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OFFICE OF PREVENTION, PESTICIDE AND TOXIC SUBSTANCES

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: November 11, 2008

SUBJECT: **Methamidophos:** Revision of DER (TXR 0054510) for the Developmental Neurotoxicity Study

PC Code: 101201
Decision No.: None
Petition No.: None
Assessment Type: None
TXR No.: 0054922
MRID No.: 45666401

DP Barcode: D354692
Registration No.: None
Regulatory Action: None
Case No.: None
CAS No.: 10265-92-6

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I. CONCLUSIONS

The previous DER (TXR No. 0054510) for the developmental neurotoxicity study (DNT) with methamidophos was revised to change the maternal NOAEL/LOAELs for brain cholinesterase activity. In the revised DER, the maternal LOAEL is 0.1 mg/kg/day (lowest dose tested) based on significant ($p \leq 0.05$) decrease in brain cholinesterase activity (8%↓) and the NOAEL is not established. The revised DER (TXR No. 0054922) is attached.

II. ACTION REQUESTED

Revise the previous DER (TXR No. 0054510) for the DNT study (MRID 45666401).

Rec'd in RRB 11/19/2008 aw

III. BACKGROUND

The maternal NOAEL/LOAELs have been changed subsequent to a meeting with the DNT Workgroup dated Nov. 23, 2004. However, the previous DER for the DNT study has not been changed. Therefore, it is necessary to change the NOAEL and LOAEL in the previous DER (TXR No. 0054510) for the DNT study (MRID 45666401).

IV. RESULTS/DISCUSSION

In the previous DER (TXR No. 0054510), the maternal LOAEL was 10 ppm (0.9 mg/kg/day) based on plasma, erythrocyte and brain cholinesterase inhibition in the dams and the maternal NOAEL was 1 ppm (0.1 mg/kg/day). Subsequent to a meeting with the DNT Workgroup dated Nov. 23, 2004, the maternal LOAEL was changed to 0.1 mg/kg/day (lowest dose tested) based on significant ($p \leq 0.05$) decrease in brain cholinesterase activity (8%↓) and the NOAEL was not established. Therefore, the NOAEL and LOAEL for brain cholinesterase activity in the previous DER (TXR No. 0054510) for the DNT study (MRID 45666401) are revised in this DER.

EPA Reviewer: Paul Chin, Ph.D. **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 11/12/08
EPA Secondary Reviewer: Kit Farwell, D.V.M. **Signature:** K Farwell
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 11/12/08

TXR#: 0054922

DATA EVALUATION RECORD – Supplemental

See TXR #s 0050923 (Root) and 0054510 for previous DERs

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426

PC CODE: 101201 **DP BARCODE:** D354692

TEST MATERIAL (PURITY): Methamidophos (72.3-74.2% a.i.)

SYNONYMS: *O,S*-Dimethyl phosphoramidothioate; Monitor®

CITATION: Sheets, L.P. (2002) A developmental neurotoxicity screening study with technical grade methamidophos (Monitor®) in Wistar rats. Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS. Laboratory Study No.: 00-D72-AI, February 11, 2002. MRID 45666401. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Box 4913, Hawthorne Road, Kansas City, MO

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45666401) methamidophos (72.3-74.2% a.i., Lot/batch # 803-0182) was administered to 30 female Wistar Crl:W (HAN)BR rats/dose, continuously in the diet, at dose levels of 0, 1, 10, or 30 ppm from gestation day (GD) 0 through lactation day (LD) 21. Average daily intake of the a.i. was 0, 0.1, 0.9 or 2.5 mg/kg/day (gestation) and 0, 0.2, 2.4 or 7.9 mg/kg/day (lactation), respectively. On post-natal day (PND) 4, litters were standardized to 8 pups/litter; excess pups were killed and discarded. Pups were weaned on postnatal day 21, after which time all animals received untreated diet. F1 pups were assigned to subgroups in order to evaluate brain weights, neuropathology, learning and memory, motor activity, and acoustic startle response. In addition, brain, erythrocyte, and plasma cholinesterase (ChE) activity was measured in the dams on LD 21 and the offspring on PNDs 4 and 21.

Maternal mortality, clinical signs, body weights, body weight gains, food consumption, reproductive performance, and abbreviated functional observations (AFO) were unaffected by treatment. However, **in the 1 ppm dams, significant ($p \leq 0.05$) decrease in brain cholinesterase activity was noted (8%↓).** In the 10- and 30- ppm dams, dose-dependent and significant ($p \leq 0.05$) decreases in plasma, erythrocyte, and brain cholinesterase activities were noted (50-84%↓).

The maternal LOAEL was 1 ppm (0.1 mg/kg/day) based on brain cholinesterase inhibition in the dams. The maternal NOAEL was not established.

Treatment had no adverse effects on offspring survival, food consumption, abbreviated FOB, brain weights, brain morphology, or neuropathology. No treatment-related effects were seen in the body weight or body weight gain of pups at the low and mid dose groups. At the high dose (30 ppm), pre-weaning body weights were decreased (↓8-12%; p≤0.01) on PNDs 11, 17, and 21 in the male and female pups. In addition, body weights were decreased (p≤0.05) on PND 4 (precull) in the female pups (↓8%). Pre-weaning body weight gains for male and female pups were decreased (p≤0.05) for most measured intervals between PND 0 and PND 21 (↓8-19%). Furthermore, body weights were decreased (p≤0.05) throughout most of post-weaning in both sexes (↓4-10%). In the 10 ppm pups, post-weaning body weight in the females was decreased (p<=0.05, ↓ 4-5%). This decrease is minor and its toxicological importance is considered to be equivocal. A slight but significant (p≤0.01) difference in the time to preputial separation was observed in the high-dose males. It occurred an average of 2.1 days later in the affected males than in the controls.

Treatment-related effects were observed on motor and locomotor activity in both sexes at the mid and high dose on PND 13. The non-statistically significant decreases were 25% in males and 33% in females at the mid dose and 45% in males and 27% in females at the high dose.

A consistent but not dose-dependent decrease in auditory startle reflex was seen in females at all dose levels on PND 22 and PND 38. On Day 22 the decreases were 15%, 15% and 28% at the low, mid and high dose groups, respectively. On Day 38, the decreases were 26%, 42% and 33%, at the low, mid and high dose groups, respectively. Peak startle response amplitude in all females at PND 22 and 38 reached statistical significance in one block at the low dose, two blocks at the mid dose and three blocks at the high dose. Additionally, the magnitude of the response was similar at the low and mid dose groups for PND 22 and PND 38 in females. Therefore, the low dose was determined to be an effect level.

In the passive avoidance test, nonsignificant but marked increases (76, 79 or 97%↑) in the latency to cross were seen at 1, 10 or 30 ppm, respectively. No other adverse effects were seen in the males and females of all dose groups.

Data from the water maze test showed that high-dose males had nonsignificant increases in the number of trials to criterion (15% ↑), errors (330% ↑), and time to make the correct choice (46% ↑) in the first trial of the learning phase of testing. During the memory phase of the water maze testing, high-dose males demonstrated more errors (200%↑) and longer time to achieve the goal (17% ↑). More errors (200 and 267% ↑) and longer time to achieve the goal (51 and 59% ↑)were also observed for the 1- and 10-ppm males during the learning phase. By contrast, there were no adverse effects in the females; females of all dose groups showed fewer errors and less time to make the correct choice compared to the concurrent controls. At 1 ppm, cholinesterase (ChE) activity was comparable to controls for all compartments. At 30 ppm, plasma (↓12-40%), erythrocyte (↓37-53%), and brain (↓14-43%) ChE activities were decreased (p≤0.05) relative to controls on PND 4 and PND

21 for the males and females. At 10 ppm, decreases ($p \leq 0.05$) were noted in erythrocyte ChE on PND 4 (↓20%, sexes combined); plasma and brain ChE on PND 21 in the males (↓22 and 13%, respectively); and a nonsignificant decrease in brain ChE on PND 21 in the females (↓17%).

The offspring LOAEL is 1 ppm (0.1 mg/kg/day), the lowest dose tested based on consistent decreases in peak auditory startle reflex response in females on PND 22 and PND 38. An offspring NOAEL was not established.

This study is classified **Acceptable/NonGuideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft) due to the inadequacies in the assessment of learning and memory in the offspring and the pending review of the of positive control data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

COMMENTS: The maternal NOAEL/LOAELs have been changed subsequent to a meeting with the DNT Workgroup dated Nov. 23, 2004. In the revised DER, the maternal LOAEL is 0.1 mg/kg/day based on significant ($p \leq 0.05$) decrease in brain cholinesterase activity (8%↓).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Methamidophos

Description:	Clear, colorless liquid
Lot/Batch #:	803-0182
Purity:	72.3-74.2% a.i.
Compound Stability:	The test material was stable in the diet for at least 14 days at room temperature.
CAS # of TGAI:	10265-92-6

2. Vehicle and/or positive control: Diet; corn oil, 1% (w/w)

3. Test animals (P)

Species:	Rat	
Strain:	Wistar Crl:W (HAN)BR	
Age at study initiation:	At least 12 weeks (females)	
Wt. at GD 0:	206.8-214.5 g (females)	
Source:	Charles River Laboratories (location not reported)	
Housing:	Individually, in stainless steel wire-bottomed cages except during mating, gestation, and lactation. During gestation and lactation, individual dams and their litters were housed in plastic cages.	
Diet:	Rodent Lab Chow 5001-4 "etts" (Purina Mills), *ad libitum* except during neurobehavioral testing.	
Water:	Tap water, *ad libitum* except during neurobehavioral testing	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 7/3/2000 End: Week of 10/9/2000

2. Study schedule: The test substance was administered to the maternal animals from gestation day (GD) 0 through lactation day (LD) 21. Pups were weaned on postnatal day (PND) 21, after which time all animals received untreated diet. F1 pups were assigned to subgroups (a minimum of 10/sex/dietary level, representing at least 20 litters per level) in order to evaluate abbreviated functional observations (AFO), physical landmarks, motor activity, acoustic startle response, passive avoidance, water maze, ophthalmology, brain weights, neuropathology and brain, erythrocyte and plasma cholinesterase (ChE) activity using the a modification of the Ellman method.

3. Mating procedure: Females were paired 1:1 with males of the same strain and source for a maximum of three consecutive days. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day (GD) 0, and each female was housed individually in a plastic nesting cage.

4. Animal Assignment: Mated females were randomly assigned, stratified by body weight, to dose groups as indicated in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 4.

Table 1. Study design [a]

Experimental Parameter	Sub-group	Dose (ppm) 0	1	10	30
Maternal Animals					
No. of maternal animals assigned [b]	NA	30	30	30	30
AFO (GD 6 and 20)	NA	30	30	30	30
AFO (LD 11 and 21)	NA	10	10	10	10
Reproductive activity	NA	30	30	30	30
Cholinesterase (ChE) activity	NA	10	10	10	10
Offspring					
Motor activity (PND 13, 17, 21, 60±2)	A	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Acoustic startle habituation (PND 22, 38±2, 60±2)	B	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Passive avoidance (PND 22 and 29)	C	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Water maze (PND 60±2 and 7 days later)	C	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
AFO (PND 4, 11, 21, 35±1, 45±1, 60±2)	C	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter	1pup/sex/litter
Neuropathology and morphometric analysis (PND 21)	D	10/sex	10/sex	10/sex	10/sex
ChE determination	D	remaining pups not included above	remaining pups not included above	remaining pups not included above	remaining pups not included above
Offspring (contd.)					
Brain weight (PND 75±5)	A, B, C	10/sex	10/sex	10/sex	10/sex
Ophthalmologic examination (PND 50-60)	A, B, C	10/sex	10/sex	10/sex	10/sex
Perfusion and neuropathology (PND 75±5)	A, B, C	those selected for ophthalmologic examination	those selected for ophthalmologic examination	those selected for ophthalmologic examination	those selected for ophthalmologic examination

a Obtained from pages 26 and 27 of the study report, MRID 45666401.
b Approximate
NA Not applicable

5. Dose selection rationale: Dose levels were chosen based on the results of a two-generation reproduction study in Sprague-Dawley rats (MRID 44466001) in which animals continuously received dietary concentrations of the test substance at 0, 1, 10 or 30 ppm, beginning approximately 10 weeks prior to mating. At 10 and 30 ppm, body weight was decreased in the dams and offspring during lactation. Additionally at 30 ppm, a slight increase in the incidence of cannibalization was noted. There were no effects on reproductive or litter parameters at any dosage. Significant ($p<0.05$) brain, plasma and erythrocyte ChE inhibition occurred at 10 and 30 ppm in the dams and pups. No treatment-related findings were noted at 1 ppm.

Based on the results of these reproduction studies, the doses (in ppm) presented in Table 1 were chosen for the developmental neurotoxicity study.

6. Dosage administration: All doses were administered to maternal animals continuously in the diet from GD 0 through LD 21. After PND 21, untreated feed was provided to all groups.

7. Dosage preparation and analysis: Formulations were prepared weekly by mixing appropriate amounts of test substance with corn oil (1%) and diet. Acetone served as a solvent in the diet preparation process and was allowed to evaporate. Prepared diet mixtures were retained frozen until use. Concentration of the test substance in the diet was evaluated for the 1, 10, and 30 ppm test diets that were provided to animals during study weeks 1, 2, 3, and 6. Stability (at room temperature and frozen) and homogeneity of the test substance in the diet were verified previously. Results are presented in MRID 43197901; it was stated that the test substance was found to be homogeneous and stable in the diet for at least 14 days at room temperature and 28 days under freezer conditions.

Results -

Concentration Analysis (range as % of nominal):
1 ppm: 74.3-96.0%
10 ppm: 93.2-101%
30 ppm: 79.7-103.3%

Note: Concentrations were adjusted for purity.
The analytical data indicated that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: Once daily checks for mortality, moribundity, and clinical signs were conducted for all maternal animals. In addition, detailed physical examinations were performed daily during treatment (GD 0 though LD 21).

An AFO was conducted on GD 6 and GD 20 for all maternal animals. A minimum of 10 dams/dose were also examined on LD 11 and LD 21.

	ABBREVIATED FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection and exophthalmus, 3) Urination and defecation 4) Pupillary function 5) Palpebral closure
X	Description, incidence and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence and severity of posture and gait abnormalities.
X	Description and incidence and severity of any unusual or abnormal behaviors

Body weight and food consumption were measured weekly throughout treatment. In addition, dams were weighed on LD 4. Brain, erythrocyte, and plasma cholinesterase activities were measured in the dams (10/dose) on LD 21, using a modification of the Ellman method.

b. Offspring

1) Litter observations: The day of completion of parturition was designated as postnatal day 0. Live pups were counted, sexed (ano-genital distance) and weighed on PNDs 0, 4, 11, 17, and 21. At least once daily, offspring were examined cage-side for gross signs of mortality, morbidity, and clinical signs. Detailed clinical observations were made once daily before weaning and once weekly, thereafter. Food consumption was measured weekly beginning the week of PND 28 (when pups were placed in individual housing) until termination. On day PND 4, litters were standardized to 4 pups/sex//litter; excess pups were killed and discarded.

2) Developmental landmarks: Beginning on PND 38, all male offspring were examined daily for preputial separation. Beginning on PND 29, all female offspring were examined daily for vaginal patency. The age of onset was recorded. In addition, all pups were tested for the presence of pupil constriction on PND 21.

3) Postweaning observations: Clinical observations were recorded daily for all animals. In addition, detailed clinical observations were recorded weekly during post-weaning. Body weights and food consumption were recorded weekly.

4) Ophthalmology: Animals that were selected for perfusion (minimum of 10/sex/dose level) were subjected to ophthalmoscopic examinations at approximately 50-60 days of age. The eyes of each animal were examined with a slit lamp microscope and an indirect ophthalmoscope equipped with a condensing lens.

5) Neurobehavioral evaluations

i) Abbreviated functional observations (AFO): On PND 4, 11, 21, 35 (±1 day), 45 (±1 day), and

60 (±2 days), selected pups (approximately 16/sex/dose; subset C) were observed outside the home cage according to procedures outlined for the dams by an individual who was unaware of each rat's dose group. In general, the neonates (PNDs 4 and 11) were not evaluated in the open field unless the observer considered it to be necessary.

ii) Motor activity testing: Activity was evaluated in 1 pup/sex/litter/dose group (subset A) on PNDs 13, 17, 21, and 60 (±2 days). Motor and locomotor activity were measured by testing animals in figure eight mazes. Each test session was one hour in duration, and consisted of 10-minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

iii) Acoustic startle habituation: Acoustic startle habituation testing was performed on 1 pup/sex/litter/dose group (subset B) on PNDs 22, 38 (±2 days) and 60 (±2 days). The performing laboratory provided the following information. A personal computer was used to control the operation of an integrated startle response test system (Coulbourn Instruments, Allentown, PA) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within a startle system enclosure. The enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and houses two speakers. One speaker, mounted in a central position within the ceiling of the enclosure, provided the eliciting stimulus (S2) - a 50-msec burst (0 msec rise/fall) of broad-spectrum "white" noise [approximately 120 dB(lin)]. The second speaker was mounted in a central position within the floor of each enclosure to provide prepulse stimuli (S1) as needed to test reflex modification (not performed in the present study). The enclosure houses four load cell/force transducer assemblies that are designed to measure the startle response. During the test session, animals were placed into individual restraining cages that were positioned on the top of each load cell. Stimulus (S2) intensity [dB(A) and dB(lin)], duration (msec), rise/fall time (msec), and spectral characteristics were established prior to initiating this study. In addition, S2 amplitude within the enclosure was established prior to testing animals on each test day. The test session consisted of 50 trials that began following a 5 minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10 second intervals. The response amplitude was recorded and the baseline was subtracted.

iv) Learning and memory testing: Learning and memory testing was performed on 1 pup/sex/litter/dose group (subset C). Passive avoidance (short-term retention and long-term retention) testing was performed on PNDs 24 and 31; water maze testing was performed on PND 60 (±2 days) and again seven days later. For both tests, only animals that demonstrated acquisition on the first day were tested for retention seven days later.

Passive avoidance test: After adaptation, individual animals were placed into the lighted compartment of a conditioning apparatus facing toward the light. After approximately 20 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off, signaling the end of the trial. At that time the animal was returned to the holding cage to await the next trial. If the rat failed to cross

within 180 seconds, it was returned to the holding cage and the latency assigned and arbitrary score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable. The test was repeated one week later. For the second trial, rats were placed in the illuminated side of the apparatus, given a 20 second acclimation period, and the latency to enter the dark side was recorded. Animals that either failed to reach criterion within 15 trials, or failed to cross during the first two trials during acquisition, were excluded from the retention phase of the experiment.

Water maze: The performing laboratory provided the following information. The water in the M-maze was maintained at 22 ± 1° C. The mazes used in this study were constructed of opaque Plexiglas, with corridors that were approximately five inches wide. The walls of the maze were approximately 16 inches high, with approximately 7.5 inches of water. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (±5) seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors during each trial. Animals that satisfied the above criteria within the 15 trial limit were tested for retention 7 days following acquisition. Animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment.

6) **Cholinesterase determination:** Brain, erythrocyte, and plasma cholinesterase activities were measured in the offspring on PNDs 4 and 21 using a modification of the Ellman method. On PND 4, samples were collected from culled male and female pups, representing as many litters as possible. On PND 21, samples were collected from pups in subset D (5-10 pups/sex/dose). Animals were not fasted prior to blood collection.

7) **Pharmacokinetic data:** Pharmacokinetics were not evaluated in this study.

2. Postmortem observations

a. Maternal animals: Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 24 (rats that did not deliver) or LD 21 (following weaning). Gross necropsies were not performed.

b. Offspring: F1 animals that were found dead or moribund were subjected to gross necropsy. The offspring selected for perfusion on PND 21 (subset D) and at study termination (subsets A-C), as well as those selected for fresh brain weight determinations (approximately 10/sex/group from

subsets A-C) were examined grossly.

Only the brain (with olfactory bulbs) was collected from the perfused animals on PND 21. Upon study termination, the brain and spinal cord, eyes (with optic nerves), selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected. All tissues were fixed in 10% buffered formalin. The brain from each animal was weighed, sectioned, and examined microscopically. Additionally, the following (CHECKED X) tissues, to be examined microscopically, were collected from perfused animals at study termination:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS
	BRAIN	X	**SCIATIC NERVE**
X	Forebrain		Mid-thigh
	Center of cerebrum		Sciatic Notch
	Midbrain		
X	Cerebellum		
X	Pons		
	Medulla oblongata		
	SPINAL CORD		**OTHER**
X	Cervical swelling	X	Sural Nerve
X	Lumbar swelling	X	Tibial Nerve
X	Thoracic swelling		Peroneal Nerve
		X	Lumbar dorsal root ganglion
		X	Lumbar dorsal root fibers
	OTHER	X	Lumbar ventral root fibers
X	Gasserian Ganglion	X	Cervical dorsal root ganglion
	Trigeminal nerves	X	Cervical dorsal root fibers
X	Optic nerve	X	Cervical ventral root fibers
	Eyes		
X	Cauda equina		
	Gastrocnemius muscle		

Only tissues from the control and 30 ppm groups were subjected to microscopic examination and morphometric analysis. The following brain sections were measured: 1) frontal cortex thickness; 2) parietal cortex thickness; 3) caudate putamen and underlying globus pallidus maximum cross-sectional width; 4) corpus callosum thickness; 5) hippocampal gyrus thickness; and 6) cerebellum height.

D. DATA ANALYSIS

1. Statistical analyses: In general, continuous data were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test). The level of significance was set at $p \leq 0.05$, with the exception of Bartlett's test which was set at $p \leq 0.001$.

The following data sets were analyzed by specific statistical procedures:

Parameter	Statistical test
Motor and locomotor total session activity data	ANOVA Dunnett's test
Interval motor and locomotor activity data	Repeated-Measures ANOVA (test interval and test occasion) ANOVA Dunnett's test
Acoustic startle response amplitude data, peak amplitude	ANOVA Dunnett's test
Acoustic startle response amplitude, block data	Repeated-Measures ANOVA (test block) Dunnett's test
Passive avoidance, latency data	Wilcoxon Test for time to failure
Passive avoidance, number of trials-to-criterion	Kruskal-Wallis and Wilcoxon tests for the acquisition phase Fisher's Exact Test for retention
Water maze, latency data	Univariate ANOVA Dunnett's test
Water maze, number of trials-to-criterion and number of errors	Kruskal-Wallis and Wilcoxon tests for the acquisition phase Fisher's Exact test for retention
Micropathology	Chi-Square One-tailed Fisher's Exact test

2. Indices

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index = # inseminated females/# females cohoused with males x 100

Fertility index = # pregnant females/# inseminated females x 100

Gestation index = # animals delivered/# pregnant females x 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:

Live birth index = # live pups born per litter/total # pups per litter x 100

Viability index = # live pups on day 4 pre-culling per litter/# live pups born per litter x 100

Lactation index = # live pups on day 21 per litter/# live pups on day 4 post-culling per litter x 100

3. Positive control data: It was stated that previous studies (MRID 42770301 and Bayer report #109803) with untreated animals and rats treated with substances that increase (triadimefon) or decrease (chlorpromazine) motor activity have established the sensitivity, reliability, and validity of the test procedures. Another study (MRID 45441302) has been performed to establish test norms for the appropriate ages under these conditions and the effects of perinatal exposure to a reference chemical (methimazole) on activity in animals tested at these ages. For the acoustic startle response amplitude, the investigator cited MRID 45441303[1] to establish the adequacy of this test procedure using 8OHDPAT and mCPP as reference substances that alter startle response amplitude. The investigator also cited the open literature (Bammer, 1982)[2] to establish the adequacy of the passive avoidance and water maze tests using scopolamine, which interferes with acquisition and/or retention. However, no concurrent data were provided.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical and abbreviated functional observations: No animals died during the study. Furthermore, no treatment-related clinical signs were noted in any dose group.

2. Body weight and food consumption: No treatment-related differences in body weights, body weight gains, or food consumption were noted during gestation or lactation (Table 2). Increased ($p \leq 0.01$) body weight gain was noted in the 1 ppm animals during GDs 0-20 (↑20%); however, this increase was not dose-related and was considered to be unassociated with treatment. Increased ($p \leq 0.01$) food consumption noted during LDs 7-14 in the 30 ppm dams (↑34%) was considered to be incidental.

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[1] Sheets, L.P. (2001). Historical control and method validation studies in rats for a developmental neurotoxicity screening battery. Auditory startle habituation and cognitive function (passive avoidance and water maze conditioning), Bayer Corp., Agriculture Division Report No. 109416, MRID 45441303.

[2] Bammer, G. (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: A review and some results. Neuroscience & Biobehavior Reviews 6: 247-296.

Table 2. Mean (±SE) maternal body weight and food consumption. [a]

Observations	Dose (ppm)			
	0	1	10	30
Gestation				
Mean body weight (g)				
Gestation day 0	214.5±2.65	210.9±1.94	206.8±2.87	207.7±2.60
Gestation day 6	233.3±2.63	233.4±2.64	225.1±2.76	224.0±2.89
Gestation day 13	255.6±3.01	257.9±2.83	248.9±3.04	248.2±3.38
Gestation day 20	307.5±6.24	322.7±3.45	308.1±4.76	299.5±5.98
Mean weight gain (g)				
Gestation days 0-20	93.0±5.87	111.9±2.05** (↑20)	101.3±4.10	91.8±4.89
Mean food consumption (g/animal/day)				
Gestation days 0-6	18.1±0.25	18.1±0.44	18.6±0.36	18.9±0.78
Gestation days 6-13	21.1±0.41	20.8±0.37	22.5±0.62	21.4±0.51
Gestation days 13-20	22.2±0.45	22.7±0.47	23.4±0.52	22.3±0.49
Lactation				
Mean body weight (g)				
Lactation day 0	247.0±3.44	247.1±3.00	239.8±3.27	236.2±3.33
Lactation day 4	258.2±3.49	255.8±3.22	252.1±3.18	247.4±4.35
Lactation day 7	266.8±3.23	270.1±3.01	260.1±3.00	257.6±3.94
Lactation day 14	288.5±4.46	294.8±3.32	284.1±3.62	280.5±4.34
Lactation day 21	291.7±3.26	289.5±3.37	285.3±3.72	279.7±3.68
Mean weight gain (g)				
Lactation days 0-21 [b]	44.7	42.4	45.5	43.5
Mean food consumption (g/animal/day)				
Lactation days 0-7	43.8±2.76	45.0±3.12	45.5±3.28	61.8±9.46
Lactation days 7-14	63.7±2.60	65.6±2.36	68.4±4.18	85.3±6.30** (↑34)
Lactation days 14-21	75.5±2.19	79.5±3.07	77.4±3.28	75.4±2.38

a Data obtained from Tables 3 through 4 and 6 through 7, pages 61 through 64 and 67 through 70 of the study report, MRID 45666401; n = 22-30.

b Calculated by the reviewers.

** Statistically different from control, $p \leq 0.01$.

3. **Test Substance Intake:** Based on maternal food consumption, body weight and dietary analyses, the doses expressed as mean daily mg test substance/kg body weight during the gestation and lactation periods are presented in Table 3. Accordingly, the average daily intake of methamidophos reported by the study investigator during gestation was 0, 0.1, 0.9 or 2.5 mg/kg/day and 0, 0.2, 2.4 or 7.9 mg/kg/day during lactation in animals receiving diets containing nominal concentrations of 0, 1, 10 or 30 ppm, respectively.

Table 3. Mean maternal test substance intake (mean mg/kg body weight/day ±SE). [a]

Period	Dose (ppm)		
	1	10	30
Gestation			
GD 0-6	0.1±0.00	0.9±0.01	2.5±0.10
GD 6-13	0.1±0.00	1.0±0.02	2.6±0.06
GD 13-20	0.1±0.00	0.9±0.01	2.4±0.05
Lactation			
LD 0-7	0.2±0.01	1.8±0.13	7.3±1.20
LD 7-14	0.2±0.01	2.6±0.15	9.1±0.69
LD 14-21	0.2±0.01	2.7±0.11	7.3±0.29

a Data obtained from Table 8, pages 71 through 73 of the study report, MRID 45666401.

4. Reproductive performance: Reproductive performance appeared to be unaffected by treatment with the test substance (Table 4).

Table 4. Reproductive performance. [a]

Observation	Dose (ppm)			
	0	1	10	30
Number mated	30	30	30	30
Number of litters	23	23	23	23
Intercurrent deaths	0	0	0	0
Mean (±SE) gestation duration (days)	21.6±0.11	21.5±0.11	21.5±0.11	21.6±0.12
Mating index (%)	100	100	100	100
Fertility index (%)	83.3	100	96.7	90.0
Gestation index (%)	92.0	76.7	79.3	85.2
Incidence of dystocia	NR	NR	NR	NR

a Data obtained from pages Table 1, page 58 in the study report, MRID 45666401.
NR Not reported

5. AFO: No treatment-related effects on AFO were noted.

6. Maternal postmortem results

Cholinesterase ChE) determination: In the 1 ppm dams, significant ($p \leq 0.05$) decrease in brain cholinesterase activity was noted (8%↓). In the 10- and 30- ppm dams, dose-dependent and significant ($p \leq 0.05$) decreases in plasma (↓50-77%), erythrocyte (↓64-84%), and brain (↓63-83%) ChE activities were noted (Table 5).

Table 5. Cholinesterase activity in dams on LD 21. [a]

Parameter	Dose (ppm)			
	0	1	10	30
Plasma (IU/mL)	0.56±0.08	0.53±0.09 (↓5)	0.28±0.08* (↓50)	0.13±0.05* (↓77)
Erythrocyte (IU/mL)	1.21±0.17	1.07±0.10 (↓12)	0.44±0.17* (↓64)	0.19±0.17* (↓84)
Brain (IU/g)	12.9±0.3	11.9±0.5* (↓8)	4.8±0.7* (↓63)	2.2±0.3* (↓83)

a Data obtained from Table CHE3-SUM, page 869 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=10.

* Significantly different from controls at p≤0.05.

B. OFFSPRING

1. Viability and clinical signs: Litter size and viability (survival) results from pups during lactation are summarized in Table 6. No treatment-related findings were noted. Similarly, no treatment-related clinical signs were noted during the pre-weaning or post-weaning periods.

Table 6. Litter size and viability. [a]

Observation	Dose (ppm)			
	Control	1	10	30
Total number born	262	272	273	270
Number born live [b]	261	271	272	270
Number born dead	1	1	1	0
Sex Ratio Day 0 (% M)[c]	55	69	56	56
Deaths Days 1-4 [cd]	0	2	4	1
Deaths Days 4-21 [ce]	0	0	0	0
Litter size (mean ± SE)	11.4±0.30	11.8±0.31	11.9±0.37	11.7±0.38
Mean number of viable pups				
Birth	11	12	12	12
Day 4 [d]	11	12	12	11
Day 4 [e]	8	8	8	8
Day 21	8	8	8	8
Live birth index (mean ± SE, %)	99.6±0.43	99.6±0.36	99.6±0.43	100±0.00
Viability index (mean ± SE, %)	99.6±0.43	99.2±0.56	99.1±0.67	97.1±1.55
Lactation index (mean ± SE, %)	99.5±0.54	98.9±0.75	100±0.00	96.2±3.28

a Data obtained from Table 9, pages 74 through 76; and Appendix X, pages 279 through 283 in the study report, MRID 45666401.

b Calculated by the reviewers from data presented in Table 9, pages 74 through 76.

c Calculated by the reviewers from individual data presented in Appendix X, pages 279 through 283. Does not include pups that were considered missing or cannibalized.

d Before standardization (culling).

e After standardization (culling).

2. Body weight: Pre-weaning body weights were decreased ($p \leq 0.01$) on PNDs 11, 17, and 21 in the 30 ppm male and female pups (↓8-12%; Table 7a). In addition, body weights were decreased ($p \leq 0.05$) on PND 4 (precull) in the 30 ppm female pups (↓8%). As shown in Table 7b, pre-weaning body weight gains for male and female pups were decreased ($p \leq 0.05$) for most measured intervals of the high dose group between PND 0 and PND 21 (↓8-19%). Pre-weaning body weights and body weight gains were comparable to controls in the 10 and 1 ppm groups.

Table 7a. Mean (±SE) pre-weaning pup body weights (g). [a]

PND	Dose (ppm)							
	0	1	10	30	0	1	10	30
	Males				Females			
0	5.9±0.08	5.9±0.08	5.9±0.11	5.8±0.08	5.6±0.07	5.6±0.08	5.5±0.09	5.5±0.07
4 [b]	9.0±0.17	9.0±0.18	8.9±0.20	8.5±0.19	8.7±0.16	8.7±0.18	8.6±0.20	8.0±0.18* (↓8)
4 [c]	9.0±0.18	9.0±0.20	8.9±0.20	8.5±0.19	8.7±0.16	8.6±0.19	8.6±0.20	8.1±0.18
11	21.7±0.30	22.1±0.44	21.2±0.33	19.2±0.50** (↓12)	21.2±0.38	21.3±0.39	20.5±0.32	18.6±0.49** (↓12)
17	33.1±0.50	34.3±0.54	32.8±0.36	30.4±0.57** (↓8)	32.3±0.46	32.9±0.54	31.8±0.39	29.5±0.55** (↓9)
21	43.3±0.76	44.1±0.81	42.9±0.60	39.2±0.81 (↓9)	42.2±0.68	42.3±0.64	41.2±0.62	37.9±0.77** (↓10)

a Data obtained from Table 12, pages 83 through 86 in the study report, MRID 45666401.. Percent difference from controls is presented parenthetically; n=23.
b Before standardization (culling).
c After standardization (culling).
* Statistically different from control, $p \leq 0.05$
** Statistically different from control, $p \leq 0.01$

Table 7b. Mean (±SE) pre-weaning pup body weight gains (g). [a]

PND	Dose (ppm)							
	0	1	10	30	0	1	10	30
	Males				Females			
0-4	3.2±0.13	3.2±0.14	3.0±0.16	2.6±0.16* (↓19)	3.2±0.13	3.1±0.12	3.0±0.15	2.6±0.15** (↓19)
4-11	12.6±0.21	13.1±0.29	12.3±0.20	10.7±0.35** (↓15)	12.5±0.27	12.6±0.24	11.9±0.21	10.5±0.35** (↓16)
4-17	24.1±0.44	25.3±0.40	23.9±0.33	22.0±0.45** (↓9)	23.6±0.39	24.2±0.40	23.2±0.37	21.4±0.44** (↓9)
4-21	34.3±0.66	35.1±0.66	34.0±0.51	30.7±0.68** (↓10)	33.5±0.59	33.6±0.49	32.6±0.50	29.8±0.67** (↓11)
11-17	11.4±0.28	12.2±0.16	11.5±0.23	11.2±0.21	11.1±0.21	11.6±0.19	11.4±0.23	11.0±0.23
11-21	21.6±0.52	22.0±0.42	21.7±0.38	20.0±0.42 (↓7)	21.0±0.38	21.0±0.30	20.8±0.36	19.3±0.44* (↓8)
17-21	10.2±0.40	9.8±0.35	10.1±0.35	8.8±0.33* (↓14)	9.9±0.35	9.4±0.23	9.4±0.34	8.3±0.31** (↓16)

a Data obtained from Table 13, pages 87 through 91 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=23

* Statistically different from control, p≤0.05 ** Statistically different from control, p≤0.01

Body weights generally showed a significant decrease (p≤0.05) throughout post-weaning in the 30 ppm males (↓7-10%, except for PND 15) and the 10 and 30 ppm females (↓4-10%; Table 8). Post-weaning body weights were comparable to controls in the 10 ppm males and in the 1 ppm males and females.

Table 8. Mean (±SD) post-weaning pup body weights (g). [a]

PND [M/F]	Dose (ppm)							
	0	1	10	30	0	1	10	30
	Males				Females			
8/9	75.3±5.1	76.9±7.4	73.0±10.7	67.8±7.0* (↓10)	74.9±6.8	73.3±7.4	71.4±8.5* (↓5)	67.9±7.0* (↓9)
29/30	211.0±15.4	211.1±19.8	208.7±17.4	196.3±14.9* (↓7)	157.4±12.3	155.0±13.5	151.8±12.3* (↓4)	147.8±10.1* (↓6)
50/51	311.5±23.9	305.6±27.7	305.8±28.7	288.4±21.7* (↓7)	196.9±16.7	193.7±17.3	189.3±16.7* (↓4)	188.4±14.2* (↓4)

a Data obtained from pages Table 15, pages 94 through 96 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically.

* Statistically different from control, p≤0.05

3. Developmental landmarks

a) Sexual maturation: Sexual maturation data are presented in Table 9. No treatment-related differences in the time to preputial separation or vaginal patency were noted. A slight increase ($p \leq 0.01$) in the time to preputial separation was noted in the 30 ppm males (↑5%).

Table 9. Mean (±SE) age of sexual maturation (days). [a]

Parameter	Dose (ppm)			
	0	1	10	30
N (M/F)	68/68	67/69	69/65	65/67
Preputial separation (males)	45.9±0.30	46.1±0.35	46.7±0.42	48.0±0.44** (↑5)
Vaginal opening (females)	38.9±0.71	38.7±0.63	40.4±0.79	39.9±0.71

a Data obtained from Table 14, pages 92 and 93 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically.

** Statistically different from control, $p \leq 0.01$

b) Physical landmarks: On PND 21, all litters were examined for the presence of pupil constriction which was found to be present in 100% of all treated and control pups.

4. Behavioral assessments

a) Abbreviated functional observations: No treatment-related effects were noted

b) Motor activity: Motor activity data are presented in Table 10. As shown, non-statistically significant differences in motor activity means were observed at 10 and 30 ppm on PND 13 (↓25 - 45% in males and 33-27% in females). Non-significant decreases were also observed at PND 17 for the male pups treated with 10 and 30 ppm (15% and 22% ↓, respectively); values were slightly higher than control for all male treatment groups at PNDs 21 and 60 . For the females, PNDs 17 and 60 values were higher than control but ≈10 % less than control for all treatment groups at PND 21.

In agreement with the motor activity data, locomotor activity results (Table 11) also showed decreased activity for male and female PND 13 pups at 10 and 30 ppm. Values for the males ranged from a 36% decrease at 10 ppm to a 50% decrease at 30 ppm; a17 % decrease was also observed at 30 ppm on PND 17. For the females decreases were 36 and 50% at 10 and 30 ppm, respectively, for PND 13. Responses for the females were generally comparable or slightly higher than control for the remaining PND intervals. The lack of statistical significance was likely due to the extreme variation in the data with standard deviations frequently higher than the mean. Nevertheless, the study investigator considered the effects on motor and locomotor activity at 10 and 30 ppm to be treatment related. The study author also claimed that these findings are "consistent with their relatively undeveloped ambulatory skills and sensory function (e.g., eyelids)". However, this assertion does not account for the marked differences between the control and high- and mid-dose animals at this young age.

Table 10. Mean (±SD) motor activity data for F1 pups in Subset A. [a]

Test Day	Dose (ppm)			
	0	1	10	30
Males				
PND 13	113±84	146±164	85±86 (↓25)	62±49 (↓45)
PND 17	289±235	223±183 (↓23)	246±149 (↓15)	225±164 (↓22)
PND 21	378±224	416±170	388±166	405±166
PND 60	570±170	632±127	547±209	602±211
Females				
PND 13	101±106	93±99	68±81 (↓33)	74±40 (↓27)
PND 17	216±111	241±207	279±187	234±165
PND 21	425±137	373±160 (↓12)	384±142 (↓10)	381±130 (↓10)
PND 60	770±242	802±268	836±324	840±204

a Data obtained from Table 19, pages 182 through 184 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=15-16. Motor activity was measured as the number of beam interruptions that occurred during the test session.

Table 11. Mean (±SD) locomotor activity data for F1 pups in Subset A. [a]

Test Day	Dose (ppm)			
	0	1	10	30
Males				
PND 13	14±19	17±43	9±15 (↓36)	7±13 (↓50)
PND 17	58±39	47±44 (↓19)	57±41 (↓2)	48±39 (↓17)
PND 21	87±44	90±38	99±43	101±54
PND 60	352±113	403±88	341±123	371±148
Females				
PND 13	14±19	13±24	9±19 (↓36)	7±8 (↓50)
PND 17	53±37	53±46	62±36	58±48
PND 21	111±30	87±38	102±42	111±51
PND 60	419±114	418±179	429±178	423±130

a Data obtained from Table 20, pages 186-187 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=16. Locomotor activity was measured by eliminating consecutive counts for a given beam.

Habituation data for locomotor activity are summarized in Table 12 and indicate that the habituation was apparent for all ages except PND 13. As shown, activity for both sexes at this age was very low

from interval 1 (measuring 2 for all treatment groups versus 4 for the control group males) to interval 6 (measuring 1-2 for all treatment groups vs. 2 for the control group). Similarly, females showed low activity with no improvement over the 60-minute session.

Table 12. Summary of Interval Locomotor Activity Data – Habituation (mean ±S.D.). [a]

Interval		Dose (ppm)			
		0	1	10	30
		Males			
PND 13	1	4±6	2±2	2±4	2±3
	2	3±5	4±10	1±2	0±1
	3	3±5	1±1	3±10	0±1
	4	1±2	3±6	1±1	1±2
	5	3±6	6±24	0±1	3±9
	6	2±3	2±4	1±3	1±1
PND 17	1	20±13	19±14	16±7	14±10
	2	8±7	8±9	8±6	10±8
	3	10±8	5±9	7±10	7±8
	4	7±8	5±9	8±10	5±9
	5	8±11	5±8	11±12	5±9
	6	5±9	5±11	9±10	7±10
PND 21	1	39±12	41±12	42±12	41±13
	2	16±8	19±9	19±11	18±11
	3	14±11	12±10	13±11	13±11
	4	7±9	9±9	11±9	11±11
	5	6±9	5±7	7±8	10±11
	6	5±8	4±6	7±9	9±11
PND 60	1	80±33	83±20	75±17	77±31
	2	65±21	72±30	53±18	61±38
	3	63±25	72±32	54±26	61±32
	4	54±33	67±26	61±32	62±30
	5	46±22	65±19	50±31	58±22
	6	45±24	43±22	49±24	51±27

Females					
PND 13	1	3±5	3±3	1±2	2±2
	2	3±4	3±8	1±2	1±2
	3	1±2	2±5	2±5	1±3
	4	2±6	2±3	1±2	1±2
	5	2±4	3±7	3±8	2±3
	6	3±8	1±2	1±4	1±39
PND 17	1	17±11	17±13	18±11	15±12
	2	7±8	8±7	9±5	8±8
	3	9±13	6±8	9±7	5±6
	4	6±9	6±9	8±9	11±12
	5	8±13	6±10	7±10	8±11
	6	6±10	10±14	11±13	11±17
PND 21	1	46±9	40±14	45±13	41±11
	2	19±8	20±7	18±7	18±10
	3	19±9	11±8	14±9	17±8
	4	13±8	9±8	10±10	12±10
	5	8±6	3±6	9±10	12±10
	6	6±7	5±9	6±10	12±12
PND 60	1	97±14	90±26	94±23	85±23
	2	70±22	64±32	71±30	69±35
	3	70±33	64±40	73±42	72±35
	4	65±28	67±46	70±36	66±29
	5	62±27	69±36	68±45	53±25
	6	55±26	65±42	53±26	62±27

a Data obtained from Tables 22, pages 198 through 205 of the study report, MRID 45666401. Habituation was evaluated as a decrease in activity over six consecutive intervals (10 sec each) of the test session.

c) **Auditory startle reflex habituation:** There were no adverse effects on the males of any treatment group at any PND. However, consistent but not dose-dependent decreases were seen in females at all dose levels on PND 22 and PND 38 (Table 13). At 1 ppm, although not statistically significant, decreases in mean peak amplitude were seen on Day 22 (15%) and on Day 38 (26%). At

10 ppm, the decreases were significant on PND 38 (47% ↓, block 1 and 42%↓, mean). However, using the 28% decrease as a "modest" indication of a treatment-related response, it was noted that amplitude data for the 30- ppm PND 38 females ranged from a 27%↓ to a significant 41%↓ in four of five trials. PND 38 peak amplitude values for the females in the 10-ppm group ranged from a nonsignificant 37% to a significant 47% decrease in all blocks with a significant mean peak amplitude of 42%. A 25 to 32 % decrease over 4 of the 5 trials, with a mean peak amplitude of 26 percent decrease was also recorded for PND 38 low-dose females. Decreased ($p \leq 0.05$) mean peak amplitude was noted in the 30 ppm females on PND 22 (↓28%) Amplitude at this time point was decreased during all blocks (↓20-26%), and was significantly decreased ($p \leq 0.05$) during blocks 1 and 2 (↓33-36%). In addition, decreases were seen for the PND 60 females of the mid- and high-dose group (23-31% ↓–all trials and 33-44% ↓–all trials, respectively). Peak startle response amplitude in all females at PND 22 and 38 reached statistical significance in one block at the low dose, two blocks at the mid dose and three blocks at the high dose. Additionally, the magnitude of the response was similar at the low and mid dose groups for PND 22 and PND 38 in females. Therefore, the low dose was determined to be an effect level.

Table 13. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.) for Rats in Subset B. [a]

Block		Dose (ppm)			
		0	1	10	30
		Males			
PND 22	1	43±16	46±13	44±13	37±18
	2	36±16	40±17	38±15	36±19
	3	31±12	42±18	39±19	35±19
	4	30±14	35±19	39±17	31±17
	5	28±12	35±14	31±11	26±11
	Mean	34±13	40±14	38±12	33±16
PND 38	1	110±77	112±56	124±52	113±56
	2	100±69	121±46	114±66	100±67
	3	78±45	105±54	108±72	76±47
	4	69±53	85±48	79±52	67±35
	5	60±36	74±40	77±45	59±27
	Mean	83±51	99±45	100±51	83±43
PND 60	1	303±205	311±128	329±244	247±134

Block		Dose (ppm)			
		0	1	10	30
	2	254±196	309±192	308±246	230±176
	3	213±168	246±162	249±223	192±141
	4	177±138	192±118	242±201	171±141
	5	146±88	196±111	194±159	161±117
	Mean	218±152	251±135	264±203	200±129
Females					
PND 22	1	58±16	44±14* (↓24)	47±14 (↓19)	37±15* (↓36)
	2	52±15	44±13 (↓15)	42±19 (↓19)	35±17* (↓33)
	3	46±16	40±12 (↓13)	40±18 (↓13)	36±23 (↓22)
	4	40±16	38±13 (↓5)	37±19 (↓8)	32±20 (↓20)
	5	39±15	36±11 (↓8)	36±19 (↓8)	29±18 (↓26)
	Mean	47±13	40±11 (↓15)	40±16 (↓15)	34±18* (↓28)
PND 38	1	107±52	73±46 (↓32)	57±25* (↓47)	63±53* (↓41)
	2	89±61	67±39 (↓25)	54±29 (↓39)	59±56 (↓34)
	3	67±29	48±28 (↓28)	42±18 (↓37)	49±38 (↓27)
	4	64±31	48±39 (↓25)	37±18 (↓42)	41±36 (↓36)
	5	52±27	45±26 (↓14)	28±12 (↓46)	45±35 (↓13)
	Mean	76±33	56±33 (↓26)	44±15* (↓42)	51±39 (↓33)
PND 60	1	154±80	146±125	118±60 (↓23)	102±75 (↓34)
	2	154±106	135±92	107±65 (↓31)	87±47 (↓44)
	3	114±63	132±89	92±71 (↓19)	77±52 (↓33)
	4	92±51	100±62	78±83 (↓15)	62±36 (↓33)
	5	98±44	96±78	68±39 (↓31)	66±53 (↓33)
	Mean	123±62	122±81	93±58 (↓24)	79±47 (↓36)

a Data obtained from Tables 23 and 24, pages 206 through 215 of the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n = 15-16.

* Significantly different from controls at $p \leq 0.05$.

d) Learning and memory testing:

- **Passive avoidance performance**

Passive avoidance data are presented in Table 14. Although not statistically significant, marked increases (76, 79 or 97%↑) in the latency to cross were observed at 1, 10 or 30 ppm, respectively in

the PND 22 first session, Trial 1 for the treated males. No increases in the time to cross were recorded for the males during the second trial of Session 1 or during the memory trials of Session 2 (Table 13). By contrast, females showed a nonsignificant 41 to 23% ↓ in the latency to cross for Trial 1 of the PND 20 first session and a 25-18% ↓ in latency for PND 67 Trial 1 of the memory segment at the low to the high doses, respectively. The response in females would not be considered adverse. No other treatment-related effects were seen in the passive avoidance test.

Table 14. Passive avoidance performance (mean ± S.D.)In F1 Rats in Subset C. [a]

Session/Parameter		Dose (ppm)			
		0	1	10	30
Males					
Session 2 Retention PND 20 ± 2	Trials to criterion	3.6±0.8	3.8±1.2	3.6±1.0	4.0±1.3
	Latency trial 1 (sec)	18.6±12.8	32.9±23.6 (↑76)	33.3±42.9 (↑79)	36.7±40.7 (↑97)
	Latency trial 2 (sec)	150.2±38.1	155.7±52.2	150.4±52.3	136.8±61.0
	Failed to learn	0	0	0	0
Session 2 Retention PND 65 ± 2	Trials to criterion	2.1±0.3	2.1±0.4	2.8±1.0	2.8±0.9
	Latency trial 1 (sec)	169.8±34.2	164.6±44.5	160.2±37.3 (↓6)	152.1±42.4 (↓10)
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	165.4±28.1* (↓8)	172.1±30.7
Females					
Session 1 Learning PND 29 ± 2	Trials to criterion	3.6±1.0	3.8±1.5	3.7±0.8	3.6±1.0
	Latency trial 1 (sec)	31.9±43.3	18.7±9.3 (↓41)	22.3±17.9 (↓30)	24.7±15.2 (↓23)
	Latency trial 2 (sec)	152.9±42.4	141.6±58.7	145.3±51.7	166.6±29.4
	Failed to learn	0	0	0	0
Session 2 Retention PND 67 ± 2	Trials to criterion	2.2±0.6	2.8±1.4	2.5±0.8	2.6±0.7
	Latency trial 1 (sec)	179.4±2.4	134.9±61.9 (↓25)	146.7±54.3 (↓18)	147.5±57.1 (↓18)
	Latency trial 2 (sec)	179.3±2.6	171.5±34.0	180.0±0.0	172.2±25.1

a Data extracted from Table 25, pages 216 through 218 of the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=15-16.

* Significantly different from controls at p≤0.05.

- **Water maze testing**

Data from the water maze performance are summarized in Table 15 and indicate that males of the high-dose group showed nonsignificant increases in the number of trials to criterion (15%↑), errors (330%↑) and duration of time to make a correct choice (47%↑) in Trial 1, Session 1 and increases in

the number of errors (200%↑) and duration (50%↑) in Trial 2. These findings were accompanied by more errors (200 and 267% ↑) and longer time to make the correct choice (51 and 59%↑) for the mid-and low-dose groups, respectively. It was also noted that single males from the mid- and high-dose groups failed to achieve the correct goal, and were excluded from the retention phase of testing. PND 67 retention data for the 30-ppm males demonstrated increases in errors (200%↑) and duration (17%↑) (Trial 1); no consistent pattern was seen for the mid- or low-dose males. Trial 2 retention data showed a marked improvement as indicated by a reduction in errors (40%↓) and less time to achieve the correct goal (17 %↑ compared to the Trial 1 data). It is noteworthy that the greater number of errors and more time to succeed in all male treatment groups of Trial 1 of the water maze test paralleled the marked increases in latency observed in the males of the passive avoidance test at all doses during the learning phase.

By contrast, females of the mid- and high-dose group demonstrated a significant ($p \leq 0.05$) decrease in errors (62%↓at 10 ppm and 54%↓ at 30 ppm), accompanied by nonsignificant but 50% decreases in duration in Trial 1 of Session 1 and 40 to 20% fewer errors and 35 to 38% decreases in time to make the correct choice for Trial 2, respectively. For the retention phase, there were nonsignificantly fewer errors and less time (63 and 37%, respectively) for the 30-ppm females in Trial 1; values for the second trial were comparable to the control. With the exception of the significant ($p \leq 0.05$) decreases in errors and time during Session 1, Trial 1 for the mid- and high-dose females, no statistical significance was ascribed to any of the findings discussed above for either the males or the females.

Table 15. Water maze performance (mean ± S.D.) In F1 Rats in Subset C. [a]

Session/Parameter		Dose (ppm)			
		0	1	10	30
Males					
Session 1 Learning PND 20 ± 2	Trials to criterion	7.6±2.8	8.1±2.8	7.2±3.3	8.7±2.8 (15%↑)
	Trial 1 - Errors	0.3±0.6	0.8±0.7 (267%↑)	0.6±1.1 (200%↑)	1.0±1.0 (330%↑)
	Trial 1 - Duration (sec)	12.9±6.0	20.5±15.3 (59%↑)	19.5±17.4 (51%↑)	18.9±12.4 (47%↑)
	Trial 2 - Errors	0.4±0.9	1.2±1.3 (300%↑)	0.3±0.6	0.8±1.2 (200%↑)
	Trial 2 - Duration (sec)	11.9±11.2	23.6±21.2 (98%↑)	9.9±6.9	17.8±14.6 (50%↑)
	Failed to meet criterion	0	0	1	1
Session 2	Trials to criterion	5.6±1.4	5.2±0.4	5.5±1.3	6.1±1.7 (9%↑)

Session/Parameter		Dose (ppm)			
		0	1	10	30
	Trial 1 - Errors	0.1±0.3	0.4±0.8 (400%↑)	0.1±0.4	0.2±0.4 (200%↑)
	Trial 1 - Duration (sec)	6.3±4.7	9.3±8.5 (48%↑)	7.0±5.2	7.4±6.5 (17%↑)
	Trial 2 - Errors	0.5±1.3	0.0±0.0	0.0±0.0	0.2±0.4 (40%↓)
	Trial 2 - Duration (sec)	6.3±8.2	3.5±1.5	3.3±1.0	5.6±4.1 (11%↑)
Females					
Session 1	Trials to criterion	7.9±3.0	7.9±2.4	8.1±3.3	7.6±2.2
Learning PND 29 ± 2	Trial 1 - Errors	1.3±1.0	1.0±1.0	0.5±0.5* (↓62)	0.6±0.9* (↓54)
	Trial 1 - Duration (sec)	26.7±17.3	21.9±15.9 (↓18)	13.3±7.8 (↓50)	13.4±10.1 (↓50)
	Trial 2 - Errors	1.0±1.0	0.8±0.9 (↓20)	0.6±0.7 (↓40)	0.8±1.2 (↓20)
	Trial 2 - Duration (sec)	18.1±13.1	13.6±8.4 (↓25)	11.8±6.8 (↓35)	11.2±8.4 (↓38)
	Failed to meet criterion	0	0	0	0
Session 2	Trials to criterion	5.5±0.5	6.8±3.3	5.7±1.3	5.4±1.0
Retention PND 67 ± 2	Trial 1 - Errors	0.8±1.0	0.5±0.9	0.7±1.3	0.3±0.6 (↓63)
	Trial 1 - Duration (sec)	12.4±7.3	9.3±9.0	10.8±8.9 (↓13)	7.8±7.3 (↓37)
	Trial 2 - Errors	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.8
	Trial 2 - Duration (sec)	3.7±1.4	3.8±1.4	3.8±2.5	4.0±4.1

a Data obtained from Table 26, pages 219 through 221 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=15-16.

* Significantly different from controls at p≤0.05.

5. Postmortem results

a) Brain weights: No treatment-related differences in absolute or relative (to body weight) brain weights were noted on PND 21 or at study termination (Table 16). A slight decrease (p≤0.05) in absolute brain weight was noted in the 30 ppm females at termination (↓4%). This finding was considered to be minor and not to be toxicologically important.

Table 16. Mean (±SD) brain weights of perfused F1 animals. [a]

Parameter	Dose (ppm)			
	0	1	10	30
Males				
PND 21				
Terminal body weight (g)	41.5±3.7	43.1±3.9	42.6±3.7	39.6±4.1
Brain weight (g)	1.389±0.065	1.360±0.079	1.396±0.068	1.347±0.074
Brain-to-body weight ratio (%)	3.374±0.281	3.169±0.261	3.297±0.312	3.477±0.352
Termination				
Terminal body weight (g)	306.3±21.3	306.8±18.0	298.8±31.1	288.5±20.0
Brain weight (g)	1.802±0.069	1.830±0.075	1.845±0.066	1.780±0.038
Brain-to-body weight ratio (%)	0.590±0.033	0.599±0.046	0.623±0.063	0.620±0.046
Females				
PND 21				
Terminal body weight (g)	40.2±3.3	41.4±3.9	40.3±4.2	36.8±3.9
Brain weight (g)	1.322±0.080	1.338±0.043	1.347±0.035	1.284±0.077
Brain-to-body weight ratio (%)	3.305±0.297	3.255±0.295	3.366±0.305	3.510±0.273
Termination				
Terminal body weight (g)	207.6±25.8	186.1±16.2	195.6±14.7	191.9±14.4
Brain weight (g)	1.710±0.044	1.684±0.047	1.695±0.033	1.646±0.042* (↓4)
Brain-to-body weight ratio (%)	0.834±0.094	0.910±0.066	0.871±0.058	0.863±0.080

a Data obtained from Tables OW1K-SUM and OW2K-SUM, pages 898 through 903 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n =10.

b) Neuropathology

1) Cholinesterase determination: At 30 ppm, plasma (↓12-40%), erythrocyte (↓37-53%), and brain (↓14-43%) ChE activities were decreased ($p \leq 0.05$) relative to controls on PND 4 and PND 21 for the males and females (Table 17). At 10 ppm, decreases ($p \leq 0.05$) were noted in erythrocyte ChE on PND 4 (↓20%, sexes combined); plasma and brain ChE on PND 21 in the males (↓22 and 13%, respectively); and a nonsignificant decrease in brain ChE on PND 21 in the females (↓17%). ChE activity was comparable to controls for all compartments in the 1-ppm group.

Table 17. Cholinesterase activity in F1 Pups[a]

Parameter	Dose (ppm)			
	0	1	10	30
PND 4 (culled pups; sexes combined)				
Plasma (IU/mL)	0.58±0.07	0.60±0.05	0.55±0.06 (↓5)	0.51±0.06* (↓12)
Erythrocyte (IU/mL)	1.32±0.28	1.28±0.29	1.06±0.26* (↓20)	0.78±0.27* (↓41)
Brain (IU/g)	4.4±0.7	4.4±0.6	4.2±0.4 (↓5)	3.8±0.5* (↓14)
PND 21 (Subset C)				
Males				
Plasma (IU/mL)	0.55±0.05	0.56±0.05	0.43±0.08* (↓22)	0.36±0.11* (↓35)
Erythrocyte (IU/mL)	1.39±0.27	1.47±0.29	1.23±0.24 (↓12)	0.87±0.42* (↓37)
Brain (IU/g)	11.2±0.5	10.9±0.3	9.8±0.8* (↓13)	7.4±1.4* (↓34)
Females				
Plasma (IU/mL)	0.52±0.07	0.57±0.06	0.48±0.10 (↓8)	0.31±0.11* (↓40)
Erythrocyte (IU/mL)	1.39±0.40	1.34±0.25	1.17±0.21 (↓16)	0.66±0.35* (↓53)
Brain (IU/g)	11.6±0.3	11.4±0.3	9.6±0.5 (↓17)	6.6±1.2* (↓43)

a Data obtained from Tables CHE1-SUM and CHE2-SUM, pages 863 through 867 in the study report, MRID 45666401. Percent difference from controls is presented parenthetically; n=18-21 for PND 4; n=8-10 for PND 21.

2) Macroscopic examination: No treatment-related findings were noted.

3) Microscopic examination: No treatment-related findings were noted.

4) Morphometric evaluation: All morphometric measurements were similar between treated animals and controls (Table 18).

Table 18. Morphometric measurements.[a]

Parameter (μm)	Dose (ppm)			
	0	1	10	30
Males				
PND 21				
Frontal cortex	1.8643±0.09	NA	NA	1.9189±0.25
Caudate putamen	2.9641±0.17	NA	NA	3.0269±0.18
Parietal cortex	1.8277±0.12	NA	NA	1.8386±0.05
Corpus callosum	0.3747±0.09	NA	NA	0.3835±0.23
Hippocampal gyrus	1.5142±0.09	NA	NA	1.5413±0.13
Cerebellum	4.1947±0.44	NA	NA	4.2962±0.26

Parameter (µm)	Dose (ppm)			
	0	1	10	30
Termination				
Frontal cortex	1.9113±0.13	NA	NA	1.8659±0.06
Caudate putamen	3.5103±0.11	NA	NA	3.4986±0.20
Parietal cortex	1.9336±0.11	NA	NA	1.9003±0.07
Corpus callosum	0.4392±0.06	NA	NA	0.4431±0.05
Hippocampal gyrus	1.7388±0.09	NA	NA	1.7753±0.14
Cerebellum	4.6433±0.52	NA	NA	4.3887±0.53
Females				
PND 21				
Frontal cortex	1.9380±0.07	NA	NA	1.8749±0.09
Caudate putamen	3.0407±0.09	NA	NA	2.9953±0.19
Parietal cortex	1.8218±0.06	NA	NA	1.8158±0.10
Corpus callosum	0.3464±0.02	NA	NA	0.3054±0.06
Hippocampal gyrus	1.4226±0.10	NA	NA	1.4362±0.13
Cerebellum	3.9951±0.34	NA	NA	4.1326±0.41
Termination				
Frontal cortex	1.8418±0.05	NA	NA	1.7944±0.07
Caudate putamen	3.4885±0.11	NA	NA	3.4471±0.13
Parietal cortex	1.8837±0.06	NA	NA	1.8175±0.09
Corpus callosum	0.4744±0.05	NA	NA	0.4391±0.07
Hippocampal gyrus	1.5610±0.13	NA	NA	1.5881±0.11
Cerebellum	4.2355±0.43	NA	NA	4.0438±0.36

a Data obtained from Tables BM1-SUM and BM2-SUM, pages 907 through 916 in the study report MRID 45666401; n=9-10.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Administration of methamidophos in the diet resulted in decreased maternal and offspring plasma, erythrocyte, and brain cholinesterase activities at 10 and 30 ppm. No systemic toxicity was observed in the maternal animals. Systemic toxicity in the 10 ppm offspring was evidenced by decreased post-weaning body weight and decreased motor activity on PND 13. Systemic toxicity in the 30 ppm offspring was evidenced by decreased pre-weaning and post-weaning body weights, decreased motor activity on PND 13, decreased startle amplitude on PND 22 (females), and a delay in the preputial separation. The maternal and offspring NOAEL was 1 ppm.

B. REVIEWER COMMENTS

Maternal mortality, clinical signs, body weights, body weight gains, food consumption, reproductive performance, and abbreviated functional observations (AFO) were unaffected by treatment.

However, **in the 1 ppm dams, significant (p≤0.05) decrease in brain cholinesterase activity was noted (8%↓).** In the 10- and 30- ppm dams, dose-dependent and significant (p≤0.05) decreases in plasma, erythrocyte, and brain cholinesterase activities were noted (50-84%↓).

The maternal LOAEL was 1 ppm (0.1 mg/kg/day) based on brain cholinesterase inhibition in the dams. The maternal NOAEL was not established.

Treatment had no adverse effects on offspring survival, food consumption, abbreviated FOB, brain weights, brain morphology, or neuropathology. No treatment-related effects were seen in the body weight or body weight gain of pups at the low and mid dose groups. At the high dose (30 ppm), pre-weaning body weights were decreased (↓8-12%; p≤0.01) on PNDs 11, 17, and 21 in the male and female pups. In addition, body weights were decreased (p≤0.05) on PND 4 (precull) in the female pups (↓8%). Pre-weaning body weight gains for male and female pups were decreased (p≤0.05) for most measured intervals between PND 0 and PND 21 (↓8-19%). Furthermore, body weights were decreased (p≤0.05) throughout most of post-weaning in both sexes (↓4-10%). In the 10 ppm pups, post-weaning body weight in the females was decreased (p<=0.05, ↓ 4-5%). This decrease is minor and its toxicological importance is considered to be equivocal. A slight but significant (p≤0.01) difference in the time to preputial separation was observed in the high-dose males. It occurred an average of 2.1 days later in the affected males than in the controls.

Treatment-related effects were observed on motor and locomotor activity in both sexes at the mid and high dose on PND 13. The non-statistically significant decreases were 25% in males and 33% in females at the mid dose and 45% in males and 27% in females at the high dose.

A consistent, but not dose-dependent decrease in auditory startle reflex was seen in females at all dose levels on PND 22 and PND 38. On Day 22 the decreases were 15%, 15% and 28% at the low, mid and high dose groups, respectively. On Day 38, the decreases were 26%, 42% and 33%, at the low, mid and high dose groups, respectively. Peak startle response amplitude in all females at PND 22 and 38 reached statistical significance in one block at the low dose, two blocks at the mid dose and three blocks at the high dose. Additionally, the magnitude of the response was similar at the low and mid dose groups for PND 22 and PND 38 in females. Therefore, the low dose was determined to be an effect level.

In the passive avoidance test, nonsignificant but marked increases (76, 79 or 97%↑) in the latency to cross were seen at 1, 10 or 30 ppm, respectively. No other adverse effects were seen in the males and females of all dose groups.

Data from the water maze test showed that high-dose males had nonsignificant increases in the number of trials to criterion (15%↑), errors (330%↑), and time to make the correct choice (46%↑) in the first trial of the learning phase of testing. During the memory phase of the water maze testing, high-dose males demonstrated more errors (200%↑) and longer time to achieve the goal (17%↑). More errors (200 and 267%↑) and longer time to achieve the goal (51 and 59%↑) were also observed for the 1- and 10-ppm males during the learning phase. By contrast, there were no adverse effects in the females; females of all dose groups showed fewer errors and less time to make the correct choice

compared to the concurrent controls. At 1 ppm, cholinesterase (ChE) activity was comparable to controls for all compartments. At 30 ppm, plasma (↓12-40%), erythrocyte (↓37-53%), and brain (↓14-43%) ChE activities were decreased ($p \leq 0.05$) relative to controls on PND 4 and PND 21 for the males and females. At 10 ppm, decreases ($p \leq 0.05$) were noted in erythrocyte ChE on PND 4 (↓20%, sexes combined); plasma and brain ChE on PND 21 in the males (↓22 and 13%, respectively); and a nonsignificant decrease in brain ChE on PND 21 in the females (↓17%).

The offspring LOAEL is 1 ppm (0.1 mg/kg/day), the lowest dose tested based on consistent decreases in peak auditory startle reflex response in females on PND 22 and PND 38. An offspring NOAEL was not established.

This study is classified **Acceptable/Non guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 (draft). This study is classified as non-guideline due to the deficiencies listed below.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted:

- Inadequate assessment of learning and memory in the offspring.
- Physical landmarks (incisor eruption, eye opening) were not evaluated

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Chemical Name: Methamidophos

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HED File Code: 13000 Tox Reviews
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