HDT) groups and included lacrimation, tremors, piloerection, hunchback, labored breathing, rales,
staggered gait, eye opacity, corneal sloughing, and keratitis; decreased pregnancy rate, body weights, and food consumption; and increased mortality (800 ppm group only). Developmental toxic effects were observed mostly in the 600 and 800 ppm groups and included significant decreases in the percentage of pups born alive, survival of pups, and body weights (bwt). Two abnormalities noted only in the 800 ppm group were missing tail and laceration on back.

Classification of Study: Acceptable

EXPERIMENTAL PROCEDURES

This assay was conducted according to the procedure of L.B. Russell, et al. (Mutation Res., 86:355-379, 1981), in which T-strain male and C57Bl/6 strain female mice are used as parents. The study was started on March 2, 1986 (first mating day) and was completed on May 26, 1986 (termination of all surviving animals).

Females with copulation plugs were assigned to groups as follows:

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Number of Females Originally Assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet control</td>
<td>134</td>
</tr>
<tr>
<td>Acephate 50 ppm</td>
<td>158</td>
</tr>
<tr>
<td>Acephate 200 ppm</td>
<td>158</td>
</tr>
<tr>
<td>Acephate 600 ppm</td>
<td>169</td>
</tr>
<tr>
<td>Acephate 800 ppm</td>
<td>137</td>
</tr>
<tr>
<td>ENU, positive control, 50 mg/kg bwt</td>
<td>139</td>
</tr>
</tbody>
</table>

During mating weeks 1 through 4, the assignment to groups was random using a computer-generated scheme. During mating weeks 5 and 6, the assignment procedure was altered to accommodate the addition of a study group (600 ppm) and to increase the likelihood of having a sufficient number of pups born in each group for assessing mutagenic potential (see Attachment I for details):

Acephate was administered in diet (Powdered Purina Certified Rodent Chow #5002) during gestation days (gd) 8.5 through 12.5. Because acephate was unstable in diets at room temperature (72 ± 5 °F), the diets were replaced on gd 10.5. Positive control was administered as a single intraperitoneal injection on gd 10.5.

The dose levels used were based on a pilot study (Project No. 22352, reviewed separately). Males of breeding age were obtained from Hazleton Laboratories America, Rockville, MD and
females from Charles River Breeding Laboratories, Raleigh, NC. The females were 11 weeks old when mated, housed singly and identified by ear and cage tags. The acclimation period was 14 days. Feed (with or without acephate) and tap water were provided in unrestricted amounts. The animal feed was analyzed for nutritional components and unintentional additives (pesticides and PCB). Tap water was also analyzed for unintentional additives.

All dams were allowed to litter normally. The mutagenic potential of acephate was determined from the number of pups having recessive color coat spots on lactation days 14 and 28. The following maternal and litter data were also obtained:

**Maternal Data**

1. Toxic signs and mortality during gestation and lactation.
2. Mean daily food consumption during gd 0.5 to 8.5, 8.5 to 13.5, 13.5 to 17.5, and 17.5 to 23.5.
3. Mean body weights during gd 0.5, 8.5, 13.5, 17.5, and 23.5, and lactation day 7.
4. Pregnancy rate.
5. Duration of gestation.
6. Histopathology: eyes were examined (at sponsor's request) from arbitrarily selected non-pregnant females in the diet control and each of the acephate-treated groups. All other tissues were discarded.

**Litter Data**

1. Size of litters and external abnormal morphology (live and dead pups) on lactation days 1, 7, 14, and 28.
2. Litter weight (live and dead pups) on lactation days 1 and 7.
3. Individual body weights on lactation days 14 and 28.
4. Sex of pups, determined on lactation day 14.
5. Percent of live pups born and survival until termination of the study.

Maternal and litter data were analyzed statistically as detailed in Attachment II. All females that did not litter on gd 23.5 were weighed, killed, and discarded. All surviving animals were sacrificed after lactation day 28.
RESULTS

Of the 895 female mice initially assigned to this study, 854 were included and 41 were excluded from the report submitted to EPA as follows:

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Number of Females Included</th>
<th>Excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet control</td>
<td>129</td>
<td>5</td>
</tr>
<tr>
<td>Acephate 50 ppm</td>
<td>150</td>
<td>8</td>
</tr>
<tr>
<td>200 ppm</td>
<td>151</td>
<td>7</td>
</tr>
<tr>
<td>600 ppm</td>
<td>164</td>
<td>5</td>
</tr>
<tr>
<td>800 ppm</td>
<td>129</td>
<td>8</td>
</tr>
<tr>
<td>Positive control (ENU)</td>
<td>131</td>
<td>8</td>
</tr>
</tbody>
</table>

The animals were excluded for the following reasons:

1. Two females in the 800 ppm group died prior to gd 8.5 (the first day of the 5-day treatment interval) and their deaths were therefore not due to treatment.

2. The remaining 39 females littered on or before gd 17.5 and it was suggested that they might have conceived before a copulation plug was detected and therefore were not actually treated during gd 8.5 through 12.5.

Mutagenic Potential of Acephate

Acephate was not mutagenic in this assay. There was no significant increase in the number of pups with recessive coat spots (RCS) when the treated groups were compared with the concurrent diet control group. However, there was a significant increase ($p < 0.01$) in the number of pups with RCS in the positive control group (see Attachment III for details).

Maternal Effects

Toxic signs were observed only in the 600 and 800 ppm groups and included lacrimation, tremors, piloerection, hunchback, labored breathing, rales, staggered gait, cloudy eye, or corneal sloughing. In the 600 ppm group, toxic signs were observed in 43 percent of pregnant and 69 percent of nonpregnant mice. The incidence of toxic signs in the 800 ppm group was 83 percent in pregnant and 90 percent in nonpregnant mice.

Mortality was increased significantly only in the 800 ppm group, compared to the diet control group. A total of 30 females (23.25%; $p < 0.01$) died during the course of the study, 8 during gd 8.5 to 13.5 (treatment interval) and 22 during gd 14.5 and 18.5, inclusive.
Pregnancy rate—Significant (p < 0.05) decreases in pregnancy rates were observed only in the 200 and 600 ppm groups, compared to the diet control group. Pregnancy rates in the diet control, 50, 200, 600, 800 ppm, and positive control groups were 44.96, 34.00, 29.14, 31.10, 35.66, and 37.40%, respectively.

(Pregnancy rate was calculated from number of females that littered or were found pregnant at necropsy divided by total number of females included in each study group.)

Body weights—Significant (p < 0.01) decreases in mean body weights were observed in the 600 and 800 ppm acephate-treated groups and in the positive control group, when these animals were compared with the diet control group, as follows:

- Acephate-treated pregnant females, on gd 13.5 and 17.5, and on lactation day 7.
- Acephate-treated nonpregnant females, on gd 13.5, 17.5, and 23.5.
- Positive control group (pregnant and nonpregnant females), on gd 13.5 and 17.5.

Food consumption—Compared to the diet control group, significant (p < 0.05 or p < 0.01) decreases in mean daily food consumption were observed in the 600 and 800 ppm acephate-treated groups, as follows:

- Pregnant females (600 ppm group), during gd 0.5 to 17.5.
- Pregnant females (800 ppm group), during gd 8.5 to 17.5.
- Nonpregnant females (both groups), during gd 8.5 to 23.5.

Duration of gestation—With the possible exception of the 800 ppm dosage, the remaining levels of acephate tested, as well as ENU, had no effect on the duration of gestation. In the diet control, positive control (ENU) and the 200 ppm acephate-treated groups, gestation period lasted from 18.5 to 20.5 days; and in the 50 and 600 ppm acephate-treated groups, from 18.5 to 21.5 days. Most of the females in the 800 ppm acephate group littered during gd 18.5 to 21.5, but 2 females (out of 34 or 5.88%) littered during gd 22.5, which might possibly be attributed to acephate.

Histopathology—Only eyes ("a representative sample") were examined histologically, at sponsor's request, subsequent to notification that ocular lesions (cloudiness and corneal sloughing) were noted grossly during the course of the study in the 600 and 800 ppm acephate-treated groups. The incidence of these lesions was not reported.
Eyes from the following females, all nonpregnant and sacrificed on gd 23.5, were examined:

<table>
<thead>
<tr>
<th>Acephate ppm</th>
<th>Number of Mice Used</th>
<th>Number of Eyes Exam.</th>
<th>Gross Observation of Eyes</th>
<th>Number of Eyes With Keratitis and Severity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>8</td>
<td>All normal</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>5</td>
<td>All normal</td>
<td>1 mild</td>
</tr>
<tr>
<td>200</td>
<td>4</td>
<td>4</td>
<td>All normal</td>
<td>1 minimal</td>
</tr>
<tr>
<td>600</td>
<td>32</td>
<td>32</td>
<td>11 normal</td>
<td>9 (4 minimal, 3 mild, and 2 moderate)</td>
</tr>
<tr>
<td>800</td>
<td>4</td>
<td>6</td>
<td>11 recovered**</td>
<td>2 mild</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 cloudy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 corneal sloughing</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 recovered</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 corneal sloughing</td>
<td></td>
</tr>
</tbody>
</table>

*Degree of inflammation was graded for severity as follows: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; and 5 = severe.

**Eyes with previous cloudy and/or corneal sloughing observations but appeared normal at necropsy.

Focal keratitis and one incidence of diffuse keratitis (600 ppm group) were the only corneal lesions observed histopathologically. The testing laboratory concluded that 1) in no case was there histological evidence of damage to corneal surface epithelium; and 2) the cause of these lesions could not be definitely identified from the data at hand.

Developmental effects were as follows:

1. Significant (p < 0.01) but dose-unrelated decrease in the percentage of pups born alive in the 200, 600, and 800 ppm acephate-treated groups when compared with the diet control group. The percentage of pups born alive in the diet control, positive control, and 50, 200, 600, and 800 ppm acephate-treated groups was 99, 98, 96, 93, 91, and 94, respectively.

2. Significant (p < 0.01) but dose-unrelated decrease in the survival of pups to days 7, 14, and 28 when the treated animals (600 and 800 ppm groups) were compared with the diet control group. The percentage of pups surviving to day 28 in the diet control, positive control, and 50, 200, 600, and 800 ppm acephate-treated groups was 80, 77, 77, 77, 25, and 26, respectively.
3. Significantly (p < 0.01 or 0.05) lower mean pup body weights at lactation days 1 and 7 in the 600 and 800 ppm groups, in comparison to the diet control group.

4. Only two pups with abnormalities were observed in this study, both in the 800 ppm group. One pup had no tail and another had a laceration on the back (at birth only).

Technical acephate itself was stable at room temperature (25 °C), but it was unstable in diets. Diets containing acephate were therefore stored at -20 °C and were changed after 3 days when fed to animals. Stability of acephate under the conditions of handling, storage, dispensing, and animal room conditions, diet homogeneity, and dose level verification were determined by the sponsor and were reported separately (see Addendum dated July 28, 1986).

Quality assurance inspections of the study were conducted six times during February 11 and April 3, 1986. Final report was reviewed during September 19 through October 3, 1986.

Classification of Study: Acceptable
ACEPHATE

Page ______ is not included in this copy.

Pages 10 through 15 are not included.

The material not included contains the following type of information:

___ Identity of product inert ingredients.

___ Identity of product impurities.

___ Description of the product manufacturing process.

___ Description of quality control procedures.

___ Identity of the source of product ingredients.

___ Sales or other commercial/financial information.

___ A draft product label.

___ The product confidential statement of formula.

___ Information about a pending registration action.

X FIFRA registration data.

___ The document is a duplicate of page(s) ________.

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DATA EVALUATION REPORT

Study Type: Mutagenic (Mouse Somatic Cell Mutation Assay): Addendum

TOX Chem. No.: 2A
Proj. No.: 7-0944

Accession No.: 40209101

Test Material: Acephate Technical (SX-1102)

Synonyms: ORTHENE

Sponsor: Chevron Environmental Health Center, Inc.
Richmond, CA

Testing Facility: Residue Chemistry Laboratory
Development Research Department
Chevron Chemical Company, Richmond, CA

Title of Report: Addendum to Somatic Cell Mutation Assay in Mice with Acephate Technical (SX-1102)

Author(s): Slagowski, J.L.; and Leary, J.B.

Report Issued: July 28, 1986

Conclusions:
Homogeneity and stability of acephate in diets used in the mouse somatic cell mutation assay (dated October 1986) were adequate. The purity of the test material (active ingredient content) used in the same assay was 99 percent.

Classification:
Acceptable as Supplemental Data. These data should be considered together with those of the main study.
EXPERIMENTAL PROCEDURES

The test material (EPA Registration No. 239-2471) was analyzed for acephate (active ingredient) content on January 8, and June 25, 1986.

Homogeneity studies were conducted on diets prepared on January 16 and March 6, 1986 and containing 50, 200, or 800 ppm of technical grade acephate. A homogeneity study for the 600 ppm dose level was conducted on samples obtained on April 10, 1986. The analyses were performed on nine samples from each dose level. Samples were collected immediately after diet preparation by Hazleton Laboratories America and shipped frozen to the testing facility.

Diets were also analyzed for stability of acephate as detailed in Attachment A. Diets were prepared no more than 5 days prior to their use and were stored frozen.

RESULTS

The test material contained 98.7 and 98.9% of acephate (active ingredient) during the first and second analysis, respectively.

The results of the homogeneity analyses were acceptable. As is shown in Attachment B, the analytical concentration of acephate in most samples ranged from 90 to 99 percent of the theoretical concentration.

Acephate was stable in diets during the course of the study. The analytical acephate concentrations of the freshly prepared diets ranged from 93 to 94 percent of the theoretical concentration. Freezing for 1 to 4 days did not affect the acephate content of diets and neither did 3-day exposure to room temperature* (see Attachment A for details).

A Quality Assurance Statement has been included in this report.

Classification:

Acceptable as Supplemental Data, which should be considered together with those of the main study (Project No. 2107-141, dated October 1986).

*Diets were changed after 3 days in the main study.
The material not included contains the following type of information:

___ Identity of product inert ingredients.
___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.

X The document is a duplicate of page(s) ________.
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DATA EVALUATION REPORT

Study Type: Range-Finding for Mouse Somatic Cell Mutation Assay

TOX Chem. No.: 2A
MRID No.: None
Proj. No.: 7-0944

Accession No.: 40209101

Test Material: Acephate Technical (SX-1102); Purity: 98% (approx.)

Synonyms: ORTHENE

Study No.: 22352

Sponsor: Chevron Environmental Health Center, Inc.
Richmond, CA

Testing Facility: Litton Bionetics, Inc./Hazleton Laboratories America, Inc.*

Title of Report: Pilot Evaluation of Chevron Acephate Technical in the Mouse Somatic Cell Mutation Assay

Author: Wolfe, G.W.

Report Issued: April 1986

Conclusions:

Various toxic effects were observed in pregnant and nonpregnant mice and in pups mostly from the mid-dose (800 ppm) and high-dose (1200 ppm) groups. It was concluded (and Toxicology Branch agrees) that, because of these effects, especially high pup mortality, dose levels of 800 and 1200 ppm "could potentially impede or negate the conduct of a Mouse Somatic Cell Mutation Assay."

Classification of Study:

Acceptable as a range-finding study.

* This pilot study was started on July 28, 1985 and completed on August 27, 1985. On August 31, 1985, all facilities and staff involved in the conduct of the study were acquired by Hazelton Laboratories America, Inc.
EXPERIMENTAL PROCEDURES

Female mice of C57Bl/6 strain, 51 to 55 per dose level, were mated with T-strain male mice and then fed diets containing 0, 200, 800, or 1200 ppm of acephate during gestation days (gd) 8.5 through 12.5. Feed (Powdered Purina Certified Rodent Chow #5002), with or without acephate, and tap water were provided in unrestricted amounts. Diets were (1) prepared the day before dosing and stored at -20 °C; (2) assayed for acephate stability prior to study initiation; and (3) analyzed for homogeneity during the course of the study. The test material itself was stored at 4 °C.

Males, obtained from Hazleton Laboratories America, were at least 2 months old and females, obtained from Charles River Breeding Laboratories (Portage, MI), were 2.5 months old when mated. The acclimation period was 32 days. Mated females were randomly assigned to groups and housed singly. The study was terminated on lactation day 7. The following maternal and litter data were obtained:

Maternal Data

1. Toxic signs and mortality during gestation and lactation.
2. Mean daily food consumption during gd 0.5 to 8.5, 8.5 to 13.5, 13.5 to 17.5, and 17.5 to 23.5, and lactation days 1 to 7.
3. Mean body weights during gd 0.5, 8.5, 13.5, 17.5, and 23.5, and lactation days 1 and 7.
4. Pregnancy rate.
5. Duration of gestation.

Litter Data

1. Toxic signs and mortality.
2. Size and weight of litters and individual pups, and gross abnormalities on days 1 and 7.

Maternal and litter data were analyzed statistically as detailed in Attachment 1.
RESULTS

Compared to the control group, toxic effects were observed in pregnant and nonpregnant animals and in pups mostly from the mid-dose (800 ppm) and high-dose (1200 ppm) groups as follows:

Mid-Dose Group

1. Toxic signs: tremors, lacrimation, labored breathing, hunchback, piloerection, eye opacity, and corneal sloughing. These signs were observed during treatment and, in several instances, persisted until termination of the study.

2. Decreases (7 to 19%; p = 0.01 or 0.05) in mean body weights on gd 13.5 and thereafter.

3. Decreases (23 to 49%; p = 0.01 or 0.05) in mean daily food consumption on gd 8.5 and thereafter.

4. Decrease (12%; p = 0.01) in the number of pups born alive.

5. Decrease (25%; p = 0.01) in the survival of pups until the termination of the study.

High-Dose Group

1. Toxic signs: the same as in the mid-dose group.

2. Decreases (7 to 21%; p = 0.01) in mean body weights on gd 13.5 and thereafter.

3. Decreases (25 to 53%; p = 0.01) in mean daily food consumption on gd 8.5 and thereafter.

4. Decrease (10%) in the survival of dams.

5. Decrease (22%; p = 0.01) in the number of pups born alive.

6. Decrease (71%; p = 0.01) in the survival of pups until the termination of the study.

7. Decreases in mean pup body weights on days 1 (22%; p = 0.01) and 7 (25%; p = 0.05).

The only toxic sign observed in the low-dose (200 ppm) group was slight (7%) but significant (p = 0.05) decrease in the survival of pups until the termination of the study.
It is a well planned and reported study. Its purpose was to select dose levels of technical grade acephate for a somatic cell mutation assay. Acephate was not tested for mutagenic potential in this study. Quality Assurance Statement dated February 19, 1986 was included in the report, as were amendments to the study and individual data.

The test material, stored at 4 °C, was stable for at least 6 months. The stability and homogeneity of acephate in diets will be reported separately.

Classification of Study:

Acceptable as a range-finding study.

Attachment
The material not included contains the following type of information:

____ Identity of product inert ingredients.
____ Identity of product impurities.
____ Description of the product manufacturing process.
____ Description of quality control procedures.
____ Identity of the source of product ingredients.
____ Sales or other commercial/financial information.
____ A draft product label.
____ The product confidential statement of formula.
____ Information about a pending registration action.
X____ FIFRA registration data.
____ The document is a duplicate of page(s) ________.
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DATA EVALUATION REPORT

Study Type:  Range-Finding for Mouse Somatic Cell Mutation Assay: Addendum

TOX Chem. No.:  2A
Proj. No.:  7-0944

Accession No.:  40209101

Test Material:  Acephate Technical (SX-1102); Purity:  99%

Synonyms:  ORTHENE

Sponsor:  Chevron Environmental Health Center, Inc.
Richmond, CA

Testing Facility:  Chevron Chemical Company, Richmond, CA


Author:  Slagowski, J.L., et al.

Report Issued:  October 16, 1985

Conclusions:

Stability of acephate in diets used in the range-finding study (dated April 1986) was adequate. These diets were not assayed for homogeneity.

Classification:

Acceptable as Supplemental Data. These data should be considered together with the range-finding study.
EXPERIMENTAL PROCEDURES

Homogeneity studies were conducted on diets prepared on June 12, July 8, and July 17, 1985 and containing 200, 800, or 1200 ppm of technical grade acephate. The analyses were performed on nine samples from each dose level. Samples were collected immediately after diet preparation by Litton Bionetics, Inc. and shipped frozen to the testing facility. Diets prepared on June 12 and August 5, 1985 were also tested for stability of acephate. Duplicate or triplicate samples were collected from each batch and assayed for acephate concentration as follows: 1) immediately after diet preparation; 2) stored frozen for 1 or 5 days; and 3) after 5 days in animal cage hoppers.

RESULTS

Homogeneity Assays

All of these assays were conducted on diets prepared before the range-finding study was started and all of the results were unacceptable because of excessive variations in acephate content of samples. For diets containing 200, 800, or 1200 ppm of acephate, the coefficients of variation were 5.2 to 22.0, 23.0, and 12 to 20 percent, respectively. An acceptable coefficient of variation was 5.0 percent. Sample variations were attributed to mixing procedures.

Stability Assays

Assays conducted on diets prepared on June 12, 1985 (before the range-finding study was started) were unsatisfactory because of great variations in acephate content of samples. Assays conducted on diets prepared on August 5, 1985 (range-finding study was started on July 28, 1985) were regarded as satisfactory because the acephate content of samples was within acceptable limits. In the latter case, freshly prepared diets, those stored frozen for 5 days, and those exposed to room temperature for 5 days contained 96 to 109, 94 to 108, and 83 to 94 percent of acephate, respectively. Diets prepared on August 5, 1985 were not assayed for homogeneity of acephate.

All analytical procedures were detailed in the report and raw data were also submitted.

Classification:

Acceptable as Supplemental Data, which should be considered together with the range-finding study (Project No. 22352, dated April 1986).