DATA EVALUATION REPORT

ALDICARB

Study Type: Rat Milk Transfer Study

Prepared for:
Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:
Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer
Sanju Diwan, Ph.D.
Date 8/11/92

Independent Reviewer
Nancy MacCord, B.S.
Date 8/11/92

QA/QC Manager
Sharon Segal, Ph.D.
Date 8/11/92

Contract Number: 68D10075
Work Assignment Number: 1-31
Clement Number: 91-116
Project Officer: James Scott
DATA EVALUATION REPORT

STUDY TYPE: Rat milk transfer study

EPA IDENTIFICATION NUMBERS

Tox Chem. Number: 011A
MRID Number: 41865801
HED Project Number:
Other Number: RTI-60C-4752

TEST MATERIAL: 2-Methyl-2(methylthio) propionaldehyde o-(methylcarbamoyl) oxime

SYNONYMS: Aldicarb, technical grade

SPONSOR: Rhone-Poulenc Ag Company, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709

STUDY NUMBER: 60C-4752

TESTING FACILITY: Reproductive and Developmental Toxicity Laboratory, Research Triangle Institute, Research Triangle Park, NC 27709-2194

TITLE OF REPORT: Preliminary Evaluation of Aldicarb Excretion in the Milk of Lactating CD Rats Exposed to Aldicarb in the Diet

AUTHOR(S): Tyl, R.W., Marr, M.C., and Myers, C.B.

REPORT ISSUED: April 5, 1991

TEST DATES: June 4, 1990 - March 22, 1991

CONCLUSIONS: In a one-generation reproduction study, CD rats were fed diets containing aldicarb at 20 ppm from gestation day (GD) 7 to postnatal day (PND) 4. The mean compound intake from GD 7 to 20 and from PND 0 to 4 was 1.4 and 1.88 mg/kg/day, respectively. Treatment related marginal toxicity was noted in dams and was manifested as decreased body weight gain and food consumption. A decrease in mean pup weight per litter was also observed on PND 4. Aldicarb was metabolized to aldicarb sulfone and aldicarb sulfoxide. The detected concentrations of aldicarb and its metabolites in the milk were within the range of 10-29 ppb. The mean test material intake by pups via the milk on PND 4 was 6.74 μg/kg.
CORE CLASSIFICATION: The study is acceptable provided that the historical control data on pup mortality and reproductive indices are submitted and are found acceptable. The reviewers further recommend that the available data be reevaluated along with the results of the full reproduction study now under review in Health Effects Division. In summary, the study seems to show some transfer of aldicarb and its metabolites via rat milk but fails to replicate the finding of significant fetotoxicity.

A. MATERIALS

Test Compound

Purity: 99.7%
Description: White, crystalline solid; odorless to light sulfur smell
Lot number: 25DEQ 89
Receipt date: Not reported
Contaminants: None specified

Vehicle(s)

None used; the test material was administered in the diet. Homogeneity and stability were determined. Dose-feed formulations were 79.7-104% of target. Three of these formulations were below 90%.

Test Animal(s)

Species: Rat
Strain: Sprague-Dawley (CD)
Source: Charles River Laboratories, Inc., Raleigh, NC
Age: Sixty-one days upon receipt
Body weight of female: 227.28-307.87 g on study day 0
Information on males used: Male rats of the same strain and source were used.

B. STUDY DESIGN

This study was designed to determine the level of aldicarb and its metabolites in the milk of lactating CD rats on PND 4. Maternal animals were exposed to aldicarb in their diet at 20 ppm from GD 7 to PND 4.

Animal Husbandry: Animals were identified by a uniquely numbered ear tag upon arrival and were housed individually in cages. They were acclimatized to the laboratory environment for 4 weeks and were given Purina Rodent Chow #5002 and tap water ad libitum. The environmental conditions were as follows: light -- 12-hour light/dark cycle; temperature -- 68-75°C; relative humidity -- 40-60%; air changes -- none specified.
Mating Procedure: Following acclimation, females at approximately 13 weeks of age were mated 1:1 with males of the same strain and source. Females were checked each morning for sperm in the vaginal smear. The day on which a sperm-positive smear was found was designated day 0 of gestation.

Group Arrangement: Animals were assigned to the two study groups by a stratified randomization method designed to provide uniform mean body weights in all groups. A total of 17 and 24 pregnant females were assigned to the control and test groups, respectively.

Dosing: The test compound was fed daily in the diet ad libitum from GD 7 through PND 4. Test diets were prepared by mixing finely powdered test compound in the feed. Homogeneity and stability of the test material were determined prior to administration of the test diet. The test diet was homogeneous (≤5% variability) and was stable for at least 7 days under refrigerated conditions. Homogeneity was determined on the basis of three samples each from the right, left, and bottom portions of the blender.

The test concentration was selected on the basis of the results of an earlier range-finding reproduction study conducted by the sponsor in which a significant increase in pup mortality (37%) was observed from PND 0 to 4 when dams were fed 20 ppm aldicarb in the diet.

Observations: Animals were examined at least once daily for mortality, morbidity, and signs of toxicity. Body weights were recorded on GD 0, 7, 14, and 20. Dams with litters were weighed daily from PND 0 through 4, the day of milk collection. Mean maternal food consumption values were reported for GD 0–7, 7–14, 14–20, and PND 0–4. Prior to milking on PND 4, dams were anesthetized by intramuscular injection of Vetalar (≈200 μl/animal). Approximately 20–40 IU oxytocin (0.1–0.2 ml) was administered peritoneally to each dam 5–30 minutes prior to milk sample collection. Dams were then euthanized by CO₂ asphyxiation; necropsies were not performed.

The numbers of live and dead pups were recorded after birth. Each live pup was sexed, weighed, and examined for external abnormalities. Litters were examined daily for survival. On PND 4, 3–5 hours prior to the scheduled milk collection, pups were weighed, examined for anomalies, removed from the dam, euthanized by CO₂ asphyxiation, and discarded without necropsy.

Historical control data were not provided to allow comparison with concurrent controls.

Milk Sample–Collection and Analysis: Since most of the milk samples were less than 1 g, samples from dams were pooled to provide a sufficient quantity of rat milk for analysis. Following extraction with solvents, 10 milk samples from treated dams and 3 samples from control dams were analyzed for the concentration of aldicarb and its metabolites, aldicarb sulfoxide and aldicarb sulfone. A series of calibration standards were prepared to span the expected range of concentrations (0–50 ppb in 1 g of milk) and were used to generate a calibration curve which was then used to calculate the concentration present in the sample. Samples were analyzed
by high performance liquid chromatography (HPLC). The extraction and analytical procedures used were validated with control rat milk. Samples were "spiked" by adding carbamate spiking solution. The recovery of aldicarb and its identified metabolites in spiked samples was low (approximately 68%-77%) and was possibly related to the small size of the samples and/or to losses during the extraction procedure.

Statistical Analysis: The following methods were used:

An arcsine-square root transformation was performed on all litter-derived percentage data. Bartlett's test for homogeneity of variance was performed on all data to be analyzed by ANOVA. GLM (General Linear Models) analysis was conducted to determine the significant dose effects for selected measures.

Protocol: A protocol was provided and the deviations from protocol were justified.

Compliance

- A signed Statement of No-Data Confidentiality Claim, dated March 22, 1991, was provided.

- A signed Statement of Compliance with EPA GLPs, dated April 4, 1991, and March 2, 1991, was provided.

- A signed Quality Assurance Statement was not provided.

C. RESULTS

The following results were reported by the study authors:

1. Test Material Analysis

The concentrations of the test material in the diets ranged from 79.7% to 104% of target. Although three of the four dietary formulations were 16-20% below the target (Table 1), the study authors claimed that the objectives of the study were met. Homogeneity analyses revealed concentrations of 90.5-108% of nominal values. Analysis for stability of the test material in the diet after 7 days under refrigeration revealed an aldicarb concentration of 101% of the day 0 measurement.

2. Parental Toxicity

Mortality: No compound-related mortality was observed.

Clinical Observations: Piloerection was noted in five dams (20%) at 20 ppm from GD 16 through 21. No other significant treatment-related clinical signs were observed.

Body Weight: Summaries of mean body weight gains from selected time intervals are presented in Table 2. Compound-related decreases in body weight and weight gain were observed in the treated group. Body
weight gain in treated dams from GD 7 to 20 (p<0.01) and from PND 0 to 4 (p<0.001) was significantly lower than in the control group. A significant decreasing trend was evident in body weight gain from GD 7 to 20 (p<0.01) and from PND 0 to 4 (p<0.001). Maternal body weight (data not shown) was reduced on GD 7 (1%), GD 14 (4%), and GD 20 (5%); a significant (p<0.001) decrease (10%) was also observed on PND 4.

Food Consumption: A summary of mean food consumption data (g/animal/day) is presented in Table 3. Food consumption in treated dams was significantly lower than in the control group from GD 7 to 20 (p<0.001) and from PND 0 to 4 (p<0.01).

3. Results of Analyses of Milk Samples

One of 10 samples contained aldicarb (24.8 ppb) and its metabolites, aldicarb sulfoxide (24.6 ppb) and aldicarb sulfone (28.8 ppb). Aldicarb sulfoxide was detected in 5 of 10 samples; the mean concentration was 16.6 ppb (range: 9.7-24.6 ppb). Aldicarb sulfone was detected in 6 of 10 samples with a mean concentration of 20.9 ppb (range: 14.2-28.8 ppb).

4. Test Material Intake

The mean test material intake of aldicarb from GD 7 to 20 and from PND 0 to 4 was 1.4 and 1.88 mg/kg/day, respectively.

5. Reproductive Toxicity

The effects of dietary administration of aldicarb on reproductive parameters are summarized in Table 4. Fertility in control and treated dams was low and no statistical analysis of the data was performed. The study authors attributed the low fertility in dams to an unspecified testicular problem in Charles River male CD rats. However, no documentation was provided to substantiate this finding. A nonsignificant reduction (6-7%) in the mean litter size on PND 0 and PND 4 was noted in the treated group. Mean pup body weight per litter was significantly (p<0.01) lower (12%) in the treatment group on PND 4. Although pup loss increased approximately two-fold over the control group from PND 0 to 4 (3.88% versus 1.55% in controls), it was reported by the study authors to be within the historical control range of the reporting laboratory for CD rat pups. No data were provided regarding external malformations in pups.
TABLE 1. Results of Test Diet Analyses

<table>
<thead>
<tr>
<th>Date of Analysis</th>
<th>Mean Concentration</th>
<th>Percent Recovery of Nominal Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/17/90</td>
<td>16.4 ± 4.1</td>
<td>81.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7/24/90</td>
<td>16.7 ± 5.8</td>
<td>83.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7/31/90</td>
<td>20.8 ± 9.5</td>
<td>104.0</td>
</tr>
<tr>
<td>8/07/90</td>
<td>16.0 ± 1.4</td>
<td>79.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data extracted from Study No. 60G-4752, Tables 5, 6, 7, and 8, pages 12-15
<sup>b</sup>16-20% below the target concentration

TABLE 2. Mean Body Weight Gain (g)<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Dietary Concentrations (ppm)</th>
<th>Mean Body Weight Gain (g) Mean Body Weight Gain (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GD 0-7</td>
</tr>
<tr>
<td>0</td>
<td>40.0</td>
</tr>
<tr>
<td>20</td>
<td>35.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data extracted from Study No. 60G-4752, Table 2, pages 20-21
<sup>b</sup>Calculated by the reviewers

* p ≤ 0.01 by ANOVA and test for Linear Trend
** p ≤ 0.001 by ANOVA and test for Linear Trend

TABLE 3. Mean Food Consumption (g/animal/day)<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Dietary Concentrations (ppm)</th>
<th>Mean Food Consumption (g/animal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GD 0-7</td>
</tr>
<tr>
<td>0</td>
<td>21.2</td>
</tr>
<tr>
<td>20</td>
<td>21.9</td>
</tr>
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</table>

<sup>a</sup>Data extracted from Study No. 60G-4752, Table 4, pages 24-25
<sup>b</sup>Calculated by the reviewers

* p<0.001 calculated by the reviewers using ANOVA
** p<0.01 by ANOVA and test for Linear Trend
<table>
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<tr>
<th>Parameter</th>
<th>Dietary Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No. of matings</td>
<td>25</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>19</td>
</tr>
<tr>
<td>Fertility index--females (%)</td>
<td>76</td>
</tr>
<tr>
<td>Total number of live pups(^b)</td>
<td></td>
</tr>
<tr>
<td>PND 0</td>
<td>258</td>
</tr>
<tr>
<td>PND 4</td>
<td>254</td>
</tr>
<tr>
<td>No. dead/missing</td>
<td>4</td>
</tr>
<tr>
<td>Mean no. live pups/litter</td>
<td></td>
</tr>
<tr>
<td>PND 0</td>
<td>13.6</td>
</tr>
<tr>
<td>PND 4</td>
<td>13.4</td>
</tr>
<tr>
<td>Live birth index (%)(^c)</td>
<td>100</td>
</tr>
<tr>
<td>Viability index (%)(^d)</td>
<td>98.4</td>
</tr>
<tr>
<td>Mean pup body weight/litter (g)</td>
<td></td>
</tr>
<tr>
<td>PND 0 (male)</td>
<td>6.65</td>
</tr>
<tr>
<td>PND 0 (female)</td>
<td>6.20</td>
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<tr>
<td>PND 4 (male)</td>
<td>10.80*</td>
</tr>
<tr>
<td>PND 4 (female)</td>
<td>10.24**</td>
</tr>
<tr>
<td>PND 4 (both sexes combined)</td>
<td>10.53**</td>
</tr>
<tr>
<td>Sex ratio (% male)</td>
<td>55.4</td>
</tr>
</tbody>
</table>

\(^a\) Data extracted from Study No. 60C-4752, Table 5, pages 26-27.

\(^b\) Calculated by the reviewers, number of litters within parentheses.

\(^c\) Live birth index is calculated as: \[
\frac{\text{No. of live pups born}}{\text{No. of live and dead pups born}} \times 100
\]

\(^d\) Viability index is calculated as: \[
\frac{\text{No. of pups alive on day 4 percent}}{\text{No. of live and dead pups born}} \times 100
\]

* p<0.05 by ANOVA and test for Linear Trend

** p<0.05 by ANOVA and test for Linear Trend
D. REVIEWER'S DISCUSSION/CONCLUSIONS

1. Test Material Analyses

In general, the test material was uniformly distributed throughout the feed. Results of the stability analysis indicated that the storage conditions were adequate to ensure test material stability. However, the test diet analyses revealed concentrations of aldicarb that were 16-20% below the target concentration in three out of four samples, indicating that the pregnant dams received aldicarb concentrations of <20 ppm.

2. Test Material Intake

Aldicarb was detected in only one sample, indicating that in most samples it was metabolized to aldicarb sulfoxide and aldicarb sulfone, the latter metabolite being formed in higher quantities.

The approximate milk intake based on mean pup body weight was 1.5 ml/day. Using the mean concentrations of aldicarb and its metabolite aldicarb sulfoxide in the milk (weight of milk assayed = 1 g), the approximate intake on PND 4 was 6.74 µg/kg.

3. Parental Toxicity

Treatment-related marginal toxicity noted in dams was evident from clinical findings during gestation, and decreased mean body weight gain accompanied by decreased food consumption, primarily during the lactation period. This was possibly related to increased test-material consumption (1.88 mg/kg/day from PND 0 to 4 versus 1.4 mg/kg/day from GD 7 to 20).

4. Reproductive Toxicity

Fertility was low in the control and treated dams and no statistical analysis of data was performed. However, it did not affect the litter size on PND 0. Pup mortality (3.88%) in the treated group was considerably lower than the 37% mortality observed at 20 ppm in an earlier study by the sponsor. The lower incidence of pup mortality observed in the present study was possibly due to the lower concentration of aldicarb (<20 ppm) in the maternal diet. Pup mortality in the treated group was two-fold higher (3.88%) than in the control group (1.55%). Although it was reported to be within the historical control range for the RTI colony, no evidence was provided to substantiate this statement. Mean litter size for PND 0-4 was slightly reduced in the treated group; mean pup weight/litter was significantly lower on PND 4. These observations indicated lactational transfer of aldicarb and its metabolites, all of which may have caused caused toxicity in pups. Adverse effects from the metabolites were predominantly seen on PND 4 and possibly resulted from the increased test material consumption by the lactating mothers.
from PND 0 to 4 and the subsequent excretion of metabolites into the milk.

5. Study (Reporting) Deficiencies

Milk samples were pooled, therefore, it was not possible to examine milk transfer variation between the dams.

No historical control data on the incidence of fertility of dams and pup mortality in CD rats were provided. In addition, no statistical analysis of the pregnancy rates was conducted.

However, the above deficiencies did not affect the outcome of the study.

E. **CLASSIFICATION:** The study is acceptable provided that the historical control data on pup mortality and reproductive indices are submitted and are found acceptable.

F. **RISK ASSESSMENT:** Not applicable
lactational transfer of aldicarb and its metabolites, all of which may have caused toxicity in pups. Adverse effects from the metabolites were predominantly seen on PND 4 and possibly resulted from the increased test material consumption by the lactating mothers from PND 0 to 4 and the subsequent excretion of metabolites into the milk.

5. **Study (Reporting) Deficiencies**

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No historical control data on the incidence of fertility of dams and pup mortality in CD rats were provided. In addition, no statistical analysis of the pregnancy rates was conducted.

However, the above deficiencies did not affect the outcome of the study.

E. **CLASSIFICATION**: The study is acceptable provided that the historical control data on pup mortality and reproductive indices are submitted and are found acceptable.

F. **RISK ASSESSMENT**: Not applicable
EPA Correspondence No. 91-101
April 18, 1991

Mr. Sephr Haddad
U. S. Environmental Protection Agency
Office of Pesticide Programs-
Reregistration Division Special Review Branch
Crystal Station No. 1
2805 Jefferson Davis Highway
Arlington, Virginia 22202

Dear Mr. Haddad:
RE: Aldicarb Rat Milk Study

On February 28, 1990, Rhône-Poulenc advised EPA that in a range finding reproduction study of aldicarb in rats, increased pup mortality was observed between postnatal days (pnd) 0 and 4 in litters of dams exposed to 20 ppm aldicarb in the diet. The reported mortality at 20 ppm was 37% for this range-finding study.

Rhône-Poulenc had previously conducted milk transfer studies in cows and goats. However, since the composition of rat milk is higher in both protein and fat contents, we decided to conduct a new study at Research Triangle Institute (RTI) to determine the magnitude of aldicarb, and metabolites, excreted in the milk of lactating rats following dietary exposure.

The RTI study was conducted with an aldicarb dietary concentration of 20 ppm to be consistent with the range-finding study. Control dams received undosed diet. The control group was used to provide a comparison of pup mortality and also to provide "blank" milk for aldicarb background levels and for analytical spike recovery determinations.

Results of the aldicarb excretion study in rats revealed that one of the 10 milk samples contained detectable levels of aldicarb and both metabolites; 4 of 10 samples contained no parent compound, but had detectable levels of both aldicarb metabolites; and 1 of 10 samples contained only aldicarb sulfone. Four of the 10 samples from aldicarb-treated dams contained no detectable aldicarb or aldicarb metabolite residues. The detected concentrations for all three forms in the milk were within a range from 9 to 29 ppb. This range is comparable to that observed in ruminants fed aldicarb at comparable dose levels.
Pup mortality on postnatal days 0-4 in the rat aldicarb excretion study was 1.55% for the control litters and 3.88% for aldicarb-exposed litters. This is in contrast to the 1% (control) and 37% (20 ppm dose group) pup mortality observed during the range finding reproduction study.

Four copies of the newly completed aldicarb lactating rat study are appended for Agency review. If you have any questions please feel free to contact me promptly.

Sincerely,

Warren A. Davis
Registration Manager
EPA Correspondence No. 90-67  
February 26, 1990

Mr. Bruce Kapner  
U.S. Environmental Protection Agency  
Office of Pesticide Programs  
Registration Division Special Review Branch  
Crystal Mall Building No. 2  
1921 Jefferson Davis Highway  
Arlington, Virginia 22202

Dear Mr. Kapner:

RE: TEMIK® brand Aldicarb

The following information is being submitted to EPA pursuant to the Agency's interpretation imposed on registrants by section 6(a)(2) of FIFRA. We believe the information in this report does not constitute additional factual reporting regarding unreasonable adverse effects within the meaning of 6(a)(2).

In order to maintain registrations for aldicarb-containing products in California, Senate Bill 950 required that we generate a new reproduction study. The following is a summary of interim data from a dose range-finding study for a 2-generation rat reproduction study to be initiated during March, 1990. The results of the range-finding study are expected to be available during August, 1990. The definitive report for the 2-generation study should be completed during July 1991.

Female Sprague-Dawley rats received 0, 0.5, 1.0, 10, 20, 30, or 50 ppm aldicarb technical in the diet from gestation day 6 through lactation, until F1 postnatal day 21. F1 pups received the same dose level of aldicarb in the diet for four weeks beginning on postnatal day 21 (the day of weaning). Dams in the 50 ppm dose group showed clinical signs (tremors), beginning with the second week of dosing and continuing throughout the study. Maternal body weight was reduced at 50 ppm through gestation and lactation, and through gestation at 30 ppm. Dam plasma cholinesterase levels were reduced at 10 ppm and above.

The length of gestation, number of litters, number of pups per litter, pup body weight at birth, and frequency of stillbirth were comparable for all dose groups. Pup mortality was increased between postnatal days 1 and 4 for litters in the 20, 30, and 50, ppm dose groups, and slightly increased in the 10 ppm dose group. By postnatal day 4, no pups survived in the 50 ppm dose group. Pup viability between postnatal day 4 and 21 was somewhat reduced in the 10, 20, and 30 ppm dose groups.
although all pups in the 10 ppm dose group dying during this time period were from a single litter.

The cause of the early postnatal pup mortality is unclear at this time. Exposure of the pups to aldicarb between lactation days 1 and 4 could come from one primary (a) and three secondary (b, c, d) sources:

(a) lactational exposure through the milk;
(b) the dam grooming the pups immediately after eating;
(c) the dam retrieving the pups in her mouth immediately after eating
(d) possible contamination of the nesting site with aldicarb residues

The latter three sources are impossible to quantify, both as a "dermally applied dose" level and as an "absorbed dose". Data are available for aldicarb residues in cow and goat milk, but the fat, protein, and water composition of rat milk is dramatically different and results of ruminant studies should not be extrapolated to rats.

Currently there is no information on the dose levels of aldicarb received by the pups during early lactation from the primary or secondary sources. During the first two weeks following weaning, pups in the 20 and 30 ppm dose groups showed tremoring and increased reactivity to external stimuli, however, pups in these two dose groups consumed as much or more aldicarb on a mg/kg/day basis as the dams in the 50 ppm dose group (see attachment). By postweaning week 4, pups in these two dose groups were no longer showing clinical signs. These results indicate that weanling rats are comparable to, or perhaps less sensitive than, adult rats to the acutely toxic effects of aldicarb.

Sincerely,

Warren A. Davis
Registration Manager
### PPM and MG/KG/DAY Dose Equivalents - DAMS and PUPS

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<tr>
<th></th>
<th>DAMS</th>
<th></th>
<th>PUPS</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
<td>mg/kg/day</td>
<td>ppm</td>
<td>mg/kg/day</td>
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<td>0</td>
</tr>
<tr>
<td>0.5</td>
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<td>0.1</td>
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<td>0.1/0.13</td>
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<tr>
<td>10</td>
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<td>10</td>
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<td>50</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*a Dam food consumption increases throughout lactation. The first number is dam compound consumption from lactation D0-4, the second number is the mean from D0-14. Compound consumption from D14-21 is excluded because dam consumption cannot be separated from consumption by pups.*

*b Mean pup compound consumption for postweaning days 0-7, for individually housed pups.*