OPP#00562

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

MEMORANDUM

SUBJECT: Carbofuran; Guideline Requirement 83-6; Developmental Neurotoxicity Study in Rats; ID #090601; Reregistration Case #0101

Tox.Chem No.: 160A
MRID No.: 43378101
DP Barcode No.: D208441
Submission No.: S475390

TO: Margaret Rice, PM Team #62
Reregistration Branch
Special Review and Reregistration Division (7508W)

FROM: William Dykstra, Ph.D., Toxicologist
Review Section I
Toxicology Branch I William Dykstra 1/24/96
Health Effects Division (7509C)

THRU: Roger Gardner, Section Head, Toxicologist
Review Section I
Toxicology Branch I Roger Gardner 1/26/96
Health Effects Division (7509C) KB 2/6/96

ACTION REQUESTED: The Registrant, FMC Corporation, has submitted a new developmental neurotoxicity study in Sprague-Dawley rats as 6(a)(2) Data. The study is being submitted in response to a FIFRA '88 DCI. The Toxicology Branch (TB-I) has been requested to review the study and prepare a DER.

CONCLUSIONS: The developmental neurotoxicity study is acceptable and fulfills the Guideline Requirement for 83-6. This study was well conducted and reported.

EXECUTIVE SUMMARY: Groups of 24 impregnated Sprague-Dawley strain rats were fed diets containing 0, 20, 75, or 300 ppm (1.70-1.73, 4.95-6.91, or 8.57-31.38 mg/kg/day) technical carbofuran from gestation day 6 and continuing through lactation day 10. Clinical signs and mortality were recorded daily and body weight and food consumption taken weekly throughout gestation and lactation.

Litters were observed daily for dead pups. Litters were culled to eight pups on Postnatal Day (PND) 4. Pinna detachment, incisor eruption, eye opening, vaginal patency, and preputial separation were evaluated for all surviving pups. Motor activity, auditory startle response and swimming, learning and memory evaluations were measured on one pup/sex/litter.

Other selected pups were sacrificed on PNDs 11 and 60, and body and brain weights were measured. Brain, spinal cord, sciatic nerve, and skeletal muscle tissues from 6/sex/group pups, which were sacrificed on PND 11 and 60, were also prepared for microscopic examination.

The maternal toxicity NOEL was 20 ppm. At the LEL of 75 ppm, there was decreased body weight gain and decreased food consumption during gestation days 6-10. During this same time, high dose females lost weight and had highly significantly decreased food consumption. The body weight of the high dose group was also significantly less than controls from gestation Days 10-20.

The NOEL for developmental neurotoxicity was 20 ppm. At the LEL of 75 ppm and also at the high dose, there was increased pup mortality during the first 4 days of lactation which produced highly significantly decreased viability indices in comparison to controls ($p < 0.01$). Live birth indices were comparable between control and treated groups, and although the mid and high dose groups had lower weaning indices than controls, the differences were not statistically significant.

Pup body weight was highly significantly decreased at birth from 7- 16% and during the entire lactation period up to 25-38% in the mid and high dose groups ($p < 0.01$).

The NOEL for developmentally delayed parameters is 20 ppm and the LEL is 75 ppm with the effects being delay of vaginal patency and preputial separation.

Evaluations of auditory startle performed on PND 22 and 60±2 did not demonstrate any treatment-related effects at any treated dose in comparison to controls.

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Evaluations performed on PND 13, 17, 22, and 60±2 did not demonstrate any treatment-related effects in motor activity. The NOEL for swimming angle development is 20 ppm.

Learning and memory were measured by water "Y" maze time trials performed on PND 24, 25, 30, 60, 61, and 65. The NOEL for learning and memory is the 20 ppm dose level.

There were no treatment-related effects in gross necropsy results in examined pups at day 11, 60, or post-day 60. There were no compound-related effects on absolute and relative brain weight directly attributable to carbofuran, or neurological histopathology of the brain, spinal cord, and peripheral nerves in Day 11 and Day 60 pups.

Reviewed by: William Dykstra, Ph.D. Toxicologist
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I

William Dykstra 1/24/96
Roger Gardner 1/26/96

DATA EVALUATION REPORT

STUDY TYPE: 83-6; Developmental Neurotoxicity Study in Rats

TOX. CHEM NO: 160A

MRID NO.: 433781-01

TEST MATERIAL: Carbofuran Technical

SYNONYMS: Furadan

STUDY NUMBER: 93-4506; FMC #A93-3746

SPONSOR: FMC Corporation

TESTING FACILITY: Pharmaco LSR, Inc. Laboratories

TITLE OF REPORT: A Developmental Neurotoxicity Study of Carbofuran in the Rat Via Dietary Administration

AUTHOR(S): Kathyrn S. Ponnock, Ph.D.

REPORT ISSUED: August 30, 1994

EXECUTIVE SUMMARY: Groups of 24 impregnated Sprague-Dawley strain rats were fed diets containing 0, 20, 75, or 300 ppm (1.70-1.73, 4.95-6.91, or 8.57-31.38 mg/kg/day) technical carbofuran from gestation day 6 and continuing through lactation day 10. Clinical signs and mortality were recorded daily and body weight and food consumption taken weekly throughout gestation and lactation.

Litters were observed daily for dead pups. Litters were culled to eight pups on Postnatal Day (PND) 4. Pinna detachment, incisor eruption, eye opening, vaginal patency, and preputial separation were evaluated for all surviving pups. Motor activity, auditory startle response and swimming, learning and memory evaluations were measured on one pup/sex/litter.

Other selected pups were sacrificed on PNDs 11 and 60, and body and brain weights were measured. Brain, spinal cord, sciatic nerve, and skeletal muscle tissues from 6/sex/group pups, which were sacrificed on PND 11 and 60, were also prepared for microscopic examination.

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The maternal toxicity NOEL was 20 ppm. At the LEL of 75 ppm, there was decreased body weight gain and decreased food consumption during gestation days 6-10. During this same time, high dose females lost weight and had highly significantly decreased food consumption. The body weight of the high dose group was also significantly less than controls from gestation Days 10-20.

The NOEL for developmental neurotoxicity was 20 ppm. At the LEL of 75 ppm and also at the high dose, there was increased pup mortality during the first 4 days of lactation which produced highly significantly decreased viability indices in comparison to controls ($p < 0.01$). Live birth indices were comparable between control and treated groups, and although the mid and high dose groups had lower weaning indices than controls, the differences were not statistically significant.

Pup body weight was highly significantly decreased at birth from 7- 16% and during the entire lactation period up to 25-38% in the mid and high dose groups ($p < 0.01$).

The NOEL for developmentally delayed parameters is 20 ppm and the LEL is 75 ppm with the effects being delay of vaginal patency and preputial separation.

Evaluations of auditory startle performed on PND 22 and 60±2 did not demonstrate any treatment-related effects at any treated dose in comparison to controls.

Evaluations performed on PND 13, 17, 22, and 60±2 did not demonstrate any treatment-related effects in motor activity. The NOEL for swimming angle development is 20 ppm.

Learning and memory were measured by water "Y" maze time trials performed on PND 24, 25, 30, 60, 61, and 65. The NOEL for learning and memory is the 20 ppm dose level.

There were no treatment-related effects in gross necropsy results in examined pups at day 11, 60, or post-day 60. There were no compound-related effects on absolute and relative brain weight directly attributable to carbofuran, or neurological histopathology of the brain, spinal cord, and peripheral nerves in Day 11 and Day 60 pups.

Core Classification: **ACCEPTABLE**

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1. Quality Assurance Statement: A Certification of Good Laboratory Practice was signed by the Study Director, Dr. Kathyrn S. Ponnock, and dated August 30, 1994. Quality Assurance Inspections and Audit Dates were signed and dated by Michael Caulfield, Quality Assurance Group Leader.

2. Test Material: Carbofuran technical; Lot Number: I-80523; Purity: 99.1%; Description: light tan to white crystalline solid

3. Dietary Analyses: Samples of the treated diets were assayed for homogeneity, stability and concentration on Study Weeks 0, 1, 2, 3, and 4.

4. Animals: 96 female Sprague-Dawley rats {Crl:CD™(SD)BR}, approximately 51 days old, weighing 202.8 to 294.5 grams were used in the study. The rats were individually caged prior to and after mating and had free access to pelleted Purina Certified Rodent Chow #5002 and tap water. Females were housed with untreated males of the same strain in a 1:1 ratio. The day of finding plug and/or sperm was considered gestational day 0 (GD 0). On GD 20, mated females' cages were fitted stainless steel floor pans containing dry bedding in preparation of delivery of pups. Maternal animals and pups remained in this type of cage through the lactation period. Room temperature and lighting were under controlled conditions.

5. Methods: Females were assigned to the following groups based on a weight-independent random allocation schedule:

Treatment Group	Number of Females per Group
Control	24
Carbofuran at:	
20 ppm	24
75 ppm	24
300 ppm	24

Mated females received the appropriate control or treated diets from GD 6 to PND 10 ad libitum. Clinical observations were made twice daily without knowledge of treatment groups from GD 0 through sacrifice. Dam body weights were recorded on GD 0, 6,

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10, 15, and 20; and LD 0, 4, 11, and 21. Food consumption was measured during the following intervals: GD 0-6, 6-10, 10-15, and 15-20. Parturition was observed and the day on which all pups were delivered was designated PND 0.

6. Type and Frequency of Observations and Analyses of Pups:

All pups were examined twice daily for clinical signs, pup abnormalities and mortality. Body weights were recorded on PND 0, 4, 11, 17, and 21. All pups were examined externally for malformations and sexed on PND 0, 4, 11, 17, and 21. On PND 4, each litter was reduced to 4 male and 4 female pups. Remaining non-selected pups were necropsied and discarded. Mean pup live birth, viability, and weaning indices were calculated for control and treated groups as follows:

$$\text{Pup Live Birth Index} = \frac{\text{total pups born alive}}{\text{total pups born}} \times 100$$

$$\text{Pup Viability Index} = \frac{\text{total pups alive Day 4 (pre-cull)}}{\text{total pups born alive}} \times 100$$

$$\text{Pup Weaning Index} = \frac{\text{total pups alive at Day 21}}{\text{total pups alive Day 4 (post-cull)}} \times 100$$

The completion of the following parameters within each litter was determined for the lactation day (PND) when they occurred: pinna detachment, eye opening, and incisor eruption. Animals retained post-weaning were observed to identify the day on which vaginal patency (females) and preputial separation (males) occurred.

BEHAVIORAL ASSESSMENT

(1) Open Field Motor Activity:

The test was conducted on PND 13, 17, 22, and 60 (±2) on 1/sex/litter. Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length and consisted of 12 5-minute intervals. Details of the methodology are appended to this report.

(2) Auditory Startle Habituation:

The test was performed on PND 22 and 60 (±2) on 1/sex/litter. The test was designed to assess

sensorimotor reflexes and habituation to redundant, nonsignificant stimuli. The response to an auditory stimulus was measured using a Startle Response Screening System. The mean response amplitude on each block of 10 trials (5 blocks of 10 trials/session) in the presence and absence of a prepulse stimulus was determined and recorded. Details of the methodology are appended to this report.

(3) Swimming/Learning and Memory:

The Swimming test was performed on PND 6, 8, 10, 12, and 14 on 1/sex/litter. In this test, each pup was given a 15 second trial in a tank filled with water and rated for direction, angle and paddling. Learning and memory were assessed on PND 24 and 60 on 1/sex/litter. Each pup was given six consecutive time trials in a water Y-maze. The time required to find the correct arm of the maze was recorded. This measured the acquisition phase of learning. On PND 25, 30, 61, and 65, each animal was evaluated for two more time trials which measured short- and long-term memory. Details of the methodology are appended to this report.

7. Necropsy:

Parental females were euthanized, necropsied and given a complete post-mortem examination. Abnormal tissues were saved in 10% formalin. Dead and discarded pups were similarly necropsied and examined for abnormalities. Abnormal tissues were saved in 10% formalin.

On PND 11, one pup/sex/litter were necropsied and body and brain weights recorded. Additionally, 6 animals/sex/group from the animals sacrificed on PND 11 had brains fixed in 10% neutral buffered formalin, embedded in paraffin and stained with hematoxylin and eosin, Luxol Fast Blue and Sevier-Munger stain.

On PND 60, one pup/sex/litter were necropsied and body and brain weights recorded. Additionally, 6 animals/sex/group from the animals sacrificed on PND 60 were anesthetized with an intraperitoneal injection of sodium pentobarbital and perfused with phosphate-buffered saline followed by 4% paraformaldehyde in the same buffer. Following perfusion, the brain, spinal cord and three peripheral nerves (sciatic, tibial, and sural) were removed and post-fixed in 4%

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paraformaldehyde in phosphate buffer. Brains and spinal cords were embedded in paraffin and stained with the same stains used in PND 11 pups.

All brains and spinal cords were sectioned to include the following structures: cerebral cortex, basal ganglia, hippocampus, midbrain, cerebellum, brain stem, cervical, thoracic and lumbar cord (cross and longitudinal sections). Peripheral nerves were embedded in plastic, sectioned at one micron and stained with toluidine blue.

8. Statistical Analyses:

Statistical analyses were done by a one-way analysis of variance (ANOVA) using the F-distribution to assess significance. Dunnett's test was used to determine which means were significantly different from the control. For nonparametric data, the Kruskal-Wallis test was used followed by the Dunn test to determine which treatments were significantly different from control. Results were considered to be statistically significant if $P \leq 0.05$ or ≤ 0.01, two-sided. A trend analysis was used to determine if there was a significant trend ($P \leq 0.05$) with increasing dosage across all treatment groups.

RESULTS

There were no deaths or abortions during the study. There were no compound-related clinical signs during gestation or lactation in the dams. During gestation and lactation, the incidence and frequency of the following clinical signs occurred without relationship to treatment: alopecia, lacrimation, chromodacryorrhea, broken incisor, scabs, and unthrifty coat. There were no treatment-related effects in mated females which delivered live pups or gestation length. The number of pregnant females with live pups out of the 24 mated females per group was 23, 23, 24, and 23 for the control, low, mid, and high-dose groups, respectively. The mean length of gestation was 22.1, 21.8, 21.9, and 21.8 days for the control, low, mid, and high-dose groups, respectively.

During gestation days 6-10, the control group gained 14 grams while the mid dose group gained only 1 gram and the high dose group had a body weight loss of 27 grams. Food consumption was also highly significantly reduced

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in the mid and high dose groups during gestation days 6-10 due to unpalatability. However, the decreased weight gain and weight loss seen in the mid and high dose dams during gestation days 6-10 are considered evidence of significant maternal toxicity. The body weight of the high dose group, but not the mid-dose group, was also significantly less than controls from gestation Days 10-20. Food consumption was increased in both mid and high doses during gestation days 10-15, signifying that the females adapted to the treated diets and, in association with the increased food consumption, body weight gain was increased during these same gestation days at the mid and high dose levels.. This finding indicates that pregnancy status was not compromised during this period. During gestation days 15-20, maternal toxicity was seen as decreased body weight gain in the presence of increased food consumption for the high dose dams. The highly significant decreased high dose pup weights at lactation day 0 are consistent with decreased dam weight gain during gestation days 15-20. Lactation body weight gains were comparable up to treatment day 10 (weight days 0-11 of lactation) and the high dose group had increased weight gain from lactation day 11-21 suggesting a rebound effect in the high dose group dams.

MEAN BODY WEIGHT

DOSE (PPM)	0	20	75	300
GEST. DAY				
0	240	243	244	240
6	268	268	269	263
10	282	280	270	235**
15	312	308	301	281**
20	385	376	371	346**

** = $p < 0.01$

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AVERAGE MATERNAL BODY WEIGHT CHANGES (grams)

Dose	Control	Low	Mid	High
Gestation Days				
0 - 6	28 (23)	25 (23)	25 (24)	23 (23)
6 - 10	14	12	1**	-27**
10 - 15	30	27	31	46**
15 - 20	73	69	70	65*
Lactation Days				
0 - 4	17 (23)	14 (22)	9 (24)	21 (10)
4 - 11	13 (23)	15 (23)	18 (21)	18 (9)
11 - 21	-2 (23)	-3 (23)	6 (21)	33**

* = P < 0.05 ( ) = number of litters
** = p < 0.01

AVERAGE MATERNAL FOOD CONSUMPTION (g/kg/day)

Dose	Control	Low	Mid	High
Gestation Days				
0 - 6	94	94	90	92
6 - 10	86	85	64**	29**
10 - 15	85	85	92*	102**
15 - 20	87	86	92*	105**

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COMPOUND INTAKE DURING GESTATION (mg/kg/day)

Dose	Control	Low	Mid	High
Gestation Days				
6 - 10	0	1.70	4.77	8.57
10 - 15	0	1.70	6.91	30.68
15 - 20	0	1.73	6.91	31.38

SUMMARY OF REPRODUCTIVE STATUS AND WEANING

The number of females with live births was comparable between control and treated groups. Increased pup mortality during the first 4 days of lactation was observed at both the mid and high dose as highly significantly decreased viability indices in comparison to controls. Live birth indices were comparable between control and treated groups, and although the mid and high dose groups had lower weaning indices than controls, the differences were not statistically significant. Therefore, following birth, if pups were able to survive the first 4 days of lactation, survival during the remainder of the lactation period was highly probable. These data are shown below as the percent pup mortality during lactation days 1-4 and 5-21.

Pup body weight was highly significantly decreased at birth from 7- 16% and during the entire lactation period up to 25-38% in the mid and high dose groups. During the post weaning period, mid and high dose pup body weights were significantly lower than controls for 2-5 intervals of the weekly weighing and weight gain for the entire period was 7-14% less than controls for these groups.

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	CONTROL	LOW	MID	HIGH
Females	24	24	24	24
Females with live pups Day 0 Postpartum	23	23	24	23
Females with live pups Day 21 Postpartum	23	23	21	9
Total Pups (M/F)	342(169/165)	335(165/168)	354(180/164)	330(161/141)
Live Pups on Day 0	334(169/165)	333(165/168)	344(180/164)	302(161/141)
Dead Pups on Day 0	8	2	10	28
Live Pups/Litter	14.5	14.5	14.3	13.1
% Live Pups	97.7	99.4	97.1	91.5
Live Pups after culling	184	178	182	75
Pup Deaths (% pup deaths)				
Postnatal Days 1 - 4	5(1.3)	19(5.7)	57(16.6)	201(66.6)
Postnatal Days 5 - 11	23(12.5)	25(14.0)	42(23.0)	20(26.6)
Postnatal Days 12 - 17	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Postnatal Days 18 - 21	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Postnatal Days 5 - 21	23(12.5)	25(14.0)	42(23.0)	20(26.6)
Individual Pup Live Birth, Viability and Weaning Indices				
Live Birth Index	97.7	99.4	97.7	91.9
Viability Index	98.5	94.7	83.4**	33.8**
Weaning Index	87.0	84.8	72.9	69.6
Live Pup Weight (g)				
Day 0	6.2	6.0	5.8**	5.2**
Day 4	9.6	9.0	7.4**	7.0**
Day 11	24.7	23.2	18.6**	15.2**
Day 17	38.9	38.4	31.8**	26.2**
Day 21	51.1	48.7	40.9**	35.6**

* = $P < 0.05$
** = $P < 0.01$

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AVERAGE BODY WEIGHT OF PUPS IN POSTWEANING

	Weeks								
Dose	1	2	3	4	5	6	7	8	GAIN
Control	97	134	151	197	242	278	296	318	221
Low	96	128	143	191	237	271	291	314	218
Mid	83	112	130	174	218	256	263**	273*	190
High	69	85**	111	155*	197*	235*	254*	274	205

* = $p < 0.05$

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BEHAVIORAL ASSESSMENT

MEAN DEVELOPMENTAL LANDMARK DATA

Mean Day to Criteria						
Dose ppm	Pinna Detachment	Upper Incisor Eruption	Lower Incisor Eruption	Eye Opening	Vaginal Patency	Preputial Separation
0	2.52	10.89	11.85	13.93	31.92	44.96
20	3.00**	10.92	12.25**	14.15**	32.28	45.51
75	2.93**	10.97	12.50**	14.29**	32.85**	47.94**
300	3.19**	11.00	12.33**	14.33**	34.93**	48.88**

** = $p < 0.01$

Based on statistical analysis, no NOEL is present at 20 ppm for pinna detachment, lower incisor eruption, and eye opening. Each of these developmental delays at 20 ppm is highly statistically significant in comparison to controls.

The Study Authors state that "Although deliveries occur throughout a 24-hour period, it is only possible to monitor these deliveries from approximately 8:30 am until 4:30 pm. Further, evaluations for developmental landmarks are performed once each day. As such, on any given day of lactation, there can be more than a twelve hour age difference in pups. Thus, one-half day delays in the appearance of certain developmental landmarks are not considered to be biologically significant and are not adverse effects of treatment." The individual pup data are not listed in the study report and only litter means are listed. Therefore, it is not possible to determine more precisely when the individual developmental landmarks occurred. However, a statement by the Study statistician, Dr. Mark J. Nicolich, notes that "The three treated groups showed retarded development for pinna detachment, lower incisor eruption, and day of eye opening; the mid and high dose groups showed developmental retardation for vaginal patency and preputial separation." Also, the historical control data provided by the testing laboratory showed the following:

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Mean Day to Criteria				
Historical Control	Pinna Detachment	Upper Incisor Eruption	Lower Incisor Eruption	Eye Opening
I	2.7	9.7	11.0	13.7
II	2.7	9.6	10.9	13.8
III	2.5	9.5	10.8	13.9

It can be seen that the statistically significant means for the developmental landmarks (pinna detachment, lower incisor eruption, and eye opening) for the 20, 75, and 300 ppm groups are all increased in comparison to the historical controls and concurrent controls. Additionally, it is also noted that the historical controls are in close agreement with the study controls for the developmental landmarks which are statistically significantly increased in the treated groups. If the study authors' argument that the highly statistical findings in the treated groups can be dismissed as not being biologically significant due to timing, then no explanation is provided for the mathematical similarity in days for same developmental landmarks of both the concurrent and historical control data. Shown below are the increases in days in developmental delays for the treated groups in comparison to controls.

Mean Increase (Days) in Developmental Delay in Comparison to Control					
Dose ppm	Pinna Detachment	Lower Incisor Eruption	Eye Opening	Vaginal Patency	Preputial Separation
0	-	-	-	-	-
20	+0.48	+0.40	+0.22	+0.36	+0.55
75	+0.41	+0.65	+0.36	+0.93	+2.98
300	+0.67	+0.48	+0.41	+3.01	+3.92

It can be seen from this Table that the increases for pinna detachment and lower incisor eruption are not dose-related and the slight, dose-related increase in eye opening does not reflect the large magnitude of the

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15-fold increase in carbofuran from the low dose of 20 ppm to the high dose of 300 ppm. Therefore, due to the slight magnitude of the treated findings in comparison to control and the absence of a dose-related occurrence in the data, the developmental delays in pinna detachment, lower incisor eruption, and eye opening at all treated doses are not considered toxicologically significant. The NOEL for developmentally delayed parameters is 20 ppm and the LEL is 75 ppm with the effects being delay of vaginal patency and preputial separation.

MEAN AUDITORY STARTLE VALUES

Evaluations of auditory startle performed on PND 22 and 60±2 did not demonstrate any treatment-related effects at any treated dose in comparison to controls. However, there were differences, as expected, in maximum response, time to maximum response, and average response over each of the 5 blocks (10 trials per block) with the response decreasing over time. Additionally, at PND 60, there was reduced response with the prepulse stimulus in comparison to the response without the prepulse stimulus but the finding occurred in controls as well as in treated groups.

MEAN MOTOR ACTIVITY VALUES

Evaluations performed on PND 13, 17, 22, and 60±2 did not demonstrate any treatment-related effects in motor activity. Group means at the treated doses were comparable to controls by sex, although females showed a lower activity level when compared to males. At all measurement intervals, control and treated groups had decreases in activity as the one-hour session progressed.

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SUMMARY OF SWIMMING DEVELOPMENT EVALUATIONS and WATER "Y" MAZE TIME TRIAL RESULTS

MALES

Summary of Swimming Angle Development Data

Dose ppm	Day 6	Day 8	Day 10	Day 12	Day 14
0	0	1	2	2	3
20	1	1	2	2	3
75	0	1	1*	2	2*
300	0	0*	1*	1*	2*

FEMALES

Summary of Swimming Angle Development Data

Dose ppm	Day 6	Day 8	Day 10	Day 12	Day 14
0	1	1	2	2	3
20	1	1	2	3	3
75	0*	0*	1*	2	2*
300	0*	0*	1*	2*	2*

* = consistently decreased

Angle
0 = head submerged
1 = nose at top of surface
2 = nose and top of head above and ears below the surface
3 = top of head, ears and nose above surface

At PND 14, a maximum score of "3" was attained for swimming angle development in males by 15/23 in the control group, 14/22 at 20 ppm, 9/19 at 75 ppm, and 2/7 at 300 ppm. In females, a maximum score of "3" was achieved by 14/22 in controls, 14/21 at 20 ppm, 8/22 at 75 ppm, and 3/8 at 300 ppm. Therefore, the NOEL for swimming angle development is clearly 20 ppm.

Learning and memory were measured by water "Y" maze

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time trials performed on PND 24, 25, 30, 60, 61, and 65. At 300 ppm, in both sexes, on PND 24 acquisition and memory were affected as shown by the increase in mean time trials for all six trials in comparison to controls. Differences were statistically significant for trials 3 and 6 in males and trials 5 and 6 in females. In males, trials 3 and 6 were 4.85 vs. 8.79 and 4.26 vs. 12.53 seconds for control vs. 300 ppm group, respectively. In females, trials 5 and 6 were 5.66 vs. 47.69 and 9.27 vs. 31.86 seconds for control vs. 300 ppm group, respectively. Also occurring in females at 75 ppm on PND 24, but not later, was a statistically significant increase in time trials 5 and 6 in comparison to controls. On PND 25, males and females at 300 ppm had increased times for trial #1 in comparison to controls, although only males were statistically significant. On PND 30, at 300 ppm in comparison to controls, males were significantly increased in trial #1, but females did not show any increase at 300 ppm. On PND 60, at 300 ppm in comparison to controls, males were significantly increased in trial #2, but the results in females were comparable between control and treated groups. Control and treated groups of both sexes at PND 61 and 65 had comparable trial times and no treatment-related findings were evident. For the second measurement period (PND 60, 61, and 65), both control and treated groups performed the trials at less time (seconds) when compared to the first measurement period (PND 24, 25, and 30). These data suggest that learning and memory were delayed rather than permanently impaired. The NOEL for learning and memory is the 20 ppm dose level.

ORGAN WEIGHTS

Severe body weight and, concomitant, brain weight decreases were present in both sexes at the mid and high dose levels in day 11 pups. It is apparent from the increased relative brain weights in comparison to the absolute brain weights that the decreased absolute brain weights in the mid and high dose reflect the severe body weight decreases at these doses rather than a specific manifestation of direct brain toxicity of carbofuran. These day 11 pups were severely delayed developmentally (decreased body weights at day 0-21 of lactation) and this generalized effect included decreased organ, including brain, as well. In fact, since the relative brain weight was increased at the mid and high dose, the brain was not affected as much

as other organs, which is consistent with the reviewer's previous experience. This interpretation is further buttressed by the body and brain weight data of day 60 pups. At day 60, in males, body weight decreases were not associated with brain weight decreases in these more mature animals.

		Control	Low	Mid	%	High	%
FEMALES							
Body Weight	(grams)	24.3	21.9	18.6**	-23	13.9**	-43
Brain	(grams)	1.010	.947	.893*	-12	.760**	-25
% B.W.		4.20	4.38	4.83**	+15	5.56**	+32
MALES							
Body Weight	(grams)	24.8	23.5	18.2**	-27	14.4**	-42
Brain	(grams)	1.025	1.001	.876**	-15	.805**	-21
% B.W.		4.15	4.28	4.90**	+18	5.77**	+39

		Control	Low	Mid	%	High	%
FEMALES							
Body Weight	(grams)	227.1	212.3	216.0		211.0	
Brain	(grams)	1.784	1.792	1.762		1.724	
% B.W.		0.79	0.85	0.82		0.83	
MALES							
Body Weight	(grams)	352.5	354.4	301.1**	-15	290.9*	-17
Brain	(grams)	1.957	1.975	1.872		1.795	
% B.W.		0.56	0.56	0.64		0.62	

* = $p < 0.05$

** = $p < 0.01$

Necropsy

There were no treatment-related gross necropsy findings in PND 11 or 60 pups.

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Histopathology

PND 11 PUPS

Dose	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
Number Necropsied	12	11	12	10	10	11	6	3
Brain								
Number Examined	6	6					6	3
Not Remarkable	6	6					6	3

PND 60 PUPS

Dose	Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
Number Necropsied	19	17	17	18	17	14	9	11
Brain								
Number Examined	6	6					6	6
Not Remarkable	4	2					5	3
Ventricles, slightly dilated	2	4					1	3
Spinal Cord								
Number Examined	6	6					6	6
Not Remarkable	6	6					6	6

Sciatic Nerve				
Number Examined	6	6	6	6
Not Remarkable	6	6	6	6
Sural Nerve				
Number Examined	6	6	6	6
Not Remarkable	6	6	6	6
Tibial Nerve				
Number Examined	6	6	6	6
Not Remarkable	6	6	5	6
Axonal Swelling, minimal	0	0	1	0

There were no treatment-related histological findings. The one male day 60 pup with minimal axonal swelling of the tibial nerve was not considered treatment-related, since other peripheral nerves were without change and the finding was an single instance out of six tibial nerves examined at the high dose. The slightly dilated brain ventricles in both sexes occurred more frequently in controls than high dose animals and was, therefore, not considered related to exposure to carbofuran.

Analyses of Diets

Analysis of homogeneity low, mid, and high-dose levels showed for samples dated 7/12/91 that the top, middle and bottom analyses for the low-dose were 75, 85, and 82% of target doses; for the mid-dose were 95, 94, and 91% of targeted doses, and for the high-dose were 94, 97, and 94% of targeted doses for the top, middle and bottom of the sample, respectively.

Analysis of homogeneity of the top, middle and bottom levels of pretest mixes showed that the top, middle and bottom analyses for the 20 ppm dose level had a mean of 97.3% of the target dose and for the 300 ppm dose, the mean for each level of mix was 102% of the targeted

dose. These values are ±10% of each other and within ±15% of the nominal doses.

Summary of concentration analyses of samples of weeks 1, 2, 3, and 4 showed averages of duplicate analyses which fell within ±10% of targeted doses for the low, mid, and high-dose levels, except for the 20 ppm level at week 2 which was 113% of nominal levels.

Results of storage stability sample analysis showed that for low and high dose levels kept at room temperature for 7 and 14 day (14 days frozen) intervals, the 20 ppm dose level had a recovery mean of 108% and the 300 ppm dose level had a recovery mean of 104% at 7 day room temperature (14 day frozen) interval. At the 14 day room temperature interval, the 20 ppm level had a recovery mean of 98.3% and the 300 ppm level had a recovery mean of 104%.

DISCUSSION

This study was well conducted and reported. The NOEL for developmental neurotoxicity and maternal toxicity was 20 ppm. At maternally toxic doses of 75 and 300 ppm, there were severe effects on pup survival during days 1-4 of lactation. Pup body weight was also severely affected at the mid and high dose during Lactation Days 1-21, as well as during the post-lactation period. Delayed developmental effects were seen in both sexes during lactation. Although there were no effects in auditory startle or motor activity, swimming angle was affected at both the mid and high dose. Learning and memory were delayed, but did not appear to be impaired, at the mid, but mostly the high dose.

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