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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

October 21, 2005

MEMORANDUM

Subject: EPA Id No.: 058001. Azinphos methyl: Review of the acute (2003, MRID No.: 46162101), repeat dosing (2004, MRID No.: 46239001) and maternal gestational exposure (2004, MRID No.: 46291101) comparative cholinesterase inhibition studies.

TXR # 0052325
DP Barcode No.: D298150
PC Code: 058001

From: John Doherty 10/21/05
ReRegistration Branch III
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To: Diane Isbell
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Background and Conclusion

ReRegistration Branch III (RRBIII) has reviewed three non-guideline studies attempting to determine the relative sensitivity of adult and neonatal rats to the inhibitory effects of azinphos methyl on plasma cholinesterase (ChE) and red blood cell (RBC) and brain acetylcholinesterase (AChE). In summary, it was demonstrated by statistical analysis that both the acute and repeat dose studies where adults were compared with 11 day old pups following a single dose (2003, MRID No.: 46162101) or a repeat dose (2004, MRID No.: 46239001) that the pups are approximately 3-4 times more sensitive than the adults. When dams were dosed during gestation only and their pups delivered by sectioning, a dose of 1.2 mg/kg/day resulted in 27% inhibition of the dams RBC AChE but no effects on the RBC AChE in the fetuses.

The three studies were reviewed in one DER and a copy of this DER is attached. These non-guideline studies were classified as Acceptable/Non-Guideline. They are further identified in the accompanying table. The following comments are also being provided.

Comments:

1. Statistical analysis by to determine the benchmark dose for inhibition of ChE by HED staff.

The plasma cholinesterase and RBC and brain acetylcholinesterase data from both the acute and repeat dose studies were reassessed statistically by Mr. Philip Villanueva of HED's Chemistry and Exposure Branch. Mr. Villanueva's report dated May 26, 2005 is attached.

2. Impact of the Comparative ChE studies on risk assessments for azinphos methyl.

The conclusion that the acute and repeat dose comparative ChE studies demonstrates a 3-4 fold increase in sensitivity of the pups relative to the adults indicates that the FQPA additional safety factor needs to be retained for risk assessments for azinphos methyl for dietary and residential uses. Since the difference in the extent of inhibition between the pups and adults is considered minor but still present, RRBIII recommends that the FQPA safety factor be reduced to 3 X rather than retaining the full 10 X.

Summary Table.

Studies	Executive Summary
Non-Guideline. Single dose comparative ChE inhibition study. Bayer Crop Sciences. Report No.: 200737. December 19, 2003. MRID No.: 46162101. Non-Guideline. Repeat dose comparative ChE inhibition study. Bayer Crop Sciences. Report No.: 200989. March 29, 2004. MRID No.: 46239001. Non-Guideline. Maternal and fetal ChE assessment following gestation exposure. Bayer Crop Science. Report No.: 201018. May 14, 2004. MRID No.: 46291101.	In a series of special studies, Azinphos-methyl (90.2 - 92.4% a.i., batch no. 2-03-0278, in water with methylcellulose and Tween 80) was administered by gavage to Wistar rats to determine the sensitivity of plasma, RBC and brain cholinesterase to neonatal rats relative to adults to inhibition (ChEI) by azinphos methyl (AZM). In the time-course evaluation study (2003, MRID 46162101), for adults, single doses of 6 mg/kg for male or 3 mg/kg for female and for post natal day 11 (PND 11) pups doses of 2 mg/kg of AZM were given to10 adult or pups/sex. In the acute study (2003, MRID # 46162101), doses of 0, 0.6, 1.1 or 2 mg/kg of AZM were given to groups of six young adult rats/sex (males were also given 3.3 mg/kg), and doses of 0.0, 0.26, 0.49 or 1 mg/kg were given to groups of 10 PND 11 pups/sex. In a 11-day repeat dosing study (2004, MRID # 46239001), groups of six adult rats/sex were given daily nominal doses of 0, 0.25, 0.54, 1 or1.6 mg/kg/day of AZM on 11 consecutive days and groups of 12 PND 11 pups/sex were given a daily nominal dose of 0, 0.24, 0.51, or 1 mg/kg/day. In a third study (2004, MRID # 46291101), groups of 12 pregnant dams were fed diets containing 0, 3, 10, or 15 ppm from gestation (GD) 0-20. Average test material intake was 0, 0.2, 0.9, and 1.2 mg/kg/day of AZM, respectively. *Systemic effects.* No deaths, body weight changes or clinical signs were reported in any of the studies. *ChEI-time course.* In adult males, peak ChEI (52-61%) from all sources was seen at 1.5 hours or at the first assessment time and some recovery was apparent after 3 hours. In females, the first assessment time was at 45 minutes and peak inhibition for plasma (43%) and brain (54%) ChE was attained at this interval and some recovery was apparent as early as 1.5 hours with plasma (28%) and brain (40%) showing less inhibition. However, for RBC inhibition in adult females, although 41% at 45 min was 51% at 3 hours. In pups, 51%-82% inhibition occurred within 45 min to 1.5 hours and remained high (61% to 80%) at 3 hours for all three enzyme species. For subsequent studies, 45- 50 minutes was selected as the time to peak effect. *Acute dosing.* Based on extent of inhibition at the LOAEL, RBC ChE was the most sensitive indicator of AZM exposure. RBC ChEI (47% in males at 1.1 mg/kg and 46% at 2 mg/kg in females) whereas a higher dose of 2 mg/kg was needed to produce statistically significant inhibition in plasma (48%) and brain (21%) in males. Plasma or brain ChE in females did not reach statistical significance at 2 mg/kg. In contrast, plasma (15-20%), RBC (31- 34 %), and brain (15-20%) ChE were statistically significantly inhibited in both male and females pups at 1 mg/kg. For acute exposure, the adult LOAEL is 1.1 mg/kg based on RBC ChEI. The adult NOAEL is 0.6 mg/kg. The LOAEL for neonatal rats is 1.0 mg/kg based on plasma, RBC, and brain ChEI. The neonate NOAEL is 0.49 mg/kg. *11-day repeat dosing.* In adults, statistically significant inhibition of RBC AChE was demonstrated at 1 mg/kg/day for males (41%) and females (53%). However, a dose of 1.6 mg/kg/day was required to produce significant plasma (33 to 42% in both sexes) or brain (23 to62%) inhibition. In pups, statistically significant RBC (29% in males) and brain (9% in males and 8% in females) inhibition of ChE was noted at 0.24 mg/kg/day or the lowest dose tested. Brain ChEI showed a progressive increase in both sexes with dose supporting the possibility that it is biologically significant at 0.24 mg/kg/day. Plasma ChE in both sexes and RBC ChE in females attained statistical significance at 0.5 mg/kg/day. For repeated exposure, the adult LOAEL is 1.0 mg/kg/day based on RBC ChE inhibition; the adult NOAEL is 0.54 mg/kg/day. The LOAEL for neonates is 0.24 mg/kg/day based on RBC ChEI; the NOAEL was not determined. *Gestational exposure.* The only treatment-related change was in RBC ChEI at 15 ppm (27%) in dams. There was no inhibition noted in the fetuses suggesting that inhibitory forms of AZM are not secreted in the dams milk. **Both the acute and repeat dose studies imply that the pups are more sensitive than the adults.** It is noted, however, that dose responses, or expected more inhibition at higher doses, was not always attained in this study resulting in problems with attempting to determine the benchmark dose for inhibition of ChE as well as limiting confidence in assigning NOAEL and LOAELs for this study. This study is classified **Acceptable/Nonguideline** for the determination of plasma, RBC, and brain ChEI following treatment with AZM in adult, neonatal and fetal rats.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF PREVENTION,
PESTICIDES AND
TOXIC SUBSTANCES

May 26, 2005

MEMORANDUM

SUBJECT: BENCHMARK DOSE ANALYSES OF THE AZINPHOS-METHYL COMPARATIVE CHOLINESTERASE SINGLE DOSE TOXICITY STUDY AND THE AZINPHOS-METHYL COMPARATIVE CHOLINESTERASE REPEAT DOSE TOXICITY STUDY. PC Code: 058001. DP Barcode D311115.

FROM: Philip Villanueva
Mathematical Statistician
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THRU: David J. Miller
Chief
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TO: John Doherty
Toxicologist
ReRegistration Branch 3
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In response to a request from RRB3, benchmark dose (BMD) analyses of cholinesterase inhibition data from the azinphos-methyl (AZM) comparative cholinesterase single (gavage) dose toxicity study (MRID# 46162101) and the AZM comparative cholinesterase repeat (gavage) dose toxicity study (MRID# 46239001) were performed to determine the extent to which neonate rats are more sensitive to AZM dosing than adult rats. The results of these BMD analyses indicate that for repeat and single dosing, neonates can be up to 3-4 times more sensitive to AZM than adults.

Detailed Analysis

Benchmark dose analysis attempts to model the dose-response relationship with a dose-response curve that can be described by a mathematical function. The dose-response curve which is estimated

based on the experimental observations interpolates the magnitude of the response for any dose within the experimental dosing range. Various mathematical models can be used to model this dose-response curve. Once a BMD model has been selected, the dose associated with a specified response (i.e. benchmark response, BMR) is determined. The BMR is expressed as a certain percent change in the control group response (i.e., background). The dose resulting in the BMR is termed the "benchmark dose". Generally, the dose resulting in a BMR of X% is referred to as the BMD_X. The corresponding lower 95% confidence limit on the BMD_X is the $BMDL_X$.

BMD analyses of cholinesterase inhibition (ChEI) data from the AZM comparative cholinesterase single (gavage) dose toxicity study (MRID# 46162101) and the AZM comparative cholinesterase repeat (gavage) dose toxicity study (MRID# 46239001) were performed using EPA's OPCumRisk program. The exponential function used for modeling the effect of AZM on cholinesterase (ChE) activity was:

$$y = B + (A - B) \times e^{-m*dose}$$

where y is ChE activity, *dose* is the dose of AZM in mg/kg/day, m is the dose scale factor, A is background ChE activity, and B is the limiting high-dose ChE activity. Both y (ChE activity) and *dose* were extracted from the above referenced toxicity studies. The equation for the exponential model reflects the observation that ChE activity decreases to a limiting value (B) as dose increases. The model has three parameters to be estimated: m (dose scale factor), A (background), and B (limiting high-dose ChE activity). The OPCumRisk program can be obtained at www.epa.gov/pesticides/cumulative/EPA_approach_methods.htm. OPCumRisk utilizes the same dose-response model (i.e., exponential model) as utilized in the Preliminary OP Cumulative Risk Assessment (CRA). This method has been previously evaluated by the FIFRA SAP (www.epa.gov/scipoly/sap/2001/index.htm). For the revised OP CRA, the exponential model was expanded to include a "low dose shoulder." The low dose shoulder corresponds to the portion of the dose-response curve where the response of the low dose group is similar to that of the control group. This low dose shoulder is not modeled in the OPCumRisk program. For AZM there is evidence that a low dose shoulder may exist for some endpoints. However the inclusion of a low dose shoulder in the decreasing exponential model would generally tend to increase the BMD (i.e. increase the dose at which the specified inhibition is expected to be observed). So generally, the BMD values computed for these analyses represent conservative estimates of those which would be calculated if a low dose shoulder were to be considered.

The calculated BMD values represent the dose at which a 10% reduction in ChE activity compared to background activity is expected. For each ChEI data set, parameters were estimated including all dose groups. The OPCumRisk program utilizes a decision algorithm for selecting from among various options for the exponential model. Generally the model is fitted until an adequate p-value for the χ^2 goodness of fit (GoF) is obtained. The decision algorithm is provided below.

1. If the p-value for the GoF statistic is greater than 0.05, then the model's fit was considered adequate and the parameter estimates were used.

2. Otherwise (that is, if the p-value was less than 0.05, or no estimates resulted because the model did not converge), the horizontal asymptote was set to zero and the model was refit to the data.

3. If the p-value was still less than 0.05, or there was no model fit at all, then the highest dose was dropped and the model was refit with the horizontal asymptote set to zero until either the p-value exceeded 0.05, or there are only three doses remaining.

Although the user can specify options that are not consistent with default decision algorithm utilized by OPCumRisk, all BMD values provided in these analyses are based on the default decision algorithm. The decision algorithm and technical details of the "basic" exponential model used in this BMD analysis can be obtained at www.epa.gov/scipoly/sap/2001/september/rpfappendix1.pdf.

BMD values were calculated for the brain, plasma, red blood cell (RBC) ChEI data from both the single and repeated dose comparative ChE toxicity studies. Table 1 contains summary information from the OPCumRisk model runs for the AZM comparative ChE single dose toxicity study. For the single dose study, the decreasing exponential model adequately fit (goodness-of-fit p-value< 0.05) both the neonate and adult data for all three compartments (brain, plasma, and RBC). However the estimated plasma BMD_{10} for adult males is outside of the experimental dose range. BMD estimates which are outside of the experimental dose range should be considered suspect and generally discarded.

Table 2 contains summary information from the OPCumRisk model runs for the AZM comparative ChE repeat dose toxicity study. For the repeat dose study, the decreasing exponential model did *not* adequately fit (goodness-of-fit p-value> 0.05) some of the neonate and adult data for some of the compartments (brain, plasma, and RBC). Additionally, some of the BMD_{10} values were outside of the experimental dose range. However based on the BMD values that are valid, it can be concluded that the various subpopulations are generally more sensitive (i.e. have lower BMD values) to repeat dosing than single dosing.

For both repeat and single dosing regiments, RBC ChE is the most sensitive endpoint compared to brain and plasma ChE. Based on female brain ChE data for single dosing and the male RBC ChE data for repeat dosing, the $BMDL_{10}$ values indicate that neonates can be up to 3-4 times as sensitive to AZM than adults. Since adequate models fits were not attained or BMD estimates were outside of the experimental dose range for many of the ChE datasets from the repeat dose toxicity study, the same comparison between neonates and adults could not be made for the brain and plasma compartments.

DATA EVALUATION RECORD

AZINPHOS-METHYL (GUTHION®)/058001

Study Type: SPECIAL STUDY, CHOLINESTERASE INHIBITION [NON-GUIDELINE]
MRID 46162101 (time course, adult and neonate rats; adult/neonatal rat comparative sensitivity, single dose); MRID 46239001 (adult/neonatal comparative sensitivity, repeat dose); MRID 46291101 (adult/fetal comparative sensitivity)

Prepared for
Health Effects Division
Office of Pesticide Programs
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1801 Bell Street
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Prepared by
Toxicology and Hazard Assessment Group
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Task Order No. 51-2004

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Disclaimer

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

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EPA Work Assignment Manager: P. V. Shah, Ph.D. **Signature** _____
Registration Action Branch 1, Health Effects Division (7509C) **Date** 10/16/05

TXR#: 0052325

DATA EVALUATION RECORD

STUDY TYPE: Special Studies. Effects on Cholinesterase in Adult, Fetal, and Juvenile Wistar Rats, Companion Studies to a Developmental Neurotoxicity Study (Report No. G200115, 2002).

PC CODE: 058001 **DP BARCODE:** D298150, D301855
SUBMISSION NO.:

TEST MATERIAL (PURITY): Azinphos-methyl (90.2% - 92.4%)

SYNONYMS: *O,O*-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methyl] phosphorodithioate; Guthion®

CITATIONS: Sheets, L. P. (2003) Cholinesterase inhibition in young-adult and neonatal (11-day-old) Wistar rats treated by gavage with a single dose of technical grade azinphos-methyl (GUTHION®). Bayer CropScience LP Toxicology, 17745 South Metcalf Avenue, Stilwell, Kansas 66085-9104. Report No.: 200737, Study Nos.: 02-P12-MO, 02-N12-MP, 02-P12-NM, and 03-D12-0Y. December 19, 2003. MRID 46162101. Unpublished

Sheets, L. P. (2004) Cholinesterase inhibition in young-adult and neonatal (11-day-old) Wistar rats after repeated exposure by gavage to technical grade azinphos-methyl (GUTHION®). Bayer CropScience LP Toxicology, 17745 South Metcalf Avenue, Stilwell, Kansas 66085-9104. Report No.: 200989, Study Nos.: 02-N12-MX and 03-D12-TH. March 29, 2004. MRID 46239001. Unpublished

Sheets, L. P. (2004) Maternal and fetal cholinesterase activities in Wistar rats following dietary exposure during gestation to technical grade azinphos-methyl (GUTHION®). Bayer CropScience LP Toxicology, 17745 South Metcalf Avenue, Stilwell, Kansas 66085-9104. Report No.: 201018, Study No.: 03-D72-TI. May 14, 2004. MRID 46291101. Unpublished

SPONSOR: Bayer CropScience LP, 2 T.W. Alexander Drive, Research Triangle Park, North Carolina 27709

EXECUTIVE SUMMARY: In a series of special studies, Azinphos-methyl (90.2 - 92.4% a.i., batch no. 2-03-0278, in water with methylcellulose and Tween 80) was administered by gavage to Wistar rats to determine the sensitivity of plasma, RBC and brain cholinesterase to neonatal

rats relative to adults to inhibition (ChEI) by azinphos methyl (AZM). In the time-course evaluation study (2003, MRID 46162101), for adults, single doses of 6 mg/kg for male or 3 mg/kg for female and for post natal day 11 (PND 11) pups doses of 2 mg/kg of AZM were given to10 adult or pups/sex. In the acute study (2003, MRID # 46162101), doses of 0, 0.6, 1.1 or 2 mg/kg of AZM were given to groups of six young adult rats/sex (males were also given 3.3 mg/kg), and doses of 0.0, 0.26, 0.49 or 1 mg/kg were given to groups of 10 PND 11 pups/sex. In a 11-day repeat dosing study (2004, MRID # 46239001), groups of six adult rats/sex were given daily nominal doses of 0, 0.25, 0.54, 1 or1.6 mg/kg/day of AZM on 11 consecutive days and groups of 12 PND 11 pups/sex were given a daily nominal dose of 0, 0.24, 0.51, or 1 mg/kg/day. In a third study (2004, MRID # 46291101), groups of 12 pregnant dams were fed diets containing 0, 3, 10, or 15 ppm from gestation (GD) 0-20. Average test material intake was 0, 0.2, 0.9, and 1.2 mg/kg/day of AZM, respectively.

Systemic effects. No deaths, body weight changes or clinical signs were reported in any of the studies.

ChEI-time course. In adult males, peak ChEI (52-61%) from all sources was seen at 1.5 hours or at the first assessment time and some recovery was apparent after 3 hours. In females, the first assessment time was at 45 minutes and peak inhibition for plasma (43%) and brain (54%) ChE was attained at this interval and some recovery was apparent as early as 1.5 hours with plasma (28%) and brain (40%) showing less inhibition. However, for RBC inhibition in adult females, although 41% at 45 min was 51% at 3 hours. In pups, 51%-82% inhibition occurred within 45 min to 1.5 hours and remained high (61% to 80%) at 3 hours for all three enzyme species. For subsequent studies, 45- 50 minutes was selected as the time to peak effect.

Acute dosing. Based on extent of inhibition at the LOAEL, RBC ChE was the most sensitive indicator of AZM exposure. RBC ChEI (47% in males at 1.1 mg/kg and 46% at 2 mg/kg in females) whereas a higher dose of 2 mg/kg was needed to produce statistically significant inhibition in plasma (48%) and brain (21%) in males. Plasma or brain ChE in females did not reach statistical significance at 2 mg/kg. In contrast, plasma (15-20%), RBC (31- 34 %), and brain (15-20%) ChE were statistically significantly inhibited in both male and females pups at 1 mg/kg. For acute exposure, the adult LOAEL is 1.1 mg/kg based on RBC ChEI. The adult NOAEL is 0.6 mg/kg. The LOAEL for neonatal rats is 1.0 mg/kg based on plasma, RBC, and brain ChEI. The neonate NOAEL is 0.49 mg/kg.

11-day repeat dosing. In adults, statistically significant inhibition of RBC AChE was demonstrated at 1 mg/kg/day for males (41%) and females (53%). However, a dose of 1.6 mg/kg/day was required to produce significant plasma (33 to 42% in both sexes) or brain (23 to62%) inhibition. In pups, statistically significant RBC (29% in males) and brain (9% in males and 8% in females) inhibition of ChE was noted at 0.24 mg/kg/day or the lowest dose tested. Brain ChEI showed a progressive increase in both sexes with dose supporting the possibility that it is biologically significant at 0.24 mg/kg/day. Plasma ChE in both sexes and RBC ChE in females attained statistical significance at 0.5 mg/kg/day. For repeated exposure, the adult LOAEL is 1.0 mg/kg/day based on RBC ChE inhibition; the adult NOAEL is 0.54 mg/kg/day. The LOAEL for neonates is 0.24 mg/kg/day based on RBC ChEI; the NOAEL was not determined.

Gestational exposure. The only treatment-related change was in RBC ChEI at 15 ppm (27%) in dams. There was no inhibition noted in the fetuses suggesting that inhibitory forms of AZM are not secreted in the dams milk.

Both the acute and repeat dose studies imply that the pups are more sensitive than the adults. It is noted, however, that dose responses, or expected more inhibition at higher doses, was not always attained in this study resulting in problems with attempting to determine the

benchmark dose for inhibition of ChE as well as limiting confidence in assigning NOAEL and LOAELs for this study.

This study is classified **Acceptable/Nonguideline** for the determination of plasma, RBC, and brain ChEI following treatment with AZM in adult, neonatal and fetal rats.

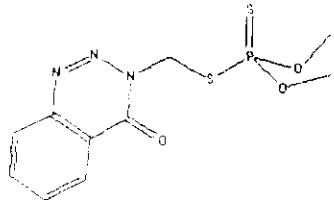
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Azinphos-methyl

Description:	yellow solid
Lot/Batch #:	2-03-0278
Purity:	90.2% - 92.4 % a.i.
Compound Stability:	stable for study duration
CAS # of TGAI:	86-50-0
Structure:	



2. **Vehicle and/or positive control:** Equal volumes (10 mL/kg) of the vehicle (0.5% methylcellulose and 0.4% Tween 80 in deionized water) were given to control animals in the gavage studies (MRID 46162101 and 46239001). In the dietary study (MRID 46291101), acetone was used to facilitate mixing of the test substance and allowed to evaporate. Acetone minus the test substance was added to the control diets in the same manner.

3. **Test animals:**

Species:	rat	
Strain:	Wistar Hannover Crl:WI (Glx/BRLHan) IGS BR	
Age and wt. at study initiation:	Virgin females to be mated - 12 weeks; males to be mated - 12-15 weeks. Males and females in the adult studies, 8-10 weeks. Neonatal study males and females - 11 days. Body weight of females in the dietary study at gestation day 0, 214.7-222.7g. Other body weights were not supplied.	
Source:	Charles River Laboratories, Inc. Raleigh, NC	
Housing:	Stainless steel with wire mesh floors or polycarbonate cages. Bed-O-Cobs corn cob bedding was used in polycarbonate cages during gestation and lactation.	
Diet:	Purina Mills Rodent Lab Chow 5002 in meal form, Purina Mills Laboratories Inc., St. Louis, MO., *ad libitum*	
Water:	tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	12-13/hr
	Photoperiod:	12 hrs light/dark
Acclimation period:	At least 6 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates**: MRID 46162101: Adult rats: Start : December 15, 2002; End: December 17, 2002;
PND-11 neonates: Start: February 17, 2003; End February 18, 2003
MRID 46239001: Adult rats: Start: October13, 2002; End: October 24, 2002;
PND-11 neonates: Start: November 3, 2003 End: November 15, 2003
MRID 46291101: Start: September 23, 2003; End: October 26, 2003

2. **Study design:** Animals were randomly selected for the treatment groups shown in Table 1. The neonate (PND 11) rats were selected from litters obtained from 10 dams. The litters were culled randomly to yield 4 pups of each sex if possible and the treatment groups were composed of one pup per sex from each litter within the limits of pup availability. The females in the dietary study (MRID 46291101) were examined for evidence of mating prior to group assignments. Cholinesterase (ChE) activity was measured in plasma, RBCs, and brain.

TABLE 1. Study design

MRID	Dose(s) (mg/kg/day)	No. of animals/sex/dose	Treatment and termination (cholinesterase activity measured in plasma, RBCs, and brain)
46162101 Acute	A. adults: M: 0, 6; F: 0, 3 pups: 0, 2 [a]B. Adults: M: 0, 0.5, 1.0, 2.0, 3.0; F: 0, 0.5, 1.0, 2.0 PND 11 M&F: 0, 0.25, 0.5, 1.0	A. Adults: 6 M/6 F; PND 11 pups: 10 M/10 F B. Adults: 6 M/6 F; PND 11 pups: 10 M/10 F	A. Adults: Single oral gavage dose; termination at: 1.5, 3.0, 8.0, 24 hours (males); 45-50 minutes, 1.5, 3.0, 8.0 hours (females); PND 11 pups: single oral gavage doses; termination at 45-50 minutes, 1.5, 3.0 hours B. Adults: Single oral gavage doses; termination at: 45-50 minutes; PND 11 pups: single oral gavage doses; termination at 45-50 minutes
46239001 Repeat	[b]Adults: 0, 0.25, 0.5, 1.0, 1.5; PND 11 pups: 0, 0.25, 0.5, 1.0	Adults: M&F: 11; PND 11 pups: M, 12; F, 11	Daily gavage doses for 11 days; termination at 45-60 minutes after last dose
46291101 Gestation	0, 0.2, 0.9, 1.2 (0, 3, 10, 15 ppm)	12 F	Administered in feed to pregnant dams, GD 0-20; terminated on GD 20

Data from pp. 17, 28, 30, 32, & 52 of MRID 46162101; p. 17 of MRID 46239001; and p. 19 of MRID 46291101.
[a]Measured concentrations were adults: 0, 0.6, 1.1, 2.0, or 3.3 mg/kg; pups: 0, 0.26, 0.49, or 1.0 mg/kg.
[b]Measured concentrations were adults: 0.0, 0.25, 0.54, 1.0, or 1.6 mg/kg/day; pups: 0.0, 0.24, 0.51, or 1.0 mg/kg/day.
PND = postnatal day.
GD = gestation day

3. **Mating procedure:** Females were paired on a 1:1 basis with males of the same strain. Each morning following pairing, the females were checked for vaginal plugs and vaginal smears were prepared from each female and examined for spermatozoa. The day a vaginal smear tested positive for spermatozoa was designated GD 0.

4. **Dose selection rationale:** Acute doses were selected by the sponsor based on a previous acute study in rats (Report no. 106365, MRID 43360301, 1994). In the acute study, brain

ChE was significantly inhibited at 1 mg/kg only in the pups, so the maximum dose chosen for the adults was 1.5 mg/kg and was reduced to 1.0 mg/kg for the pups in the 11-day studies.

5. **Dosage administration:** All single or multiple doses by gavage were administered to the adult males and females, mated dams, and selected offspring at a volume of 10 mL/kg calculated from the most recent body weight.

6. **Dosage preparation and analysis:** In the gavage studies, the formulations were prepared by suspending the Azinphos-methyl in a solution of 0.5% methyl cellulose and 0.4% Tween 80 in deionized water (w/v %) and stirring constantly during use. Concentrations were adjusted for a dose volume of 10 mL/mg body weight. The stability and homogeneity of the mixtures were verified in an oral neurotoxicity study (MRID 43360301) and in a previous 11-day study (Study no. 02-N12-MX). Each concentration used was analyzed for Azinphos-methyl content and the analytically confirmed dose was utilized in the report. In the feeding study, the Azinphos-methyl was dissolved in acetone to facilitate mixing in the diet; the acetone was allowed to evaporate. Control diets were prepared with acetone alone and treated in the same manner. The diets were prepared weekly and analyzed for Azinphos-methyl content using a liquid chromatographic method. The homogeneity and stability of the test substance was evaluated as part of a Developmental Neurotoxicity study (Report no. G200115, 2002); these data were not included in the current report.

Results -

Homogeneity analysis: Samples of the 0.025 mg/ml solution used for the 0.25 mg/kg gavage dose were reported to have a coefficient of variation of 0.7%. No additional data on homogeneity were given.

Stability analysis: The concentrations used to achieve gavage doses of 1.0 to 40 mg/kg were said to have no appreciable decrease in concentration after 8 or 9 days of storage (data not included). The concentration used for the 0.25 mg/kg dose had no appreciable decrease in concentration after 19 days of storage at room temperature (data not included.). No stability data were given for the dietary concentrations.

Concentration analysis: In the single-dose gavage study (MRID 46162101), the average concentrations ranged from 101% to 121% of the nominal concentrations and in the repeat dose gavage study (MRID 46239001), the concentrations ranged from 94% to 108% (means) of the nominal concentrations. All doses in the dietary study were within 5.0% of the nominal concentrations. The substance intake values were calculated to reflect the analytically determined values in all the studies in this report.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. **Adult animals:** All animals were checked daily for clinical signs or ill health. Any animal that showed possible clinical signs was removed from the cage and a detailed

assessment was made. All animals were weighed prior to treatment with the test substance to insure the appropriate dosimetry in the gavage studies. Food consumption was not measured in the gavage studies. Daily detailed examinations were performed on the females in the dietary study during gestation and they were weighed on gestation days 0, 6, 13, and 20. Food consumption was measured on gestation days 6, 13, and 20.

b. **Offspring:** Records were maintained on the number of live and stillborn pups in each litter. The gender and body weight were determined as soon as possible after birth. Pups were also observed for possible clinical signs daily, and were weighed on lactation day 4 and before each treatment.

2. **Cholinesterase (ChE) determination:** Cholinesterase assays were done on RBC, plasma, and brain samples. The modified Ellman assay involved using 6,6'-dithio-dinicotinic acid as a coupling agent resulting in an absorbance change at 340 nm monitored on a spectrophotometer.

3. **Necropsy procedures:** The animals in the gavage studies did not undergo detailed macroscopic examination unless the investigator decided it was necessary to determine the cause of an unscheduled death. In the feeding study (MRID 46291101), females found dead, moribund, or delivering prematurely were subjected to detailed gross necropsy examination including a collection of tissues for microscopic examination. However, no animals were routinely given a detailed gross necropsy and no necropsy data were included in the reports. Animals were sacrificed by CO_2 asphyxiation. Dams were sacrificed on GD 20, and fetuses were removed from the uterine wall and sacrificed by decapitation.

4. **Statistical analyses:** The same statistical procedures were used in all the studies reviewed in this report. The ChE results from animals that were treated with Azinphos-methyl were analyzed relative to the concurrent age-matched controls. Bartlett's test was used to initially assess the ChE results for equality of variance. An Analysis of Variance (ANOVA) was used to analyze group means with equal variances followed by a Dunnett's test if a significant F-value was seen in the ANOVA. Data showing unequal variances were assessed with Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons. The statistical calculations were performed using software from either INSTEM Computer Systems or SAS. Statistical significance was flagged at $p \leq 0.05$ except for Bartlett's test which was tested at $p \leq 0.001$.

II. RESULTS:

A. MORTALITY AND CLINICAL AND FUNCTIONAL OBSERVATIONS: There were no treatment-related deaths reported in the studies. No treatment-related clinical signs of toxicity were reported in adults or in offspring. No data on clinical signs or the lack thereof were reported in MRIDs 46162101 and 46239001.

B. BODY WEIGHT AND FOOD CONSUMPTION: The body weight for each dosed group of the dams treated through gestation in the feeding study (MRID 46291101) was consistently 2% -3% less than the control group (beginning on day 0) and the body weight gain of the treated females was about 4% less than that of the control group. However, the differences were not statistically significant and were not dose related. No significant differences in food

consumption were reported. Body weight in the acute gavage studies was taken for dosimetry and was not specifically reported.

C. CHOLINESTERASE ACTIVITY:

1. **Time to peak ChE inhibition (MRID 46162101):** The time to peak ChE inhibition data are shown in Table 2. Following a dose of 6 mg/kg, the peak of ChE inhibition in young adult males (52% to 61% for plasma, RBC or brain) was observed at 1.5 hours. In females, following a dose of 3 mg/kg, plasma (43%) and brain (54%) had maximal inhibition after 45-50 minutes. However, maximal RBC inhibition (51%) was not attained until 3 hours. Considerable recovery followed at later time points. For example, after 8 hours, RBC AChEI was only 29% in males and 20% in females (not significant in either sex). Plasma ChEI was 31% in males and 0% in females. Whereas brain AChEI was 21% in males and 18% in females (both $p < 0.05$).

In PND 11 dosed with 0 or 2 mg/kg Azinphos-methyl by gavage, ChE inhibition ranged from 51% to 82% in males and 55% to 82% in females and was similar at all time points. No data to assess for possible reversible after 8 and 24 hours were generated. Based on these results, the optimum time for measurement of peak ChE inhibition was 45-50 minutes or up to 3 hours.

TABLE 2. Time-course of cholinesterase activity inhibition in plasma, RBC and brain in adult and PND 11 pups

Time after admin. ChE source	Cholinesterase activity							
	Dose: Young adults				Dose: 11-Day old pups			
	0		Males: 6 mg/kg Females 3 mg/kg		0		Both sexes: 2 mg/kg	
	Males	Females	Males	Females	Males	Females	Males	Females
45 minutes Plasma (U/L) RBC (U/L) Brain (U/g)	ND	2.03±0.52 0.91±0.17 14.7±0.3	ND	**1.16±0.40*(43%)** 0.54±0.27*(41%) **6.8±2.4*(54%)**	0.61±0.13 1.41±0.50 5.8±1.6	0.67±0.07 1.42±0.34 6.2±0.5	0.30±0.16*(51%) 0.36±0.42*(41%) 2.3±1.8*(60%)	0.27±0.09*(60%) **0.26±0.15*(81%)** 1.9±1.0*(69%)
1.5 hours Plasma (U/L) RBC (U/L) Brain (U/g)	0.46±0.11 0.92±0.23 13.2±1.7	1.88±0.39 0.80±0.15 14.3±0.8	**0.22±0.04*(52%)** **0.36±0.18*(61%)** **5.5±0.7*(58%)**	1.36±0.41*(28% 0.53±0.11*(34%) 8.6±1.3*(40%)	ND	ND	**0.25±0.04*(59%)** **0.26±0.15*(82%)** **1.3±0.5*(78%)**	0.30±0.06*(55%) **0.36±0.20*(75%)** 1.6±0.5*(74%)
3 hours Plasma (U/L) RBC (U/L) Brain (U/g)	0.46±0.02 0.89±0.09 13.2±0.6	1.95±0.61 1.03±0.09 13.6±0.4	0.28±0.07*(39%) 0.45±0.21*(49%) 6.8±0.5*(48%)	1.33±0.28(32%) **0.50±0.21*(51%)** 10.1±0.9*(26%)	ND	ND	**0.24±0.04*(61%)** **0.33±0.14*(77%)** **1.5±0.4*(74%)**	**0.22±0.07*(67%)** **0.28±0.24*(80%)** **1.4±0.4*(77%)**
8 hours Plasma (U/L) RBC (U/L) Brain (U/g)	0.39±0.06 0.73±0.10 13.0±0.6	1.62±0.44 0.85±0.37 13.7±0.6	0.27±0.05*(31%) 0.52±0.22(29%) 10.3±0.5*(21%)	1.62±0.48 (–) 0.68±0.19(20%) 11.2±1.3*(18%)	ND	ND	ND	ND
24 hours Plasma (U/L) RBC (U/L) Brain (U/g)	0.42±0.06 1.01±0.22 13.6±0.3	ND	0.33±0.06*(21%) 0.80±0.21(21%) 10.5±0.5*(23%)	ND	ND	ND	ND	ND

Data from pp. 43-57, MRID 46162101.
ND = No data - study not conducted at this time point.
* = $p \leq 0.05$.

2. **Single dose study (MRID 46162101):** The results of this study are summarized in Table 3. Plasma ChEI in adult males was dose related at doses of 1 to 3 mg/kg, attaining statistical significance at 2.0 mg/kg (48%). For adult females, plasma ChEI (23%, 16%, and 20% at 0.5, 1.0, and 2.0 mg/kg, respectively) was not statically significant at any dose and did not show a dose-response effect.

At doses of 1.0, 2.0, and 3.0 mg/kg, RBC ChEI in adult males was statistically significant and similar (46-57%) among the dose groups. RBC ChEI of 24%, 19% and 46% seen in adult females at 0.5, 1.0, and 2.0 mg/kg, respectively, is clearly treatment-related at the high dose; however, the changes seen at the lower doses are not dose related.

Brain ChEI in males attained statistical significance (21%) at the 2.0 mg/kg dose, but brain ChE activity in females was unaffected at this dose (highest dose tested in females).

For 11-day old pups, plasma ChEI attained statistical significance at 1.0 mg/kg, the highest dose tested. However, the reductions of 15% (males) and 20% (females) at the 1.0 mg/kg dose are borderline biologically significant.

Decreases in RBC ChE activity of 16% and 14% seen at 0.5 mg/kg in male and female pups, respectively, were not statistically significant, but were dose-related and were likely treatment-related changes. Statistical and biological significance was attained for RBC ChEI in both sexes (31-34%) at the 1.0 mg/kg dose.

In brain, ChEI of 15% (males) and 20% (females) at 1.0 mg/kg is considered biologically significant.

TABLE 3. Plasma, RBC, and brain cholinesterase activity in adult and PND 11 rats treated with Azinphos-methyl - acute exposures				
Dose (mg/kg) ChE source tested	ChE in young adult rats (n = 6/sex)		ChE in 11-day-old pups (n = 10/sex)	
	Males	Females	Males	Females
Control 0 mg/kg				
Plasma (U/L)	0.40±0.06	2.18±0.12	0.62±0.06	0.61±0.07
RBC (U/L)	0.89±0.10	1.00±0.17	1.40±0.24	1.45±0.19
Brain (U/g)	13.1±0.9	13.6±1.2	6.1±0.5	6.1±0.5
0.25 mg/kg				
Plasma (U/L)	ND	ND	0.63±0.07	0.60±0.05
RBC (U/L)			1.38±0.22	1.31±0.21
Brain (U/g)			6.0±0.6	6.2±0.4
0.50 mg/kg				
Plasma (U/L)	0.45±0.06	1.67±0.38 (23)[a]	0.58±0.06	0.60±0.06
RBC (U/L)	0.80±0.11	0.76±0.18 (24)	1.18±0.13 (16)	1.25±0.20 (14)
Brain (U/g)	12.6±0.7	14.2±1.4	5.7±0.6 (7)	5.8±0.5 (5)
1.0 mg/kg				
Plasma (U/L)	0.39±0.08	1.84±0.35 (16)	0.53±0.09* (15)	0.49±0.05* (20)
RBC (U/L)	0.47±0.31* (47)	0.81±0.19 (19)	0.96±0.29* (31)	0.95±0.26* (34)
Brain (U/g)	12.9±0.7	12.9±0.8 (5)	5.2±0.8* (15)	4.9±0.6* (20)
2.0 mg/kg				
Plasma (U/L)	0.21±0.03* (48)	1.74±0.43 (20)	ND	ND
RBC (U/L)	0.38±0.16* (57)	0.54±0.18* (46)		
Brain (U/g)	10.3±1.4* (21)	12.4±0.8 (9)		
3.0 mg/kg				
Plasma (U/L)	0.20±0.02* (50)	ND	ND	ND
RBC (U/L)	0.48±0.14* (46)			
Brain (U/g)	9.1±1.8* (31)			

Data from pp. 30, 32, and 59-68, MRID 46162101.
[a] Results in parenthesis are percent inhibition relative to control.
ND = no data; study not conducted at this dose level.
* = $p \leq 0.05$.

3. Repeat exposure:

a. Adult rats: The plasma, RBC, and brain ChE in young adult rats (8-10 weeks) following 11 days of single doses of Azinphos-methyl by gavage is summarized in Table 4. No ChE inhibition was seen in young adults of either sex at 0.25 mg/kg/day. Plasma ChEI was observed in adult males at 1.0 mg/kg/day (21%; NS) and in females at 1.5 mg/kg/day (42%; $p \leq 0.05$). The RBC ChE was inhibited by 17% (NS) in males at 0.50 mg/kg/day and by 41% and 53% ($p \leq 0.05$) in males and females, respectively, at 1.0 mg/kg/day, and inhibited to 49% and 83% ($p \leq 0.05$), respectively, at the 1.5 mg/kg/day dose. Brain ChE was affected only at 1.5 mg/kg/day in both sexes and was significantly inhibited by 23% in males and by 62% in females (both $p \leq 0.05$).

b. PND 11-21 neonates: Plasma ChE was not significantly inhibited in either sex at 0.25 mg/kg/day (Table 4). Statistical significance was attained for both sexes (males, 23%; females 15%) at 0.50 mg/kg/day. The values at 0.50 and 1.0 mg/kg/day showed a dose response. Pre-weaning males showed significantly inhibited ChE activity in RBCs of 29% at 0.25 mg/kg/day; the RBC ChE activity was inhibited by 12% in females at 0.25 mg/kg, but the difference was not statistically significant. Significance was attained at

1.0 mg/kg/day (58% inhibition, both sexes). Brain ChE activity was slightly, but significantly, inhibited in both sexes at 0.25 mg/kg/day (males, 9%; females, 8%; $p \leq 0.05$). However, biological significance was attained at 0.50 mg/kg/day. The ChE activity from all sources was significantly inhibited in both sexes at 0.50 mg/kg/day and at 1.0 mg/kg/day compared to the control groups. The ChE activity from RBCs was the most affected and was decreased by 83% and 78% in males and females, respectively, at 1.0 mg/kg/day.

TABLE 4. Plasma, RBC, and brain cholinesterase activity in adult and PND 11 rats - 11-day repeat exposures

Cholinesterase source	Cholinesterase activity				
	Dose (mg/kg/day)				
	0	0.25	0.5	1	1.5
Adult Males					
Plasma (U/L)	0.42 ± 0.03	0.43 ± 0.07	0.40 ± 0.07 (5)[a]	0.33 ± 0.06 (21)	**0.28 ± 0.08 (33)***
RBC (U/L)	0.95 ± 0.23	1.01 ± 0.40	0.79 ± 0.13 (17)	**0.56 ± 0.25 (41)***	**0.48 ± 0.14 (49)***
Brain (U/g)	13.5 ± 0.6	13.5 ± 0.6 (0)	12.9 ± 1.3 (4)	13.5 ± 1.3 (0)	**10.4 ± 1.8 (23)***
Adult Females					
Plasma (U/L)	1.66 ± 0.51	1.61 ± 0.44	1.90 ± 0.39	1.78 ± 0.47	**0.97 ± 0.08 (42)***
RBC (U/L)	1.11 ± 0.28	1.12 ± 0.24	1.09 ± 0.30 (2)	**0.52 ± 0.29 (53)***	**0.19 ± 0.15 (83)***
Brain (U/g)	13.8 ± 0.5	14.4 ± 0.3*	13.5 ± 0.6 (2)	13.3 ± 0.5 (4)	**5.2 ± 2.4 (62)***
PND-11 Males					
Plasma (U/L)	0.53 ± 0.04	0.52 ± 0.05 (2)	**0.41 ± 0.06 (23)***	**0.28 ± 0.05 (47)***	
RBC (U/L)	1.73 ± 0.57	**1.23 ± 0.19 (29)***	**0.73 ± 0.15 (58)***	**0.29 ± 0.10 (83)***	ND
Brain (U/g)	10.4 ± 0.4	**9.5 ± 0.6 (9)***	**7.8 ± 0.8 (25)***	**4.4 ± 0.8 (58)***	
PND-11 Females					
Plasma (U/L)	0.52 ± 0.06	0.49 ± 0.05 (6)	**0.44 ± 0.07 (15)***	**0.31 ± 0.06 (40)***	
RBC (U/L)	1.58 ± 0.40	1.39 ± 0.35 (12)	**0.67 ± 0.11 (58)***	**0.35 ± 0.15 (78)***	ND
Brain (U/g)	10.4 ± 0.3	**9.6 ± 0.4 (8)***	**7.4 ± 0.9 (29)***	**4.8 ± 1.0 (54)***	

Data from pp. 34-39 and 41-48, MRID 46239001.
[a] Results in parenthesis are percent inhibition relative to control.
n = 6 adults/sex/dose and 12 pups/sex/dose.
ND = no data; experiments were not conducted at these doses.
* = $p \leq 0.05$.

c. **Prenatal exposure to dams: gestation days 0-20 (MRID 46291101):** The ChE in plasma, RBCs, and brain from maternal rats and pooled fetuses from each dam treated with Azinphos-methyl during gestation is summarized in Table 5. RBC ChE in the maternal rats was significantly inhibited by 27% at 15 ppm. No other treatment-related changes in ChE were seen in either the maternal rats or in the pooled tissues taken from the fetuses. Without a dose-response relationship, the apparent 10% decrease in brain ChE in dams at 10 ppm was not considered treatment related.

TABLE 5. Plasma, RBC, and brain cholinesterase activity in dams and fetuses treated from gestation days 0-20				
Cholinesterase source	Mean Cholinesterase activity			
	Dietary concentration (ppm)			
	0	3	10	15
Maternal				
Plasma (U/L)	2.21 ± 0.40	1.97 ± 0.33 (11)*	1.99 ± 0.24 (10)	2.03 ± 0.36 (8)
RBC (U/L)	1.43 ± 0.31	1.41 ± 0.30 (1)	1.39 ± 0.43 (3)	**1.05 ± 0.20 (27)***
Brain (U/g)	11.1 ± 0.5	10.8 ± 0.7 (3)	10.0 ± 1.9 (10)	10.7± 0.7 (4)
Pooled 20 day fetuses				
Plasma (U/L)	0.24 ± 0.03	0.25 ± 0.02	0.24 ± 0.02 (0)	0.24 ± 0.02 (0)
RBC (U/L)	1.36 ± 0.28	1.30 ± 0.07 (4)	1.31 ± 0.15 (4)	1.32 ± 0.17 (3)
Brain (U/g)	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.1 (0)

Data from pp. 57-65, MRID 46291101.
* Results in parenthesis are percent inhibition relative to control.
n = 11 dams/dose and 11 fetuses/dose.
* = $p \leq 0.05$.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that treatment with the test material in an acute study (MRID 46162101) at single gavage doses of 6 mg/kg for adult males, 3.0 mg/kg for females, and 2 mg/kg for neonates resulted in peak cholinesterase (ChE) inhibition in plasma, RBCs, and brain in about 45 minutes after administration in all cases. Some recovery was seen with the adult rats at later time points such as 8 and 24 hours. Since no assessments at 8 and 24 hours were made for the neonates, no assessment of recovery was made. Single gavage doses of 0.5, 1.0, 2.0, and 3.0 mg/kg to adult male and/or female rats and doses of 0.25, 0.5, and 1.0 mg/kg to neonates resulted in NOAEL values of 0.6 mg/kg in adult males and females and 0.49 mg/kg in neonates (doses corrected for assayed values).

B. A LOAEL of 1.1 mg/kg in adults based on inhibited ChE in RBCs and plasma in females and RBCs in males was determined. A LOAEL of 024 mg/kg was determined for neonates based on decreased ChE in plasma, RBCs, and brain. The study author also concluded in the acute study that there were no gender-based differences in sensitivity in the adult rats or in the neonates, and there was no age-related difference in sensitivity to acute oral doses of Azinphos-methyl.

In a second study (MRID 46239001), single daily gavage doses of Azinphos-methyl were given to adult rats and PND 11 pups for 11 consecutive days. NOEL values of 0.54 mg/kg/day and <0.24 mg/kg/day were reported for adult and neonate rats (both sexes), respectively. LOEL values were 1.0 mg/kg/day in adults based on inhibited RBC ChE activity in both sexes and 0.24 mg/kg/day in neonates based on inhibited ChE activity in RBCs in males and in brain in both sexes. The author concluded there were no gender-based differences in response at either age in this study and there were no cumulative effects in adults resulting from the 11-day treatment period.

The effects of Azinphos-methyl on maternal and fetal ChE activity was tested in a feeding study in which mated female rats were fed diets containing Azinphos-methyl from gestation

day 0 - 20 (MRID 46291101). NOEL values of 0.9 mg/kg/day for female rats and 1.2 mg/kg/day for fetuses were reported. A LOAEL of 1.2 mg/kg/day was determined for females based on decreased RBC ChE activity. No significant changes in ChE activity were seen in fetal tissues.

C. **DISCUSSION AND REVIEWER COMMENTS:** The series of experiments was conducted to determine the effects of Azinphos-methyl on plasma, RBC, and brain ChE activity in male and female adult, pregnant adult, juvenile, and fetal Wistar rats. The acute time course studies showed that the maximum ChE inhibition in plasma, RBC, and brain occurs within 1.5 hours following treatment in both sexes of adult and pre-weaning rats. Some recovery was seen in the adult rats. The earliest time period (45-50 minutes) was not tested in males; but, since maximum ChE inhibition was seen at this time point in females and pre-weaning rats, the 45 minute time after Azinphos-methyl exposure was selected for the additional studies.

In the acute study, for both adults and pups, RBC ChE was the most sensitive indicator of acute Azinphos-methyl inhibition. RBC ChEI was significantly inhibited (47%) at 1.0 mg/kg in adult male. Lack of a dose response relationship for plasma ChEI in adult females precluded assignment of a clear LOAEL value but 46% inhibition was noted at 2 mg/kg. Plasma, RBC, and brain ChE were inhibited in both male (15-31%) and female pups (20-34%) at 1 mg/kg. Based on percent inhibition for each compartment, adult males were slightly more sensitive than adult females and PND 11 pups were slightly more sensitive than adults.

In the 11-day adult study, RBC ChE was the most affected by repeat-dose Azinphos-methyl treatment in adults and pups. RBC ChE showed significant inhibition (41% and 53%) at 1.0 mg/kg. At 1.5 mg/kg/day RBC (49% and 83%), plasma (33% and 42%) and brain (23% and 62%) ChE was inhibited in males and females, respectively. ChE activity was inhibited by 17% in RBCs at 0.5 mg/kg/day and in plasma by 21% at 1.0 mg/kg/day in males. These changes are likely treatment-related, but were not statistically significant, and, for these compartments are only borderline biologically significant. Brain ChE activity was inhibited only at the highest dose of 1.5 mg/kg/day in both sexes in adults.

The pups dosed for 11 days were demonstrated to be more sensitive to AZM than the adult rats. RBC and brain ChE was significantly inhibited by 29% and 9%, respectively, in males and by 12% (NS) and 8% in females at 0.25 mg/kg/day. The brain ChEI of 8% and 9% in pups that received 0.25 mg/kg/day, was statistically significant and there was a clear dose response at the next two higher doses to suggest that it was biologically significant at 0.25 mg/kg/day. The ChE activity in all compartments was significantly inhibited in both sexes of pups at 0.5 mg/kg/day and 1.0 mg/kg/day.

In both the acute and repeat dose studies, there were indications that the pups were more sensitive than the adults to the inhibitory effects of AZM. A separate statistical assessment of both the acute and repeat dose studies to determine the benchmark doses prepared by Mr. Phillip Villanueva (dated May 26, 2005) is attached.

In the 20-day feeding study from gestation days 0 through 20, there were no treatment-related clinical signs or body weight changes. The only significant effect on ChE inhibition

was seen in maternal RBC ChE at 15 ppm (~1.2 mg/kg/day). This dose compares favorably to the LOAEL of 1.1 mg/kg seen in both sexes in the single gavage dose study and to the LOAEL of 1.0 mg/kg/day for adult rats seen in the 11-day study. The effects were greater in the gavage studies than in the dietary study, which could be the result of different absorption rates. There were no treatment-related effects of Azinphos-methyl on the fetuses.

D. STUDY DEFICIENCIES:

- There was a lack of dose response or increased inhibition with increase in dose that confound the interpretation of this study to limit the confidence in assigning NOAEL and LOAELs.

- A higher dose in the maternal and fetal feeding study might have resulted in comparative fetal effects that would have been helpful. Evaluation of clinical signs was part of the study protocol, but no table of clinical signs (or the absence thereof) was included with the acute and repeat dose studies.

In a protocol review dated July 18, 2002, the protocols for azinphos-methyl (supplemental information to a developmental neurotoxicity study in rats submitted by Bayer Corporation were considered adequate for the assessment of comparative cholinesterase activity data as specified in the EPA Data-Call-In for adult and developmental neurotoxicity studies on organophosphate pesticides.

DATA FOR ENTRY INTO ISIS

Special Study

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day*	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
58001	46162101	special ChE study	rats	Acute dose (one day)	oral	gavage	0.0-3.3	0.0, 0.6, 1.1, 2.0, 3.3	0.6	1.1	Cholinesterase activity inhibition (RBC)	Adult
58001	46239001	special ChE study	rats	Repeat dose (11 days)	oral	gavage	0.0-1.6	0.0, 0.25, 0.54, 1.0, 1.6	0.54	1	Cholinesterase activity inhibition (plasma, RBC)	Adult
58001	46162101	special ChE study	rats	Acute dose (one day)	oral	gavage	0.0-1.0	0.0, 0.26, 0.49, 1.0	0.49	1	Cholinesterase activity inhibition (plasma, RBC, brain)	offspring (PND 11)
58001	46239001	special ChE study	rats	Repeat dose (11 days)	oral	gavage	0.0-1.0	0.0, 0.24, 0.51, 1.0	not determined	0.24	Cholinesterase activity inhibition (RBC)	offspring (PND 11)

* Analytically-determined concentrations

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

OFFICE OF PREVENTION,
PESTICIDES AND
TOXIC SUBSTANCES

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MEMORANDUM

SUBJECT: BENCHMARK DOSE ANALYSES OF THE AZINPHOS-METHYL COMPARATIVE CHOLINESTERASE SINGLE DOSE TOXICITY STUDY AND THE AZINPHOS-METHYL COMPARATIVE CHOLINESTERASE REPEAT DOSE TOXICITY STUDY.

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In response to a request from RRB3, benchmark dose (BMD) analyses of cholinesterase inhibition data from the azinphos-methyl (AZM) comparative cholinesterase single (gavage) dose toxicity study (MRID# 46162101) and the AZM comparative cholinesterase repeat (gavage) dose toxicity study (MRID# 46239001) were performed to determine the extent to which neonate rats are more sensitive to AZM dosing than adult rats. The results of these BMD analyses indicate that for repeat and single dosing, neonates can be up to 3-4 times more sensitive to AZM than adults.

Detailed Analysis

Benchmark dose analysis attempts to model the dose-response relationship with a dose-response curve that can be described by a mathematical function. The dose-response curve which is estimated based on the experimental observations interpolates the magnitude of the response for any dose within the experimental dosing range. Various mathematical models can be used to model this dose-response curve. Once a BMD model has been selected, the dose associated with a specified response (i.e. benchmark response, BMR) is determined. The BMR is expressed as a certain percent change in the control group response (i.e., background). The dose resulting in the BMR is termed the "benchmark dose". Generally, the dose resulting in a BMR of X% is referred to as the BMD_X. The corresponding lower 95% confidence limit on the BMD_X is the $BMDL_X$.

BMD analyses of cholinesterase inhibition (ChEI) data from the AZM comparative cholinesterase single (gavage) dose toxicity study (MRID# 46162101) and the AZM comparative cholinesterase repeat (gavage) dose toxicity study (MRID# 46239001) were performed using EPA's OPCumRisk program. The exponential function used for modeling the effect of AZM on cholinesterase (ChE) activity was:

$$y = B + (A - B) \times e^{-m*dose}$$

where y is ChE activity, *dose* is the dose of AZM in mg/kg/day, m is the dose scale factor, A is background ChE activity, and B is the limiting high-dose ChE activity. Both y (ChE activity) and *dose* were extracted from the above referenced toxicity studies. The equation for the exponential model reflects the observation that ChE activity decreases to a limiting value (B) as dose increases. The model has three parameters to be estimated: m (dose scale factor), A (background), and B (limiting high-dose ChE activity). The OPCumRisk program can be obtained at www.epa.gov/pesticides/cumulative/EPA_approach_methods.htm. OPCumRisk utilizes the same dose-response model (i.e., exponential model) as utilized in the Preliminary OP Cumulative Risk Assessment (CRA). This method has been previously evaluated by the FIFRA SAP (www.epa.gov/scipoly/sap/2001/index.htm). For the revised OP CRA, the exponential model was expanded to include a "low dose shoulder.". The low dose shoulder corresponds to the portion of the dose-response curve where the response of the low dose group is similar to that of the control group. This low dose shoulder is not modeled in the OPCumRisk program. For AZM there is evidence that a low dose shoulder may exist for some endpoints. However the inclusion of a low dose shoulder in the decreasing exponential model would generally tend to increase the BMD (i.e. increase the dose at which the specified inhibition is expected to be observed). So generally, the BMD values computed for these analyses represent conservative estimates of those which would be calculated if a low dose shoulder were to be considered.

The calculated BMD values represent the dose at which a 10% reduction in ChE activity compared to background activity is expected. For each ChEI data set, parameters were estimated including all dose groups. The OPCumRisk program utilizes a decision algorithm for selecting from among various options for the exponential model. Generally the model is fitted until an adequate p-value

for the χ^2 goodness of fit (GoF) is obtained. The decision algorithm is provided below.

1. If the p-value for the GoF statistic is greater than 0.05, then the model's fit was considered adequate and the parameter estimates were used.

2. Otherwise (that is, if the p-value was less than 0.05, or no estimates resulted because the model did not converge), the horizontal asymptote was set to zero and the model was refit to the data.

3. If the p-value was still less than 0.05, or there was no model fit at all, then the highest dose was dropped and the model was refit with the horizontal asymptote set to zero until either the p-value exceeded 0.05, or there are only three doses remaining.

Although the user can specify options that are not consistent with default decision algorithm utilized by OPCumRisk, all BMD values provided in these analyses are based on the default decision algorithm. The decision algorithm and technical details of the "basic" exponential model used in this BMD analysis can be obtained at www.epa.gov/scipoly/sap/2001/september/rpfappendix1.pdf.

BMD values were calculated for the brain, plasma, red blood cell (RBC) ChEI data from both the single and repeated dose comparative ChE toxicity studies. Table 1 contains summary information from the OPCumRisk model runs for the AZM comparative ChE single dose toxicity study. For the single dose study, the decreasing exponential model adequately fit (goodness-of-fit p-value$<$ 0.05) both the neonate and adult data for all three compartments (brain, plasma, and RBC). However the estimated plasma BMD_{10} for adult males is outside of the experimental dose range. BMD estimates which are outside of the experimental dose range should be considered suspect and generally discarded.

Table 2 contains summary information from the OPCumRisk model runs for the AZM comparative ChE repeat dose toxicity study. For the repeat dose study, the decreasing exponential model did *not* adequately fit (goodness-of-fit p-value$>$ 0.05) some of the neonate and adult data for some of the compartments (brain, plasma, and RBC). Additionally, some of the BMD_{10} values were outside of the experimental dose range. However based on the BMD values that are valid, it can be concluded that the various subpopulations are generally more sensitive (i.e. have lower BMD values) to repeat dosing than single dosing.

For both repeat and single dosing regiments, RBC ChE is the most sensitive endpoint compared to brain and plasma ChE. Based on female brain ChE data for single dosing and the male RBC ChE data for repeat dosing, the $BMDL_{10}$ values indicate that neonates can be up to 3-4 times as sensitive to AZM than adults. Since adequate models fits were not attained or BMD estimates were outside of the experimental dose range for many of the ChE datasets from the repeat dose toxicity study, the same comparison between neonates and adults could not be made for the brain and plasma compartments.

Table 1. BMD Summary for ChEI Data from the AZM Comparative ChE Single Dose Toxicity Study (MRID# 46162101)

Compartment	Sex	Subpopulation	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	B set to zero?	Drop Highest Dose Group?	GoF p-value
Brain	Male	Adult	0.88	0.70	Yes	No	0.25
		Neonate	0.63	0.43	Yes	No	0.85
	Female	Adult	1.84	1.10	Yes	No	0.20
		Neonate	0.44	0.35	Yes	No	0.09
Plasma	Male	Adult	6.51*	0.64	Yes	Yes	0.14
		Neonate	0.60	0.40	Yes	No	0.50
	Female	Adult	1.20	0.59	Yes	No	0.17
		Neonate	0.47	0.35	Yes	No	0.08
RBC	Male	Adult	0.23	0.17	Yes	Yes	0.40
		Neonate	0.26	0.19	Yes	No	0.55
	Female	Adult	0.37	0.26	Yes	No	0.32
		Neonate	0.25	0.19	Yes	No	0.70

*Benchmark dose estimate outside of experimental dose range of 0.6 to 3.3 mg/kg/day

Table 2. BMD Summary for ChEI Data from the AZM Comparative ChE Repeat Dose Toxicity Study (MRID# 46239001)

Compartment	Sex	Subpopulation	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	B set to zero?	Drop Highest Dose Group?	GoF p-value
Brain	Male	Adult	13.85*	1.40	Yes	Yes	0.45
		Neonate	0.19	0.16	Yes	Yes	0.09
	Female	Adult	1.90*	1.14	Yes	Yes	0.03**
		Neonate	0.13	0.12	Yes	No	0.05
Plasma	Male	Adult	0.37	0.28	Yes	No	0.73
		Neonate	0.16	0.14	Yes	No	0.03**
	Female	Adult	0.39	0.25	Yes	No	0.02**
		Neonate	0.20	0.17	Yes	No	0.16
RBC	Male	Adult	0.21	0.16	Yes	No	0.61
		Neonate	0.06	0.05	Yes	No	0.65
	Female	Adult	0.09	0.08	Yes	No	0.08
		Neonate	0.07	0.06	Yes	No	0.03**

*Benchmark dose estimate outside of experimental dose range of 0.25 to 1.0 mg/kg/day

**Model did not attain adequate fit to ChE data

cc: Cathy Eiden

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	PC Code: 058001
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