3-1-96

OPP#00562

DATA EVALUATION REPORT

ALDICARB

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY - RAT (83-6)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 96-1

Primary Reviewer: Carol S. Forsyth, Ph.D.	Signature:	Carol Forsyth
	Date:	3/1/96
Secondary Reviewers: Rosmarie A. Faust, Ph.D	Signature:	Rosmarie A. Faust
	Date:	3/1/96
Robert H. Ross, M.S., Group Leader	Signature:	RHR
	Date:	3/1/96
Quality Assurance: Rosmarie A. Faust, Ph.D.	Signature:	RAF
	Date:	3/1/96

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

1921

ALDICARB

Developmental Neurotoxicity Study (83-6)

EPA Reviewer:
W. Sette, Ph.D.
Science Analysis Branch (7509C)

W. Burnam, Chief
Science Analysis Branch I (7509C)

Wm Sette, Date 10/27/98

W. Burnam, Date 10/27/98

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity - Rat [§83-6]

DP BARCODE: D221458
P.C. CODE: 098301

SUBMISSION CODE: S497793,
TOX. CHEM NO.: 011A

TEST MATERIAL (PURITY): Aldicarb (98.9% a.i.)

SYNONYMS: Propanal, 2-methyl-2-(methylthio)-, *O*-((methylamino) carbonyl)oxime

CITATION: Weiler, M. (1995) Developmental Neurotoxicity Study with Aldicarb in Rats. Hazleton Wisconsin, Inc. (3301 Kinsman Boulevard, Madison, WI 53704). Laboratory project identification HWI 6224-213, October 10, 1995. MRID 43829601. Unpublished.

SPONSOR: Rhône-Poulenc Ag Company,
Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a developmental neurotoxicity study groups of 30 presumed pregnant Sprague-Dawley (Crl: CD® BR VAF/Plus®) rats were administered Aldicarb by gavage at doses of 0, 0.05, 0.10, or 0.30 mg/kg/day on gestation day (GD) 6 through lactation day 10. The offspring were not administered the test material. Clinical observations, a functional observational battery (FOB), body weights, and reproductive data were recorded for the dams (F_0). Pups (F_1) were weighed, monitored for emergence of vaginal perforation or balanopreputial separation, observed until approximately postnatal day 65, and given neurobehavioral evaluations (FOB, motor activity, learning and memory test, auditory startle response). Plasma, red blood cell, and brain cholinesterase (ChE) activities were measured in both dams and pups. Neuropathological assessment of high dose and control pups were made on day 11 and day 65.

On treatment days, tremors were observed in as many as 11 and as few as 1 F_0 dams given 0.3 mg/kg. Clinical signs seen in the FOB on gestation day 6 (GD6) in 0.3 mg/kg/day dams, were tremor (10/10), lacrimation (7/10), salivation (6/10), stained fur (7/10), hunched posture (4/10), ataxia (9/10), lip smacking (4/10), decreased body temperature and miosis (10/10). The mean number of rears was also reduced ($p \leq 0.05$) in high dose dams (0.6) as compared to controls (7.9). During lactation, tremors or a few other signs were observed on days 0-2 in a few 0.3 mg/kg dams only.

2821

Clinical signs or changes in the FOB were not observed in any other dose group or the controls.

Mean maternal body weights of the 0.3 mg/kg/day group were significantly less (6%) than the controls beginning on GD 9 and continuing until GD20. Overall body weight gain during gestation was also significantly less in the 0.30 mg/kg/day dams (17%) in comparison to controls. During lactation, body weights of these dams were significantly ($p \leq 0.05$) less than controls only on day 4. Recovery was evident in high-dose dams after cessation of treatment with day 21 body weights 100% of the control value. No differences were seen between body weights or in body weight gains of the 0.05 or 0.10 mg/kg/day groups during gestation or lactation.

Mean maternal plasma ChE (ca 80%) and red blood cell ChE (27%) activities were significantly reduced in 0.3 mg/kg dams on GD 7 and lactation day 7 as compared to controls. On GD 7, plasma ChE inhibition in 0.1 mg/kg dams was 40%, (not statistically significant), but much less later. No inhibition of ChE was seen in the brains of dams tested on lactation day 11 or in the plasma, red blood cell, or brain activity of the F_1 males or females tested on lactation days 4, 10, or 11. In many cases, less than 5 rats were used, limiting the power or sensitivity of these measures.

No statistically significant effects were observed on duration of gestation, total number of pups delivered, pup survival indices, or per cent male pups. There were no differences between treated and control groups in the emergence of balanopreputial separation or vaginal perforation.

A few treatment related effects on FOB measures and motor activity changes were observed in the 0.1 or 0.3 mg/kg pups during lactation and post-weaning. Motor activity was significantly decreased, about 30%, in both 0.1 and 0.3 mg/kg males on day 17. A 29% decrease was also seen in the 0.05 mg/kg group, but it did not reach statistical significance. No differences in motor activity were seen on days 13 and 21. There is normally, and, here in control animals, an increase in motor activity on day 17 in relation to either day 13 or day 21. Dosed males showed reduced or absent increases on day 17 in relation to days 13 or 21. At day 60, motor activity was signficantly increased in the 0.1 mg/kg males for the whole session (20%) and 30% for the 10-20 minute interval. For the 0.3mg/kg males overall activity was not significantly different (14% increase) but for the 10-20 min interval a significant 34% increase was seen.

FOB observations were made on F_1 animals on postnatal days 14, 21, 35 and 63. On days 35 and 63 high-dose F_1 males made significantly fewer rears (and fecal boli) in the open field. Number of rears were also reduced in high-dose females at day 35. Decreased hindlimb grip strength (20%) and splay (15%) were also found in mid and high dose females on Day 35.

On day 63, high dose males had increased latency to first step (12/20 waiting 9 seconds vs 3/20 controls); significantly reduced forelimb grip strength (20%); and an increased latency on the first trial (15 vs 10 secs), but not the second trial or average latency, to a heat stimulus. No consistent effects attributable to treatment were seen on startle reflexes and no differences found on

speed or errors in the learning and memory test. In summary, while many of these measures are isolated, there do appear to be a number of significant differences noted in high dose males at all time points.

Pup body weights adjusted for litter size from high-dose dams were significantly lower than controls on lactation days 0 (8%), 4 (11-13%), and 7 (males and females), lactation day 11 (males), and lactation day 17 (females). Male and female pups from 0.1 mg/kg/day dams also had significantly lower body weights than controls on lactation days 7 and 17, 8% and 6%, respectively. Adjusted body weights from the mid- and high-dose dams were not significantly different by lactation day 21, where adjusted pup body weights were 95 and 93%, respectively, for males and 95 and 94%, respectively, for females of the corresponding control value. However, unadjusted mean body weights of these F_1 male and female rats earlier given 0.1 and 0.3 mg/kg/day were still depressed consistently throughout the post-weaning period. Significant differences occurred at weeks 0, 2, and 4 for the mid- and high-dose males (6-8%; 10-15%) and high-dose females (13-7%), and at week 2 for the mid-dose females (4%). Body weight gain for 4 weeks after weaning was significantly less than controls for the mid- and high-dose males (ca 8%) but no differences in weight gains were seen in females.

Maternal toxicity at 0.30 mg/kg/day included clinical signs of toxicity such as tremor, salivation, and lacrimation, blood ChE inhibition, and 17% reduced body weight gain during gestation.
The maternal LOEL is 0.10 mg/kg/day based on plasma ChEI of 40% on GD 7.
The maternal NOEL is 0.05 mg/kg/day.

The offspring LOEL is 0.10 mg/kg/day based on reduced body weights from birth through postweaning, and on decreased motor activity on day 17 in male pups.
The offspring NOEL is 0.05 mg/kg/day.

While additional data on positive controls should be provided, this developmental neurotoxicity study in the rat is currently acceptable and satisfies the guideline requirement for a developmental neurotoxicity study (83-6) in rats.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice Statements, Data confidentiality and Flagging statements were included.

4821

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aldicarb

Description: white crystalline solid Lot/ Batch No.: 25 DEQ 89
Purity: 98.9% a.i.
Stability of compound: not provided; noted in the study that the test material was determined by the Sponsor to be stable until January 1998. CAS No.: 116-06-3

2. Vehicle and/or positive control

Reverse osmosis water was used as the vehicle and as the negative control. No positive controls were used.

3. Test animals

Species: rat
Strain: Sprague-Dawley, Crl: CD® BR VAF/Plus®
Age and weight at study initiation: ≈13 weeks; 244.7 to 355.1 g
Source: Charles River Laboratories, Inc., Portage, MI
Housing: individually in stainless-steel, screen-bottom cages; mated females (beginning at approximately GD 15) and females with pups were housed in polycarbonate cages.
Diet: Certified Rodent Diet #5002 meal, PMI® Feeds, *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions: Temperature: 19-25°C
Humidity: 50±20% Air changes: not provided
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 14 days

B. STUDY DESIGN

This study was designed to assess the developmental neurotoxicity potential of Aldicarb when administered by gavage to rats from GD 6 through lactation day 10.

1. In life dates Start: August 15, 1994; end: November 18, 1994
2. Mating

Males of the same strain and source were used for mating. One female was paired with one male and copulation determined daily by the presence of a vaginal plug or by sperm in the vaginal smear. The day this evidence of mating was found was designated as Day 0 of gestation. Pregnancy was confirmed on GD 14 or 15 by the observation of a vaginal membrane.

Animal assignment Animals in the F_0 generation were assigned using a consecutive block design to one control or one of three treatment groups (Table 1). 11 dams received 0.4 mg/kg on GDs 6 and 7. Due to one death, this dose was reduced to 0.3 mg/kg/day. Five animals/group were assigned for determination of cholinesterase (ChE) activity.

5/21

TABLE 1: F ANIMAL ASSIGNMENT

Dose Group	Dose (mg/kg/day)	Number Assigned[a]
Control	0.00	30
Low	0.05	30
Mid	0.10	30
High	0.30[b]	30

Data taken from p. 19, MRID 43829601.
[a]Includes 5 animals/group assigned for cholinesterase determination.
[b]Eleven of the high-dose group animals were given 0.4 mg/kg/day for 1 or 2 days then given 0.3 mg/kg/day; all other animals were given 0.3 mg/kg/day.

4. Validation of testing procedures

Laboratory validation of the testing procedures was not included in the study. However, the laboratory has conducted a number of validation experiments and has agreed verbally to provide this data through the registrant.

5. Dose selection rationale

No information was included in the study to explain the selection of doses. But based on the effects seen in the dams at the highest dose tested, the effects seen in other studies, chiefly ChE inhibition, from repeated oral doses of 0.05-0.1 mg/kg, and the reasonably tight spacing of these doses, the dose levels used in this study appear to be adequate.

6. Preparation and analysis of dosing solutions.

Dose concentrations were based on test material as supplied (98.9% a.i.). Dose solutions were prepared weekly and each level was prepared separately. The required volume of a stock solution was measured into a graduated cylinder and the appropriate amount of carrier was added. The cylinder was manually inverted and the solution transferred to labeled containers for daily dose administration. Samples from the low- and high-dose solutions were taken from the top, middle, and bottom to determine homogeneity. The low- and high-dose solutions were analyzed for stability following storage in a refrigerator for 7 or 11 days.

Results

Homogeneity Analysis: Samples from the top, middle, and bottom of the low dose solution ranged from 87.5 to 90.3% of theoretical and from the high dose solution ranged from 91 to 93.5% of theoretical.

Stability Analysis: After 7 or 11 days of refrigerated storage, samples from the low and high dose solutions were within 5% of their original concentrations.

6921

Concentration Analysis: The measured concentrations of test substance in the low and high dose solutions were within 11% of nominal for each of the 5 weeks that dose solutions were prepared. The analytical data indicate that the mixing procedure was adequate and that the test substance was stable in solution for the period of use.

7. Statistical analysis

Tests for homogeneity were performed using Levene's test. For heterogeneity of variance where $p \leq 0.05$, transformations were used to stabilize the variance. Analysis of variance (ANOVA) was performed if the transformation analysis indicated homogeneity. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparisons between the treatment and control groups. When no transformation established variance homogeneity, the data were examined by nonparametric techniques using the Wilcoxon-Mann-Whitney two-sample rank test. One-way ANOVA was used to analyze body weight data, litter data, days to mate, length of gestation, FOB evaluations, motor activity counts, auditory startle response data, vaginal perforation, balanopreputial separation, learning and memory data, cholinesterase activity, brain weights, brain measurements, and simple morphometrics. Pup survival indices were analyzed by parametric ANOVA with Arcsin transformation of the percentage data. One-way analysis of covariance (ANCOVA) was used to analyze pup body weights with the number of pups in the litter as the covariate. Statistical significance was set at the 0.05 and 0.01 levels.

C. METHODS

1. Maternal clinical observations and mortality

Animals were examined twice daily for clinical signs of toxicity, mortality, and moribundity. A detailed physical exam was performed when body weights were recorded on GD 0, 6, 9, 12, 16, and 20 and on lactation days 0, 4, 7, 11, 17, and 21. As soon as possible after weaning of the pups, dams were euthanatized and discarded. Females that failed to deliver were euthanatized 26 days postcopulation and the uteri examined for implantation sites. Food consumption was not recorded.

2. Litter clinical observations and mortality

Litters were observed daily for abnormal behavior, poor health, and mortalities. As soon as possible after birth and on lactation days 7, 11, 17, and 21, litter size was recorded and the pups sexed, examined for gross external malformations, and weighed individually. Pups selected for neurobehavioral evaluation were identified with a foot tattoo on lactation day 0. On lactation day 4, pups not selected for neurobehavioral evaluation or ChE determination were culled, euthanatized with Beuthanasia-D®, and discarded.

After weaning (lactation day 21), F_1 animals were weighed and observations recorded at 2-week intervals. Beginning on postnatal day 27, F_1 females were examined daily until a patent vagina was observed. Beginning on postnatal day 35, F_1 males were examined daily for balanopreputial separation (when the prepuce could be completely retracted to reveal the glans penis).

3. Neurobehavioral evaluations

a. Functional observational battery (FOB)

Ten F_1 females/group and at least 20 F_1 pups/sex/group were assigned to a battery of behavioral tests and observations. These tests were performed on the F_1 females prior to the initiation of treatment and approximately 2 hours after dosing on GD 6 and lactation day 7. Information from the sponsor stated that 2 hours was the time to maximum effect. F_1 animals were tested on postnatal days 14, 21, 35, and 63 (±2 days). See Appendix A for complete FOB testing details (pp. 241-247, MRID 43829601).

b. Motor activity A minimum of 20 F_1 pups/sex/group were assessed for motor activity on postnatal days 13, 17, 21, and 60 (±2 days). The motor activity of each animal was monitored for 40 minutes in an open field enclosure and based on the number of photobeam breaks (counts) at 2-minute intervals.

c. Auditory startle response The latency and amplitude of each animal's response to a stimulus was assessed for 20 F_1 animals/sex/group on postnatal days 22 and 60 (±2 days). Five blocks of 10 trials each were performed on each testing day.

d. Learning and memory (water maze) A minimum of 20 F_1 pups/sex/group were tested for learning and memory in a "M-shaped" water maze on postnatal days 23, 24, 25, 30, and 60 (±2 days). According to a preselected assignment, either the light on the left side or the light on the right side of the maze was lit at each trial with the escape ramp positioned on the lighted side. Animals were placed in the center channel of the maze facing the outside wall and given 60 seconds to reach the exit ramp. The number of errors (movement of all four feet into an incorrect channel) and the completion time for successful trials were recorded. The number of errors and a time of "60+" seconds were recorded for unsuccessful trials. Three trials were done for each animal on each testing day.

4. Clinical chemistry (cholinesterase determination)

Blood was collected from the lateral tail vein from 5 F0 animals/group prior to study initiation, and approximately 2 hours post-dosing on GD 7 and lactation day 7 for RBC ChE and plasma ChE activity. On lactation day 11, blood was collected from anesthetized animals by cardiac puncture and brain ChE activity was also evaluated for these animals. The selection of dams was prior to treatment initiation and females that did not deliver were not replaced for the ChE activity evaluation.

When possible, five pups/sex/group each from litters designated for ChE activity were selected for evaluation at 2 hours post-dosing of the respective dam on lactation days 4, 10, and 11. Pups were anesthetized with sodium pento-barbital, blood was collected by cardiac puncture for RBC and plasma ChE activity, and the brains were collected for ChE analysis.

5. Pathology

A necropsy was performed on all dams that died or were sacrificed intercurrently. After necropsy, all tissues were discarded.

On lactation day 11 and again after postnatal day 60, 10 pups/sex/group from separate litters were anesthetized with sodium

8821

pentobarbital, weighed, and exsanguinated. Brain weights were recorded on all pups while 6/sex/group were used for necropsy and neuropathological evaluation. The necropsy included a macroscopic examination of the external surface of the body, all orifices, the cranial cavity, external surfaces of the brain and spinal cord, and cervical, thoracic, and abdominal viscera. The following tissues were collected and immersion-fixed (day 11 pups) or fixed *in situ* by whole body perfusion with 10% phosphate-buffered formalin (day 60 animals) and processed for histological evaluation:

X	brain	X	eyes
X	pituitary	X	sciatic nerves
X	entire spinal cord	X	tibial nerves
X	cervical dorsal root	X	sural nerves
X	ganglia	X	anterior tibialis muscles
X	lumbar dorsal root ganglia	X	gastrocnemius muscles
X	trigeminal ganglia	X	macroscopic lesions
	optic nerve		

At trimming, the maximum length, width, and height of each cerebrum and cerebellum were measured with a caliper. Tissue sections from control and high-dose groups were examined microscopically for evidence of neuropathological alterations. The brains from 6 pups/sex/dose sacrificed after day 60 were evaluated for development by morphometric analyses that consisted of multiple (27 total) measurements of five sections of the brain: forebrain, caudate nucleus, thalamus/hypothalamus, midbrain, and cerebellum.

II. RESULTS

A. Body weight

Body weight data for F_0 females and F_1 pups are summarized in Tables 2 and 3, respectively. Mean maternal body weights of the 0.3 mg/kg/day group were significantly less than the controls beginning on GD 9 and continuing until parturition. GD 20 body weights of the high-dose group were 95% of the control value. Overall body weight gain during gestation was significantly (17%) less in the 0.30 mg/kg/day group as compared to controls. The greatest effect on weight gain occurred at the beginning of the treatment period as evidenced by a weight loss of 13.3 grams by the high-dose animals during the GD 6-9 interval.

During lactation, body weights of the high-dose dams were significantly less than controls only on day 4 (Table 2). Recovery was evident in the high-dose animals after cessation of treatment with day 21 body weights 100% of the controls. Initially (day 0-4) the high-dose group gained significantly ($p \leq 0.01$) less than controls, but then gained significantly more than controls for the lactation day 4-7 interval. Overall weight gain during lactation was significantly ($p \leq 0.05$) greater than the controls only for the mid-dose animals. However, there was a trend toward greater weight gain in all treated groups. No differences were seen between body weights or body weight gains of the 0.05 or 0.10 mg/kg/day groups as compared to controls during gestation or lactation.

Mean body weights of the F_1 generation 0.1 and 0.3 mg/kg/day males and females were consistently lower than the controls throughout the postweaning period. Significant differences occurred at weeks 0, 2, and 4 for the mid- and high-dose males and high-

9721

dose females, and at week 2 for the mid-dose females. Final body weights of the high-dose males and females were 90 and 93%, respectively of controls. Body weight gain was significantly ($p \leq 0.01$) less than controls for the mid- and high-dose males but no differences in weight gains were seen in females.

Table 2: Maternal Body Weights During Gestation and Lactation (g)

Day of Study	0.0 mg/kg	0.05 mg/kg	0.10 mg/kg	0.30 mg/kg
	Gestation			
GD 0	292.5 ± 20.1	290.9 ± 21.0	284.5 ± 19.7	292.5 ± 16.4
GD 6	334.0 ± 24.3	331.8 ± 24.5	323.5 ± 21.2	335.9 ± 21.0
GD 9	344.8 ± 25.7	344.0 ± 23.4	336.6 ± 26.3	322.4 ± 24.6**
GD 12	364.9 ± 26.6	361.3 ± 25.9	354.1 ± 27.7	333.0 ± 24.6**
GD 16	391.1 ± 27.4	391.7 ± 31.5	380.2 ± 29.7	364.7 ± 23.4**
GD 20	448.6 ± 33.1	456.4 ± 39.6	439.5 ± 42.3	423.1 ± 29.5*
Body weight change (GD 0-20)	156.1 ± 28.7	165.5 ± 29.5	155.0 ± 37.4	130.4 ± 25.0** (-17%)
	Lactation			
Day 0	346.2 ± 27.1	350.1 ± 28.8	339.3 ± 30.2	334.1 ± 23.0
Day 4	365.4 ± 24.8	363.2 ± 27.4	354.2 ± 31.6	339.2 ± 24.3**
Day 7	373.1 ± 22.1	375.7 ± 23.2	365.3 ± 31.6	359.8 ± 23.0
Day 11	383.9 ± 26.3	388.2 ± 23.0	383.2 ± 31.2	373.0 ± 21.9
Day 17	377.6 ± 27.7	378.2 ± 22.5	373.9 ± 29.0	370.9 ± 20.5
Day 21	355.7 ± 24.4	360.5 ± 17.3	362.9 ± 28.6	355.0 ± 20.0
Body weight change (Day 0-21)	7.9 ± 17.5	11.4 ± 21.9	24.8 ± 17.6*	18.7 ± 15.0

Data taken from Tables 7, 8, 10, and 11, pp. 74, 75, 78, and 79, respectively, MRID 43829601.
Significantly different from control, *p ≤ 0.05, **p ≤ 0.01.

11821

TABLE 2 b F1 pup body weights prior to weaning (adjusted for litter size)

	0	0.1 mg/kg	0.3 mg/kg
MALES			
DAY 0	6.93	6.89	6.34**
4	12.05	11.33	10.68**
7	19.62	18.07* (-7.9%)	17.27**
11	31.25	29.52	27.49**
17	52.11	49.46	48.82
21	68.89	65.65	64.40
FEMALES			
DAY 0	6.48	6.56	5.96**
4	11.43	10.76	9.93**
7	18.35	17.32	16.60**
11	29.28	28.20	26.58
17	50.32	47.43* (-5.7%)	47.54*
21	65.96	62.84	62.20

12921

Table 3: F. Body Weights After Weaning (g)

Postweaning interval	0.0 mg/kg	0.05 mg/kg	0.10 mg/kg	0.30 mg/kg
		Males		
Week 0	148.5 ± 20.7	145.2 ± 21.6	137.3 ± 17.1*	126.4 ± 15.2**
Week 2	287.4 ± 24.7	286.7 ± 26.9	269.2 ± 23.2**	257.2 ± 24.4**
Week 4	414.6 ± 26.7	414.3 ± 30.1	380.1 ± 30.9**(-8.3%)	372.8 ± 34.2**(-10%)
Body weight change (Week 0-2)	138.9 ± 8.5	141.5 ± 11.7	131.9 ± 13.7**	130.9 ± 13.4**
Body weight change (Week 0-4)	266.1 ± 19.7	269.0 ± 23.6	242.9 ± 29.7** (-8.7%)	246.5 ± 26.3** (-7.4%)
		Females		
Week 0	130.6 ± 17.2	128.5 ± 16.8	123.3 ± 11.0	113.3 ± 10.3**
Week 2	205.4 ± 14.9	205.1 ± 17.8	197.1 ± 15.1*	188.0 ± 13.6**
Week 4	251.3 ± 19.1	253.9 ± 21.9	243.5 ± 22.7	233.5 ± 17.1**(-7%)
Body weight change (Week 0-2)	74.8 ± 10.7	76.6 ± 12.8	73.8 ± 14.0	74.7 ± 13.1
Body weight change (Week 0-4)	120.7 ± 17.8	125.4 ± 18.7	120.2 ± 21.9	120.3 ± 17.4

Data taken from Tables 9 and 12, pp. 76-77 and 80-81, respectively, MRID 43829601.
Significantly different from control, *$p \leq 0.05$, **$p \leq 0.01$.

B. Delivery and litter data

Delivery and litter data are summarized in Table 4. No statistically significant differences were observed for any treated group as compared to control for duration of gestation, total number of pups delivered, pup survival indices, or per cent male pups. However, pup body weights from high-dose dams were significantly ($p \leq 0.01$) lower than controls on lactation days 0,

13421

4, and 7 (males and females), lactation day 11 (males), and lactation day 17 (females, $p \leq 0.05$). Male and female pups from 0.1 mg/kg/day dams also had significantly ($p \leq 0.05$) lower body weights than controls on lactation days 7 and 17, respectively (Table 2b). Recovery of pups from the mid- and high-dose dams was apparent with lactation day 21 body weights 95 and 93%, respectively for males and 95 and 94%, respectively for females of the corresponding control values.

There were no differences between treated and control groups in the rate of physical development of the pups as assessed by balanopreputial separation or vaginal perforation. In males from dams in the control, 0.05, 0.1, or 0.3 mg/kg/day groups, balanopreputial separation was observed by day 44.5, 45.5, 45.2, and 45.8, respectively; in female pups vaginal perforation was observed by day 33.4, 33.7, 32.7, and 33.1, respectively.

Table 4: Delivery and Litter Data

Observation	0.0 mg/kg/day	0.05 mg/kg/day	0.10 mg/kg/day	0.30 mg/kg/day
Females mated	30	30	30	30
Females pregnant	26	27	23	25
Duration of gestation (days)	22.1	22.0	22.1	22.4
Total live pups	335	373	288	276
Pups/litter	13.6	14.7	14.3	12.4
Stillborn	3	7	13	19
% Male pups	49	53	51	49
Pup survival (%)				
Livebirth index	99	98	96	93
Viability index	98	95	88	98
Weaning index	64	68	73	60
Pup wt/litter (g)				
Day 0				
-males	6.93[a]	6.96	6.89	6.34**
-females	6.48	6.57	6.56	5.96**
Day 4 (postcull)				
-males	12.05	11.68	11.33	10.68**
-females	11.43	11.03	10.76	9.93**
Day 21				
-males	68.89	65.94	65.65	64.40
-females	65.96	63.81	62.84	62.20

14821

Data taken from Table 13, pp. 82-86, MRID 43829601.
[a]Covariate adjusted mean based on number of pups per litter.
**Significantly different from control, $p \leq 0.01$.

C. Clinical observations and mortality

On treatment days, tremors were observed in as few as 1 to as many as 11 F dams given 0.3 mg/kg. This was accompanied by excessive salivation in some animals. Other observations in 1 or 2 high-dose animals during gestation included dry, brown material around the nose and mouth and anogenital staining of the haircoat. These clinical signs were not observed in any other dose group or the controls. During lactation, tremors were observed on days 0-2 in 1 or 2 high dose dams only. No treatment-related overt clinical effects were observed in the pups during lactation or postweaning.

D. Neurobehavioral evaluations

1. Functional observational battery (FOB)

Parameters of the FOB affected in dams on GD 6 in the cage and open arena are listed in Table 5. Treatment-related effects in the high-dose animals included tremor, excessive lacrimation and salivation, stained fur, hunched posture, gait abnormalities, ear flicking, lip smacking, fewer rears, and miosis. On lactation day 7, the most significant observation was mild tremor in 5/10 high-dose animals ($p \leq 0.01$) as compared to 0/10 controls. Ear flicking (3/10), lip smacking (4/10), and mild ataxia (3/10) were also seen.

In dams, no differences were seen between any treated groups as compared to controls for hindlimb foot splay measured on lactation day 7 or fore- or hindlimb grip strength measured on GD 6 or lactation day 7. The analgesic reflex latency was significantly ($p \leq 0.01$) increased in the high-dose F_0 females on GD 6 as compared to controls, but no differences were seen on lactation day 7. A significant ($p \leq 0.01$) decrease in body temperature was measured in high-dose dams on GD 6, but not on lactation day 7.

15421

Table 5: Functional Observational Battery Data for F$_1$ Females on Gestation Day 6

Observation	0.0 mg/kg/day	0.05 mg/kg/day	0.10 mg/kg/day	0.3 mg/kg/day
Tremor (home cage and open arena)	0/10	0/10	0/10	10/10**
Lacrimation	0/10	0/10	0/10	7/10**
Salivation	0/10	0/10	0/10	6/10
Stained fur	0/10	0/10	0/10	7/10**
Hunched posture (open arena)	0/10	0/10	0/10	4/10*
Ataxia	0/10	0/10	0/10	9/10**
Ears flick	2/10	2/10	0/10	8/10*
Lip smacking	0/10	0/10	0/10	4/10*
Mean number of rears	7.9	10.3	9.8	0.6*
Miosis	0/10	0/10	1/10	10/10**

Data taken from Table 16, pp. 95-101, MRID 43829601.
Significantly different from control; *$p \leq 0.05$; **$p \leq 0.01$; incidence data calculated by reviewer using Fisher's Exact Test.

Offspring

No differences were noted for any parameter of the FOB on the F$_1$ males and females on postnatal days 14 or 21. However, some differences were observed on days 35 and 63. On day 35, high-dose males had significantly ($p \leq 0.05$) fewer fecal boli and high-dose males ($p \leq 0.05$) and females ($p \leq 0.01$) had significantly less rears when observed in the open field (20 vs 13). Number of fecal boli and number of rears were also reduced ($p \leq 0.01$) in high-dose males at day 63 (14 vs 8 rears). Also on day 63, the latency to first step was increased for high-dose males with 12/20 waiting 9 seconds as compared to 3/20 controls.

No differences in hindlimb foot splay were observed for F$_1$ males on days 35 or 63, but the distance in females on day 35 was significantly ($p \leq 0.05$) reduced in the mid- and high-dose groups as compared to controls. F$_1$ females in the mid- and high-dose groups had significantly ($p \leq 0.05$) lower hindlimb grip strength on day 35 as compared to controls. Average forelimb grip strength was significantly ($p \leq 0.05$) reduced in high-dose males on day 63. In

16921

the analgesic reflex test, there was an increase ($p \leq 0.05$) for high-dose males during Trial 1 on day 63; Trial 2 and the average were not significantly different from controls.

2. Motor activity

There is normally, and, here in control animals, a pattern of increases and then decreases in motor activity between days 13-21. Motor activity was significantly decreased, about 30%, in both 0.1 and 0.3 mg/kg F_1 males on day 17. A 29% decrease was seen in the 0.05 mg/kg group, but it did not reach statistical significance. No differences in motor activity were seen on days 13 and 21.

At day 60, motor activity was signficantly increased in the 0.1 mg/kg males for the whole session (20%) and 30% for the 10-20 minute interval. For the 0.3mg/kg males overall activity was not significantly different (14% increase) but for the 10-20 min interval a significant 34% increase was seen.

3. Auditory startle response

The latency to maximum response was unaffected in F_1 males and females on days 22 and 60. The maximum amplitude of the response was increased in low- and mid-dose males for the second ($p \leq 0.05$) and fourth ($p \leq 0.05$, $p \leq 0.01$, respectively) sessions on day 22 and in high-dose males for the second session on day 60 ($p \leq 0.05$).

4. Learning and memory

There were no differences in average time or total errors in the water maze for any treated group of F_1 males or females as compared to their respective controls on days 23, 24, 25, 30, or 60.

E. Clinical chemistry (cholinesterase determination)

Plasma and red blood cell ChE activity of the dams is listed in Table 5. Mean maternal plasma and red blood cell ChE activities were significantly ($p \leq 0.05$) reduced in high-dose animals on gestation day 7 and lactation day 7 as compared to controls. Plasma ChE inhibition (40%) occurred in the 0.1 mg/kg/day group on GD 7 but lacked statistical significance. Greater than 10% inhibition of ChE activity was consistent in red blood cells through lactation day 11. Plasma and red blood cell activities were severely inhibited in the high dose group on treatment days but some recovery was apparent on lactation day 11, especially of red blood cell activity.

No inhibition of ChE activity was seen in the brains of dams tested on lactation day 11 or in the plasma, red blood cells or brain activity of the F_1 males or females tested on lactation days 4, 10, or 11.

Table 5: Maternal Cholinesterase Activity (mU/mL)

Day of study	0.0 mg/kg/day	0.05 mg/kg/day	0.1 mg/kg/day	0.3 mg/kg/day
	Plasma			
Predose	1003	996	1284	1092
Gestation day 7	829	724 (-13)[a]	501 (-40)	129* (-84)
Lactation day 7	665	544 (-18)	719 (+8)	124* (-81)
Lactation day 11	541	419 (-23)	594 (+10)	446 (-18)
	Red Blood Cell			
Predose	1119	874	991	746
Gestation day 7	1247	1177 (-6)	1107 (-11)	909* (-27)
Lactation day 7	1457	1437 (-1)	1224 (-16)	1061* (-27)
Lactation day 11	1209	1010 (-16)	1078 (-11)	1189 (-2)

Data taken from Tables 33-36, pp.158-161, MRID 43829601.
[a]Numbers in parentheses are per cent inhibition; %I = (treated value - control value)/control value; calculated by reviewer.
*Significantly different from control, $p \leq 0.05$.

F. Pathology

There were no treatment-related gross abnormalities observed at necropsy of the dams or of the offspring. No microscopic lesions were observed in the neurological tissues of the male or female offspring. There were scattered changes noted in peripheral nerves, but none that could be clearly attributed to treatment.

There were no differences in absolute brain weights of the F_1 animals at days 11 or 60. At lactation day 11, F_1 females in the 0.05 mg/kg/day group had significantly ($p \leq 0.05$) larger brains as compared to controls, but, no differences in brain size were seen at postnatal day 60. These measures were apparently a summation of brain length, width, and height.

Twenty seven morphometric measurements from 5 brain sections were made on 6 rats/sex/dose sacrificed on day 60. There were a few changes noted that reached statistical significance by student t tests. These included: in low dose males, 4 measures (3d,3g,4a, 4h); in low dose females, 2 measures (2d, 3a);

18921

none in mid dose males; one in mid dose females (1c); in high dose males, one measure (1a); high dose females, one measure (2d).

III. DISCUSSION

Groups of female rats were administered Aldicarb by gavage at doses of 0.0, 0.05, 0.10, or 0.30 mg/kg/day on GD 6 through lactation day 10, inclusive. The developmental neurotoxicity potential of the chemical was evaluated in the offspring through postnatal day 60. Clinical signs and effects in the FOB observed in the high-dose dams after dosing, such as tremor and salivation, are typical of severe ChE inhibition. Coincident with the clinical signs of toxicity at the high dose, body weights and body weight gains (-17%) of the treated dams were significantly lower than controls during gestation, but did not exceed the criterion of 20% usually given as a limit for developmental toxicity studies. Plasma and red blood cell ChE activities were also severely inhibited in the high-dose dams following treatment on GD 7 and lactation day 7.

In the mid dose dams that received 0.1 mg/kg, plasma ChE inhibition on GD 7 was 40%, but no plasma ChEI was seen on lactation day 7 or 11, the day after dosing. RBC inhibition for this dose group was consistently between 11-16% for these times. None of these effects were statistically significant. This however, may well have been due to the smaller number of subjects used, i.e., only 2 dams from the 0.1 mg/kg dose group on those later days. Examination of the individual plasma ChEI data from GD 6 showed that the 40% mean group decrease was a result of even larger differences in 2 dams. Thus, since the magnitude of the effect was relatively large, not the result of one animal, and generally consistent with data on non-pregnant adult rats (i.e.,70-80 % plasma ChEI in the acute neurotoxicity study), it is concluded that this is a toxicologically significant effect.

Dams in the dosed groups showed recovery after dosing: recovery in body weight was evident in the high-dose animals after cessation of treatment, with lactation day 21 body weights similar to the control values; blood ChEs were not statistically significant on Day 11. Parameters of the FOB affected in dams were greatly reduced on lactation day 7. For example, at the high dose, significant differences on GD 6 in body temperature and analgesic reflex were seen but were transient and not seen on lactation day 7. This recovery could have been due to tolerance in the animals as a result of continuous treatment for several weeks.

Last, treatment with Aldicarb did not affect duration of gestation, total number of pups delivered, pup survival indices, or per cent male pups.

In summary, there was maternal toxicity at 0.30 mg/kg/day that included clinical signs of toxicity such as tremor, salivation, and lacrimation, plasma ChE inhibition of 80% and RBC ChE inhibition of 27%, and 17% reduced body weight gain during gestation.
The maternal LOEL is 0.10 mg/kg/day based on plasma ChEI of 40% on GD 7.

19821

The maternal NOEL is 0.05 mg/kg/day.

Pup body weights adjusted for litter size from high-dose dams were significantly lower (about 10%) than controls during most of lactation Pups from 0.1 mg/kg/day dams also had significantly lower body weights than controls on some lactation days. But by day 21 adjusted body weights from the mid- and high-dose dams were not significantly different from controls.

However, unadjusted mean body weights of these F_1 male and female rats earlier given 0.1 and 0.3 mg/kg/day were still depressed consistently throughout the postweaning period. Significant differences occurred at weeks 0, 2, and 4 for the mid- and high-dose males (6-8%; 10-15%) and high-dose females (13-7%), and at week 2 for the mid-dose females (4%). In high dose males, the lower body weights were also reflected in reduced growth of these animals as indicated by lower body weight gains (8%) postweaning.

The larger brain size of the low dose F_1 females on lactation day 11 is probably not treatment-related since the value was only 102% of the control, there was no dose-response relationship, and males were not similarly affected. Also, no differences in brain size were seen at postnatal day 60.

There was no consistent pattern in morphometric measurements as a function of dose, either within one sex or between sexes. Thus, it is concluded that were no meaningful effects on these measures. Given the large number of measures made, it is not surprising to find some as statistically significant. More rigorous analyses, (correction for the large number of measures) given the marginal significance of the findings noted, i.e., none less than 0.03, would have rendered these changes less statistically meaningful.

It is unfortunate, however, that more detailed morphometric measures were not made on day 11 animals, as called for in the guideline.

ChE activity in the offspring was not affected during lactation days 7 or 10, or lactation day 11 the day after dosing. As for the dams, part of this may be due to a limited number of subjects, i.e., 3-4 subjects for some days and doses, and no more than 5 at any time. Second, these are exposures through the milk, which one might expect to be much less than *in utero* exposure. On the other hand, they were done 2 hours after a daily gavage exposure, where exposure might be expected to be greatest.

Most neurobehavioral observations of the offspring were transient or inconsistent across test days and not dose-related. In any study such as this, with a large number of measurements, some findings due to chance are to be expected. Still, certain findings seem more demonstrably or plausibly treatment related. Motor activity was significantly decreased, about 30%, in both 0.1 and 0.3 mg/kg F_1 males on day 17. A 29% decrease was seen in the 0.05 mg/kg group, but it did not reach statistical significance. No differences in motor activity were seen on days 13 and 21. Since there is normally, and, was seen here in control animals, a pattern of increases and then decreases in motor activity between days 13-

20821

21, a decrease only on day 17 is consistent with that normal pattern.

The increases seen at day 60 in high and mid dose males are less consistent, not expected, and so more difficult to interpret. A variety of other effects were also noted at different times, most often in pups from high-dose litters particularly on postnatal days 35 or 63. Chief among these were reductions in measures such as rearing, latency to first step in the open field or number of fecal boli, all in males. These contrast to the automated motor activity increases. Second, it is not clear why they would not have appeared earlier as well. Similarly effects seen on measures of grip strength or splay seemed inconsistent between days, hind limbs and fore limbs, and not readily explained coherently.
In summary, only the effects seen on day 17 in motor activity seem clearly treatment related and significant.

In summary, in the offspring the LOEL is 0.10 mg/kg/day based on reduced body weights from birth through postweaning, and on decreased motor activity on day 17 in male pups.
The offspring NOEL is 0.05 mg/kg/day.

Study deficiencies

Less than 5 subjects/dose were used at some time points for measurements of ChEs in both dams and pups. As few as 2 subjects were used. However, there appears overall to be sufficient data to show a general picture in dams broadly consistent with other studies in adults. The measures in the pups were and no persistent or significantly greater effects in offspring for the times and doses tested.

Validation of the FOB testing or other behavioral procedures for the laboratory were not included, but studies in adults have been presented publicly for this lab.

The study was unclear about timing of weaning and initiation of observations for the F_1 animals; there is a discrepancy between lactation day 21 body weights and week 0 postweaning body weights for these animals.
Overall, these deficiencies are minor and do not seriously alter the acceptability of the study.

21921